Full Length Research Paper

# Geometric diagnosis of erythrocyte morphophysiology

Catalina Correa<sup>1</sup>, Javier Rodríguez<sup>2</sup>, Signed Prieto<sup>3</sup>, Luisa Álvarez<sup>4</sup>, Benjamín Ospino<sup>5</sup>, Ángela Munévar<sup>6</sup>, Pedro Bernal<sup>7</sup>, Jessica Mora<sup>8</sup> and Sarith Vitery<sup>9</sup>

<sup>1-4,7</sup>Insight Group - Universidad Militar Nueva Granada and Centro de Investigaciones de la Clínica del Country, Bogotá, Colombia

<sup>1, 2, 8,9</sup>Research Area and Special Internship: "Mathematical and Physical Theories Applied to Medicine" - Universidad Militar Nueva Granada, Bogotá, Colombia

<sup>5,6</sup>Hospital Militar Central - Bogotá, Colombia

Abstract

Fractal geometry allows the unbiased characterization of objects irregularity. Fractal dimension has been applied to different diagnostic images in order to differentiate normality from disease. In this paper, 7 normal and 7 abnormal erythrocytes from patients and transfusion bags were analyzed. Software was developed in order to evaluate fractal dimensions and the number of spaces covered by the edge of the red blood cells in the 5x5 pixel grid in the Box Counting space. The surface occupied by the image in pixels was also analyzed in order to establish differences between normality and disease. A methodology of clinical application to diagnose erythrocyte morphophysiology was developed based on the ratio between the number of surface pixels and the number of spaces touched by the edge in the 5x5 grid. Values equal or greater than 174.7 represent normal erythrocytes, and values of 170.84 or below represent the abnormal ones. The developed methodology shows that the morphology and physiology of red cells have a geometric self-organization characterized by a mathematical proportion based on a simultaneous use of fractal and euclidian measures. This allows differentiating normality and abnormality for any particular case in an objective way.

Keywords: Fractal, erythrocyte, diagnosis, geometric, morphophysiology.

## INTRODUCTION

Fractal geometry was developed to solve the inconsistencies and paradoxes involved in the implementation of euclidian measures to irregular objects (Mandelbrot, 2000). For this purpose the fractal dimension concept and different methodologies for their calculation were developed. according to the characteristics of the measured object (Peigen et al., 1992b, c).

Its implementation has been useful in cells, tissues and irregular dynamics as the cardiac and immune system behaviour characterization (Goldberger et al., 1990, 2002; Burgos, 1996a, b; Rodriguez, 2006).

Besides, it has served as the basis for the development diagnostic methods and generalizations at of experimental and clinical levels. Such is the case of the methodology developed to differentiate healthy arteries from restenosed ones (Rodriguez et al., 2002), which served as basis to the establishment of the total number of fractal prototypes of coronary arteries. Fractal dimension has also been useful (Rodriguez et al., 2010b), in the clinical diagnosis of preneoplasic and neoplasic cells (Rodriguez et al., 2010c), or cardiac system evaluations (Rodriguez et al., 2011b) including a diagnostic method of left ventriculography (Rodriguez et al., 2012b) and an exponential law of cardiac dynamics that differentiates normality, disease and evolution between them (Rodríguez, 2011). These and other works show that in many cases the analysis of fractal measures by themselves does not allow differentiating normality from disease, being necessary to establish methodolo-

<sup>\*</sup>Corresponding Author E-mail: grupoinsight2025@yahoo.es. Phone: 0573134057252



**Figure 1.** Normal erythrocyte selected (left) and delineated with the software (right). The cells were treated with Wright dye, magnified with an increase of 100x through a microscope Axiostar Plus, and photographed with a digital camera Canon Power Shot 65, for their measurement with the developed software.

gies for further analysis.

Functional changes of the red cells are reflected in their cellular structure (Peñuela et al., 2003), for this reason, the screening methods of erythrocyte viability used nowadays for transfusions of packaged red blood qualitative morphological cells are based on characteristics, microscopically evaluated in red cells extended. This assessment depends on the subjectivity and experience of the specialist, because an objective diagnostic method has not been developed yet (Peñuela et al., 2003; Naotaka et al., 2000). Previous works demonstrate the relevance of applying fractals measures to the evaluation of the erythrocyte, finding it can be evaluated by fractal dimensions of the erythrocyte sedimentation rate (ESR) (Reuben et al., 1990; Sharma et al., 1996). The model developed by Tang et al. predicts the fractal dimension of sedimentation aggregates (Tang et al., 2002). Kuo et al., (1994) found that the ESR is an intrinsic constant characteristic of the interaction between erythrocytes and macromolecules, that can be introduced in the function of energy sedimentation law. They found that ESR allows defining symmetrical curves of sedimentation that describe a fractal dimension D and a new constant of ESR that explain the trend of the accumulation of fluid in the retraction clot. At a structural level, a characterization for red blood cells samples was developed, establishing parameters for viability evaluation; however, this method needs the measurement of several cells for the diagnostic determination (Rodriguez et al., 2008).

Since these works are based on kinetic behaviour of the samples or the evaluation of more than one cell in each sample, the purpose of this study is to develop a diagnostic method for clinical application of erythrocyte state based on the geometric analysis of morphological alterations observed in one erythrocyte. All these based on fractal and euclidean geometry, seeking an objective geometric diagnosis of erythrocyte morphophysiology.

## **METHODS**

Erythrocytes samples from patients and transfusion bags were selected. Transfusion bags were chilled to  $4^{\circ}$ C up for a period of 42 days, and diagnosed by a specialist in accordance with the conventional parameters. A total of 14 samples were selected and divided into two groups. Group A consist of 7 normal samples, three from patients with no erythrocytes alterations, with hemoglobin levels less than 9 g / dl, and a prescription of blood transfusion, one from an individual with normal diagnosis and finally three blood bags approved for transfusion. Group B consists of 7 samples diagnosed as abnormal, from 5 patients with different erythrocytes and two bags discarded by their non-viability for transfusion.

Samples were observed in an extended treaty with Wright dye, with an increase of 100x through a microscope Axiostar Plus. For each sample, a cell was chosen based on morphological features that were clearly representative for the states of normality or disease, in order to establish the mathematical characteristics of each state, which allow differentiating objectively the functionality degree for each particular red cell (see Figure 1). This methodology is based on objective and reproducible measures from representative cells of each sample, which makes the use of a large number of samples or statistical analyses unnecessary.

The cells were photographed with a digital camera Canon Power Shot 65 and transferred via serial interface to a computer. They were analyzed using software that calculates the fractal dimension using the following equation:

$$D = \frac{LogN(2^{-(K+1)}) - LogN(2^{-K})}{Log2^{k+1} - Log2^{k}} = Log_2 \frac{N(2^{-(k+1)})}{N(2^{-k})}$$

Where:

N: number of spaces that contain the edge of the object.



**Figure 2.** Abnormal erythrocyte selected (left) and delineated with the software (right). The cells were treated with Wright dye, magnified with an increase of 100x through a microscope Axiostar Plus, and photographed with a digital camera Canon Power Shot 65, for their measurement with the developed software.

**Table 1.** Fractal dimension, number of pixels touched by the red cell surface and number of spaces touched by the edge in the 5x5 pixels grid.

	_	N1	N2	N3	N4	N5	N6	N7
a	DF	0,9692	0,9030	1,0053	1,088	1,079	1,0969	1,0511
Ë	S	39.543	33.548	33.008	28.276	26.389	26.205	25.117
۶	Кр	177	160	162	147	144	150	135
		A1	A2	A3	A4	A5	A6	A7
lal	DF	1,0969	1,0095	1,1083	1,0874	1,0511	0,9970	0,9937
orn	S	17.809	30.102	24.503	27.164	24.958	21.070	18.629
Abn	Кр	150	184	154	159	180	149	138

**DF: Fractal Dimension** 

S: Number of pixels touched by the surface of red cell

Kp: Number of spaces touched by the edge in the 5x5 pixels grid

K: degree of partition of grid.

D: fractal dimension.

In addition, the number of spaces that were touched by the edge of image in the 5x5 pixels grid and the number of pixels touched by the surface of red cells were determined with the software. Subsequently, the number of pixels occupied by the surface was divided between the number of spaces occupied by the edge in the 5x5 grid and the values obtained were compared between the groups in order to establish differences between them.

This research was made according to the regulations established on articles 11 and 13 of the 1993 Colombia's Health Ministry resolution. The physical calculations are made on results of medically prescribed exams of the clinical practice, protecting the integrity and anonymity of participants. According to that, informed consents were not necessary.

#### RESULTS

Fractal dimensions presented values between 0,9030 and 1,1083, ranging between 0,9030 and 1,0969 for normal erythrocytes and between 0,9937 and 1,1083 for abnormal. The number of spaces that touch the edge of red cell in the 5x5 grid of Box Counting space ranged between 135 and 184, showing values between 135 and 177 for group A and between 138 and 184 for group B. The number of pixels touched by red cell surface were between 17.809 and 39.543, ranging between 25.117 and 39.543 in normal erythrocytes group and between 17.089 and 30.102 in abnormal group. (See table 1).

By dividing the number of pixels touched by the surface between the number of spaces touched by the edge in the 5x5 grid proportions between 118,76 and 223,40 were found. Normal values were equal to or

				C/Kn			
	N1	N2	N3	5/Kp N4	N5	N6	N7
Normal	223,40	209,67	203,75	192,35	183,25	174,7	186,05
Abnormal	A1	A2	A3	<b>A</b> 4	A5	A6	A7
ADHORMAI	118,76	163,59	159,11	170,84	138,65	141,40	134,99

**Table 2.** Ratio between the number of pixels touched by the surface and the number of spaces touched by the edge in the 5x5 pixels grid (S / Kp)

greater than 174, 7 in all cases, showing a maximum value of 223,40, while the abnormal were always equal to or less than 170,842, with a minimum value of 118,76, differentiating objectively normality and abnormality (see table 2).

# DISCUSSION

This is the first work in which a mathematical method for clinical diagnosis of the erythrocyte morphology and physiology is developed. It is based on the simultaneous use of fractal and euclidian measures, from the ratio between the number of pixels within the surface and the number of spaces of 5x5 pixels touched by the edge of red cell. This methodology improves conventional clinical diagnosis of erythrocyte viability for packaged red blood cells for transfusions establishing its functional capacity both in patient samples and transfusion bags in an objective and reproducible way, revealing a mathematical order underlying the qualitative characteristics currently observed in the extended. It is mathematically evidenced that red cells require a geometric structure that enables them to fulfill their role, meaning that any change in their structure implies a change in their normal activity.

None of the mathematic measures -fractal dimension, number of spaces covered by the edge of red cells in Box Counting grid or number of pixels touched by the surfaceevaluated by themselves, allow distinguishing normality from disease. The establishment of comparable values for measurements of the surface and the edge and their calculation. allow obtaining an objective rate measurement that takes into account the characteristics of irregular objects as well as their euclidian magnitudes, making an arithmetic counting of spaces occupied by the surface and the edge. Differences in the irregularity will vary according to the scale used for evaluation, finding that very small scales reveal irregularities that are not relevant in the differentiation between health and disease, while others do not show differences in the irregularity of both groups.

The evaluated cells were selected based on differences in morphological characteristics of normality and disease, enabling an objectively mathematical characterization that differentiate the functionality degree for each particular red cell. Thus, the use of large samples or statistical analyses is not necessary neither for the development of diagnostic methodology nor for its clinical application. It is possible to establish diagnostic differences only with a red cell taken from each sample, which can be selected as potentially pathological by the expert, thereby avoiding the characteristic impossibility of statistical analysis to make statements at an individual level.

Fractal geometry has shown its usefulness in the development of characterizations in medicine, allowing to objectively evaluate the intrinsic irregularity of structures and biological dynamics (Mandelbrot, 2000; Goldberger et al., 1990, 2002; Burgos, 1996 a, b). At a cytological level, morphological characterizations of red blood cells (Rodriguez et al., 2008), neurons (Milosević et al., 2007; Caserta et al., 1990) and bone marrow (Moatemed et al., 1998) have been developed, among others, as well as diagnostic generalizations of clinical application (Rodriguez et al., 2010 b, c; Rodriguez, 2011). At a tissue level, applications especially useful in diagnosing neoplasic alterations have been developed (Pohlman et al., 1996; Lefebvre et al., 1995; Luzi et al., 1999; Landini et al., 1993; Gazit et al., 1995, 1997; Baish et al., 2000).

However, it is not possible to establish diagnostic differences based on numerical ranges very often, so it is necessary to develop new concepts. One example is the work of Rodríguez et al., where the Intrinsic Mathematical Harmony concept allows differentiating normal and restenosed arteries, which is not possible with isolated fractal dimensions (Rodriguez et al., 2002). A similar method was applied to develop a diagnostic method of preneoplasic cervix cells, showing a geometric order underlying the cell structure, not evidenced with fractal dimensions evaluated independently (Rodriguez et al., 2010c). These types of work show that it is necessary to develop a theoretical understanding of fractal geometry and its relationship with euclidean geometry in order to establish its true scope and usefulness in medicine.

This methodology would be a generalization of the mathematical erythrocyte viability that optimizes the process of transfusion of packed red blood cells at a clinical level and it could be useful in developing automated methods. Such work is based on the search of mathematical intrinsic orders of the morphology and physiology, in order to develop an acausal perspective of Medicine. Just as in theories of modern physics as statistical mechanics (Tolman, 1979), quantum mechanics (Feynman, 1964) and chaos theory (Devaney, 1992; Peitgen, 1992a; Crutchfield et al., 1990), where causes no longer constitute the basis for revealing the order of nature. This search has allowed solutions for different medicine areas. For example, a clinical diagnostic method for cardiac dynamics was developed through the application of probability and entropy theories. It differentiates normality, chronic disease and acute disease, as well as evolution between these stages (Rodríguez, 2010a) and it has proven to be useful for the study of the heart dynamics evolution in the Coronary Care Unit (Rodriguez et al., 2011a). In molecular biology immunology, useful solutions for vaccines and development have been made. For example, there is a predictive theory of the peptides that bind to HLA class II (Rodriguez et al., 2009) or a theory that predicts the binding of malaria peptides to Red Blood Cells, with a specificity of 90% and a sensibility of 95% (Rodriguez et al., 2010a). Rodríguez et al. recently developed methods for the prediction of CD4 in HIV patients starting from the values of leukocytes and lymphocytes, based on the set theory (Rodriguez et al., 2011c) and probability theory (Rodriguez et al., 2012a). Also, methodologies for epidemics prediction have been developed, such as a method based on probability and entropy, for the prediction of malaria outbreaks in 820 municipalities from Colombia, in ranges of three weeks (Rodríguez, 2010b).

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