The aplication of the fractal analysis in oncopathology

PhD Lutfi ALIA
Center of Oncological Prevention (Centre of excellence).
Siena – Italy

Summary

Fractal analysis is an objective approach that in oncopathology is one of the important fields of application. In this study is present fractal methodologies at histological level that have been successfully applied to characterize pathological features and able to perform differential diagnosis and prognosis in oncopathology. The basic principles and prospects of fractal geometry in pathology are promising. In particular, fractal analysis is emerging as a powerful tool to perform differential diagnosis and prognosis of the patients in cancer and other malignancies as well to improve the effectiveness and safety of patient care. All fractal objects have Fractal Dimension FDs, commonly calculated with box counting. Morphometry, the measure of shapes of the structures, can be added to every imaging technique in order to obtain objective indexes. In this field, fractal analysis has been applied to histopathology, cytopathology, and electron microscopy with great success. Performing fractal analysis of tissue samples, it's possible to make differential diagnosis between the early stage of tumours and flogosis, or among the different types of Basal Cell Carcinoma, and different grade of Invasive Bladder Carcinoma, well as to investigate the subtle alterations of the nuclear patterns in human breast tumours, or to evaluate other tumours. Fractal analysis shows a high ability for automatic classification of cancer cells in urinary smears, as well as to identify prostate cancer cells, has been able to distinguish

diagnostic/prognostic classes in the invasive bladder carcinoma and myelodysplastic syndromes. In the future, fractal analysis may help with the diagnosis, understanding of pathogenesis and management of the lesions.

Keywords: *Fractal analysis, fractal dimension, oncopathology, differential diagnosis, prognosis.*

Introduction

The first work using the concept of fractal geometry in cell biology was done by Paumgartner, Losa and Weibel which examined mitochondrial and reticulum membrane surface densities and found fractal behavior. [1, 2] Subsequently, in histology, fractal geometry has proved usefulness in describing the shape of neurons, glial cells, convolutions of the brain surface, retinal vasculature and in other tissues. [1, 2, 3, 4] In effect, living structures may be described as being in a self – organizing, fluctuating steady – state far from equilibrium. Self organization and a state far from equilibrium are characte-ristics of chaotic structures. Chaotic structures present fractal geometry, so is not too astonishing that the branching pattern of the airways in the lung, or the arterial vascular pattern of the cardiovascular system [5, 6, 7], colorectal polyps [4] anal intraepithelial neoplasia [16] and in the more tumour pattern as mycosis fungoides, basal cell carcinoma, urothelial carcinoma [8], brest cancer [12], brain tumours [13] etc, have been described with fractal properties.

Like coastlines a tumour examined by light microscopy has a complex, irregular border and retains a similar level of complexity over a range of magnifications. [4, 5, 8, 11, 12] Eucledian morphometric measurements were found to be invalid outside precisely defined conditions of resolution and magni-fication. [2, 3, 5, 12] Fractal analysis was used by the authors as a numerical method of describing the irregularity of the epithelial – connective tissue interface, in an attempt to provide an objective means of classifying the irregularity of the interface. So, moderate/severe dysplasia and squamous cell carcinoma were significantly distinguished by the fractal dimension values, from the normal epithet-lium and mild dysplasia. [4] Cross and co-workers [4] also applied fractal analysis to evaluate colorectal polyps. Studying 359 cases colorectal polyps the authors demonstrated that all the examined polyps had a fractal structure and the fractal dimension between these categories.

In this study, its applied fractal dimension analysis to study human tumours at light and ultrastructural levels, and presenting data obtained studying the epithelial - connective tissue interface in basal cell carcinoma of the skin, the boundaries of invasive bladder carcinomas (urothelial neoplasia), and the lymphocytic nuclear membrane in mycosis fungoides and chronic dermatitis.

Mandelbrot's concept of fractal geometry [1] is a powerful approach in order to precisely

Material and methods

The material of this study was derived from e series of 147 cases of basal cell carcinoma of the skin, (BCC), and 27 cases of invasive bladder carcinomas (IBC) were analysed.

Histopathology

A. Basal cell carcinoma (BCC). Haematoxylin and eosin-stained paraffin sections of each case of BCC were reviewed by a single pathologist and assigned to three diagnostic categories:

1. Circumscribed basal cell carcinoma (CBCC) are tumours composed of large islands (one or more) of basaloid cells, aggregated in cohesive clusters bound together by fibrovascular stroma. The tumour margins are convex and the neoplasm grows expansively with regular from the invasion.

2. Infiltrative basal cell carcinoma (IBCC) are tumours that lack central cohesive mass of basal cell islands as seen in solid BCCs, consisting of elongated islands and cords that are widely separated spatially.

3. Mixed basal cell carcinoma (MBCC), are tumours that have a mixture of solid and infiltrative growth pattern.

Five - micron - thick sections were stained with monoclonal antibodies against human cytokeratins and used for image fractal analysis.

B. Invasive bladder carcinoma (IBC). Haematoxylin and eosin-stained paraffin sections of each case of IBC were reviewed by a single pathologist and assigned to diagnostic categories and histological grade. Histopathological diagnosis of 27 cases, presented 12 cases resulted as a low grade, 8 cases Papillary Urothelial Neoplasm of Low Malignant Potential (well-preserved cell pattern, cells with increased and uniform size, fine and even chromatin, papillary and lose cluster cell arrangement); 4 cases are Low Grade Papillary Urothelial Carcinoma, while 15 cases of IBC areas high grade: High Grade Papillary Urothelial Carcinoma (cells with increased and polymorphic size, coarse and uneven chromatin, isolated and lose cluster arrangement).

Fractal analysis

Was performed by using the box-counting method, using software Visual Basic 3.0. Briefly, ich image was converted with a net of square boxes (4 to 100 pixel),

and the number of boxes containing any part of the outline was counted. A log-log plot of the reciprocal side length of the square against the number of outline – containing squares was performed. The slope of the best linear segment of the best linear segment of the graph represented the fractal dimension of the image.

To perform computerized image analysis and to obtain morphometric fractal indices, the first challenge is the tissue/cell segmentation, to separate noise and biological features. The second step is the feature selection to isolate the tissue, the cells, or the nuclei of interest. The last important challenge is the system of evaluation, e.g. the log-log plot in order to determine the fractal dimension of the skeletonized tissue/cell/nucleus, being the fractal dimensions the exponent of a log-log plot, meaning the self-similarity of the biological structure. In a possible approach, images are digitized, while aperture settings and conditions of illumination and magnification are kept constant. Single pixel outlines of the contours of the image are automatically obtained by using grey-level threshold segmentation. x 40 magnification may be suitable to evaluate tissue, x 100 magnification to analyze the distribution of nuclei. Image analyzer can be used to obtain the skeletonized images.

Results

Linear discriminant analysis was applied by a Wilks'lambda statistic, and the results summarised in a confusion matrix, in order to evaluate the predictive significance of fractal dimension with respect to the qualitative classification of basal cell carcinoma: none of the cases of circumscribed basal cell carcinoma was placed in the infiltrative basal cell carcinoma group. The percent of correct classification was 74 %, with $p < 0, 001$. (Table 1)

Variance analysis was used to analyze the samples of invasive bladder carcinomas of different histological grades:12 cases as a low grade (Figure 1), 8 cases Papillary Urothelial Neoplasm of Low Malignant Potential; 4 cases are Low Grade Papillary Urothelial Carcinoma; while 15 cases of IBC are High Grade Papillary Urothelial Carcinoma (Figure 2).

The lowest histological grade resulted in the lowest value of fractal dimension ($p < 0, 05$), while

the cases with high grade resulted in high values of fractal dimension ($p < 0, 05$). (Table. 2)

Fractal dimension value statistically differs between low grade (well preserved cellular pattern), respectively with high grade (low-preserved cellular pattern with pleomorphic cells and coarse chromatin).

FIG. 1. Papillary Urothelial Neoplasm of Low Malignant Potential

FIG 2. High Grade Papillary Urothelial Carcinoma.

Table 1. Fractal dimension of histological outlines of basal cell carcinoma tissue, confusion matrix between actual and predicted group membership, after discriminant analysis.

	Predicted Group Membership[b]			
Group[a]	Number of cases	1	2	3
CBCC	60	51 (85 %)	9 (15 %)	0 (0 %)
MBCC	39	12 (31 %)	21 (54 %)	6 (6 %)
IBCC	48	3 (6 %)	9 (19 %)	36 (57 %)

[a] CBCC – circumscribed basal cell carcinoma; MBCC – mixed basal cell carcinoma; IBCC – infiltrative basal cell carcinoma.

[b] Present of cases correctly classified = 74 %; $p < 0, 001$. Mean value ± standard deviation.

TABLE 2. Fractal dimension of histological outlines in two different histopathological type of Invasive Bladder Carcinoma (low grade, high grade).

Low grade	High grade	
IBC = 1, 35 ± 0, 20. n = 12	IBC = 1, 50 ± 0, 17 n = 15	p < 0, 05

IBC – infiltrative bladder carcinoma.
Note: Mean value ± standard deviation

Discussion

Lowered cost and increased power of computer system, have recently brought image analysis into many histological laboratories. Image analysis in oncological pathology is a powerful tool to isolate important discriminating factors useful in the diagnostic decision.

A lot of morphometric measurements have been performed to discriminate between different diagnostic classes, examples include the measures of nuclear diameter, perimeter, area and respective form factor. [1, 3, 4, 5, 6, 7, 8]

Fractal dimension gives an objective number that is able to characterize quantitatively a lesion close to a 100% correct classification. In effect, since many years, neoplasms in organs have been studied by fractal analysis for demonstrating differences between normal, dysplastic and neoplastic cells and tissues [2, 3, 5, 7], also estimating angiogenesis [10, 13], analysing monocytes in diabetes [10] for detection of prostate cancer on histopathological samples [17], evaluating the response of anticancer therapy as well as in order to determine the prognosis of the patient, in the squamous cell carcinomas of the larynx, the first work that used fractal dimension to perform prognosis [21], studying the fractal characteristics of chromatin observed in light and electron microscopy [10], or studying glioma tissues, being the fractal indexes able to differentiate the malignant grades of that tumour, [13] as well in radiomic approaches in order to predict pathological response after chemo-radiotherapy in rectal cancer [16]. Also in our hands, fractal analysis has been very able to distinguish among diagnostic classes linked to prognosis in cancer, studying bioptic samples in mycosis fungoides [8] as well in and myelodysplastic syndromes[19]

However, all these Euclidian parameters present many problems when applied to the highly irregular histopathological structure, for example, all are dependent on scale or, in other words, on the magnification. [3, 9, 10, 11, 12]. In the recent years, in the field of oncological pathology same works on concerning diagnosis and prognosis of patients. [17, 18, 19, 20, 21, 22]

Here we present our original data concerning the great capacity of fractal analysis to distinguish among diagnostic classes in the study of tumours. Fractal analysis, performed with box-counting method, has revealed the ability of this approach to distinguish between circumscribed and infiltrative pattern of basal cell carcinoma of the skin, thus originating a non subjective method to analyze that type of malign neoplasms. Using the box-counting technique over the tissue outlines in order to evaluate the geometric complexity [3,10,12, 15, 17] or the entropy [9, 14] of the sample. Also, fractal analysis has allowed us to obtain an index that distinguishes between urothelial carcinoma low grade, with urothelial carcinoma high grade.

References

01. Paumgartner D, Losa G, Weibel E. R. (1981) Resolution affects on the stereological estimation of surface and volume and its interpretation in terms of fractal dimensions. Jour. Micros. 121, 51
02. Losa GA, Nonnenmacher F. (1996) Self-similarity and fractal irregularity in the pathologic tissues.Mod. Pathol. 9; 174 – 182.
03. Losa GA (2009). The fractal geometry of life. Rev. Biology. 102: 29-59
04. Cross S. S. (1994). The application of fractal geometry analysis to microscopic images. Micron. 25 (1): 101 – 113
05. Luzi P, Bianciardi G, Miracco C, De Santi M. M, Del Vecchio M. T, Alia. L, Tosi. P: (1999) Fractal analysis in human pathology. (1999) Annales of New York Academy of Sciences. Vol. 879, 255
06. Bianciardi G, Miracco C, De Santi M. M, Del Vecchio M. T, Alia L, Perrone A, Luzi P. (2000) Fractal dimension analysis in human pathology. Jour. of Chaos Theory and applications. 61, 41.
07. Papagianni M. (2006) Quantification of the fractal nature of mycelial aggregation in Aspergillus niger submerged culture. Microb. Cell Fact.13; 5: 5 - 8.
08. Bianciardi G, Miracco C, De Santi MM, Luzi P (2003). Differential diagnosis between mycosis fungoides and chronic dermatitis by fractal analysis. J Dermatol Science 33: 184 – 186
09. Bianciardi G, Tanganelli I, Totagiancaspro D, Brogi M, Carducci A, De Santi MM (2006) Fractal analysis of monocytes in diabetes. Clin Hemorheol Microcirc 35:269-272
10. Bianciardi G, Traversi C, Cattaneo R et al (2012) Phase transition of Microvascular Network Architecture in Human Pathologies. Theor Biol Forum 1:37 – 45
11. Rajesh L, Dey P. (2003) Fractal dimensions in urine smears: a comparison between benign and malignant cells. Anal Quant Cytol Histology 25: 181-2.
12. Losa GA, Castel C (2005). Nuclear patterns of human breast cancer cells during apoptosis: characterization by fractal dimension and (GLCM) co-occurrence matrix statistics. Cell and Tissue Research 322: 257-67.
13. Di Ieva A (2010) Angioarchitectural morphometrics of brain tumours: are they any potential histopathological biomarkers? Microvascular Res 80: 522-33. (27)

14. Farahnaz N, Gholamreza B, Shahram S, Hamidreza FI, Zuhair MH, et al. (2011) Fractal Study on Nuclear Boundary of Cancer Cells in Urinary Smears. Iranian J Pathology 6: 63-7. (28)
15. Baljekar P. N. Patil H.A. (2012) A comparison of waveform fractal dimension techniques for voice pathology classification. *Proceedings of the IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP)*, (2012), pp. 4461–4464
16. Klonowski W. P. Stepien R. Stepien, R. Sedivy, H. Ahammer nnd S. Z. Spasic. (2018) Analysis of anal intraepithelial neoplasia images using 1d and 2d Higuchi's fractal dimension methods. Fractals Vol. 26, No. 03, 1850021 (2018)
17. Yu E, Monaco JP, Tomaszewski J, Shih N, Feldman M, Madabhushi A (2011) Detection of prostate cancer on histopathology using color fractals and Probabilistic Pairwise Markov models. Conf Proc IEEE Eng Med Biol Soc 3427-30.
18. Bianciardi G, Miracco C, Lazzi S, Luzi P. (2013) Fractal analysis of epithelial-connective tissue interface in basal cell carcinoma of the skin. Current Bioinformatics 8: 357-361.
19. Bianciardi G, Luzi P (2014) Fractal Analysis of the Bone Marrow in Myelodysplastic Syndromes. Current Bioinformatics 9: 408-13.
20. Soumya Ranjan Nayak, Jibitesh Mishra. (2021) Fractal dimension based generalized box-counting techniche with application to greyscale images. Fractals. Vol 29, Nr.03, 215
21. Zbiginiew Omiotek et. Al. (2020) Fractal analysis as a method for feature extraction in detecting Osteoporetic bone destruction. Fractals. Vol 29, Nr.04, 113 - 120
22. Alejandro Moreno – Gomez, Jose H. Macorro Lopez et al. (2020). Fractal dimension analysis for assessing the health condition of a truss structure using vibration signals. Fractals. Vol 27, Nr .08, 127 - 130