ANALYSIS OF MICROVASCULAR PATTERN ON HISTOLOGICAL SAMPLE OF MYOCARDIUM USING VORONOI SEGMENTATION

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Abstract

Remodeling of a microvascular network is common part of pathological changes associated with wide spectrum of diseases. Quantitative analysis of these alterations relies often on analysis of a point-pattern on the histological slide, i.e. on sections through the microvascular network only. Common techniques are based on the estimation of the average density of points representing section through microvessels on the histological image. This approach inherently omits the information about the regularity of the pattern. Thus, we used approach based on the Voronoi segmentation and chose the best statistical model of areas of Voronoi cells surrounding microvessels on 20 samples of human myocardium. The best model is based on the log-normal distribution. Parameters of the model for given data can be estimated as a mean and a standard deviation of logarithms of areas of Voronoi cells. Moreover, these parameters can be transformed to the widely used measure called the microvascular density.

Keywords

cardiovascular pathology, quantitative histology, medical image analysis, point-pattern analysis

Introduction

Remodeling of the structure of the microvascular network, i.e. the network composed of precapillaries, capillaries and postcapillaries, and/or the structure of the wall of microvessels inside the myocardium is a common alteration associated with a wide range of diseases and pathological states including the heart failure [1], the arrhythmia [2] or the cardiological complications of the type 2 diabetes [3]. The altered structure leads usually to the alteration of the function of the vascular supply at the microscopic level which may contribute to main pathological process or to late complications.

Even in the clinical research, structure of the microvascular network is usually analyzed on histological sections, i.e. on the two-dimensional sections through the three-dimensional structure. Despite several approaches to the analysis of the section exist, the most common technique is analysis of the microvascular density (*MVD*). The *MVD* is simply the mean number of microvessels which are perpendicular to the plane of the sections on the unit area on the microscopic image [4].

Common approaches to the analysis are focused on the biological interpretation [4] and omit information about the arrangement of microvessels on the section, i.e. properties related to the arrangement or the regularity rather to the amount points. There are numerous approaches to the analysis of the point-pattern overcoming the above mentioned limitation [5], but they are used rather in the ecology than in the histology. For this work, we chosen analysis based on the Voronoi segmentation (tessellation), which was used in some other works [6].

Material and Methods

For the analysis, samples of explanted hearts from our previous study were used. Patients and methods of harvesting are described in the publication [7]. Number of samples is smaller than number of samples in the publication [7] because of new processing and staining of remaining material was applied. Due to the aim of this study, we were blinded to the clinical records. The use of these samples is in concordance with both

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informed contents and Declaration of Helsinki (2000) of the World Medical Association.

Three-step immunoperoxidase detection of CD31 was performed. After antigen retrieval with tris chelaton III buffer (pH = 8.5), the endogenous peroxidase activity and the non-specific antibody binding sites were blocked. Next, the sections were incubated with a primary antibody. Mice primary antibodies anti-human CD31 (M0823, Dako Cytomation, Glostrup Denemark) at dilution 1:800 for 60 minutes at room temperature were used. Visualization was performed using a LSAB+ peroxidase kit (Dako, Denmark).

The microscope Leica DMLB (Leica Microsystems GmbH, Wetzlar, Germany) with 40 times magnification of the objective was used to capture at least 10 non-overlapping images from each sample. Each vessel with diameter smaller 10 micrometers was assumed to be a microvessel and was manually labeled as a center-point for further analysis. In order to mitigate an inter-observer variability, only one author performed this analysis (A.P.).

Voronoi segmentation of a set D containing n points P_i called center-points is a division of the set D to n non-overlapping subsets C_i called cells or Voronoi cells. Each cell C_i contains all points of D which are closer to P_i than to other points $P_j, j \neq i$. The distance is given by a metric, the Euclidean metric is the most common metrics used in applications [8].

The segmentation was performed by a short script written in the Octave language (ver. 6.2.0). Main segmentation was carried out using the function *voronoin*. Only complete cells were used for further analysis, i.e., cells touching the border of the image were excluded. Areas of complete cells A_i from all images of the sample were merged to one datafile. Areas A_i were calculated in pixels, before further analysis, the values were transformed to square micrometers.

Beside the Voronoi analysis, common *MVD* was also calculated as a number of the microvessels perpendicular to the plane of the section per unit area of the image. This value was based on the same set of points as for *MVD* calculated indirectly from areas of Voronoi cells.

Basic idea behind use of the Voronoi segmentation to the analysis of the point-pattern is based on the analysis of the areas A_i of the cells C_i related to given center-points P_i. An analytical solution of the statistical distribution of areas A_i is not known even for the simplest case, the point-pattern generated by a Poisson's process [8]. Therefore, some approximations must be used, appropriate approximation is chosen experimentally. Common approximations are Pareto distribution, log-normal distribution, exponential distribution, and gamma distribution [9]; we used all of them.

To fitting of the areas of the cells by each model, we used the R language (ver. 4.0.4), namely the function *fitdist* from the package *fitdistrplus*. Because of values of the distribution functions were necessary for the analysis, those from packages *stats* and *rmutils* were used as well. Akaike information criterium (AIC) was used as a measure of quality of the fitting. Parameters of the best model were the searched descriptors of the microvascular pattern.

Results

We used 20 samples; 8 were from the left ventricle, 9 from the right ventricle and 3 were from the interventricular septum. Manually measured *MVDs* were in range from 252 to 1000 capillaries per square micrometer. The Voronoi segmentation was performed for all samples. Example of the segmentation of representative sample is on Fig. 1.

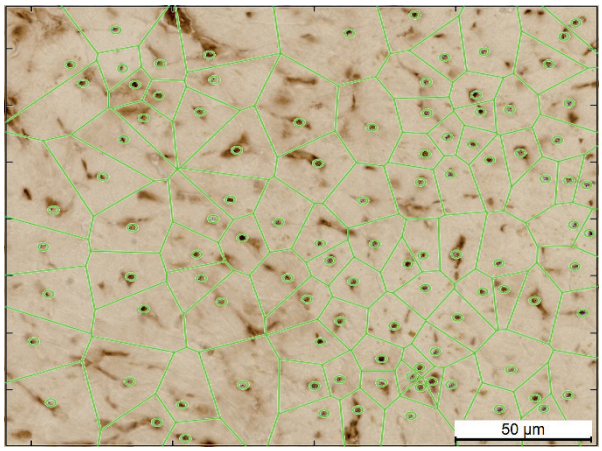

Fig. 1: Voronoi segmentation of representative sample. Green circles label the microvessels. Green polygons highlight borders of Voronoi cells. Immunostaining for CD31. Note that Voronoi cells probably crossing the border of the image are not included in the analysis.

For all samples and models, the lowest value of AIC was achieved for the log-normal distribution. Thus, we have chosen the log-normal distribution as the best model. Diagnostic plots for one sample are on Fig. 2.

Result of fitting by the gamma distribution was only slightly worse by the result of the log-normal distribution. Result of fitting by the Pareto distribution was obviously worse. Exponential distribution was the worst model, in some cases, the function *fitdist* fails in convergence.

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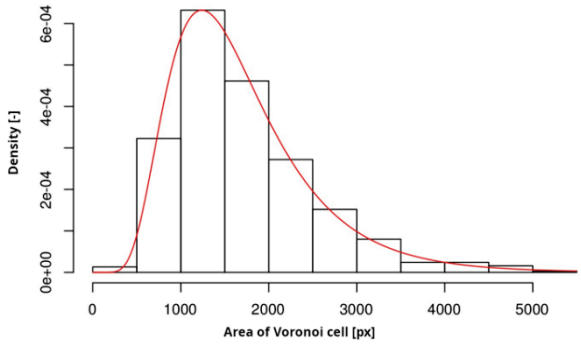

Fig. 2: Comparison of empirical and theoretical densities the log-normal model. Red lines are values according to the model, black boxes are measured data. Visually, the model is appropriate. Diagnostic plots of all samples are similarly satisfactory.

Discussion

According to our results, the best statistical model of areas A_i is the log-normal distribution. This distribution has two parameters μ and σ. Estimation of these parameters from data A_i is simple: μ is the arithmetic mean of logarithms of the areas of Voronoi cells and σ is the standard deviation of logarithms of the areas of Voronoi cells [10]:

$$\mu = \frac{1}{N}\sum_{i=1}^{N} \log A_i \qquad (1)$$

and

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N} (\mu - \log A_i)^2}. \qquad (2)$$

Mean value of the areas A_i is not the parameter μ. As a first raw moment, it can be calculated as follows [10]:

$$M_1 = \exp\left(\mu + \frac{\sigma^2}{2}\right). \qquad (3)$$

Common interpretation of M_1 is mean area of Voronoi cells. Relation with the surface density of points/microvessels, i.e. with the *MVD*, is thus the inverted value:

$$MVD(\mu,\sigma) = \exp\left(-\mu - \frac{\sigma^2}{2}\right). \qquad (4)$$

In addition to the measured value of the *MVD*, we now have the estimated *MVD(μ,σ)* from the parameters of the model. Linear correlation of these values is very good (Pearson's correlation coefficient $r = 0.9940$, $p < 2.2 \cdot 10^{-16}$) and the linear model has good adjusted coefficient of determination $R^2 = 0.9873$, but small non-zero offset and non-one slope are present, see Fig. 3 and Eq. 5:

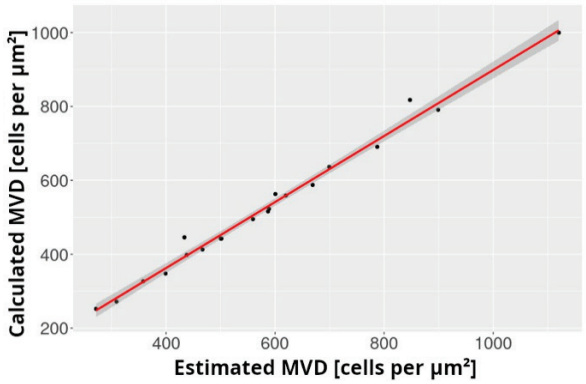

Fig. 3: Linear correlation of the estimated value MVD(μ,σ) (abscissa) and the measured value MVD (ordinate). The red line is the trendline with its 0.95 confidence interval (grey). Offset and slope of the trendline are coefficients in the Eq. 5.

$$MVD = 5.084 + 0.894 \cdot MVD(\mu,\sigma) \qquad (5)$$

The non-zero offset and non-one slope are caused by slightly different information used for the calculation. Direct measurement takes whole areas, whereas the calculation from the model excludes cells crossing the borders of the image. It means that *MVD* obtained by direct measurement cannot be compared with the *MVD* calculated from the parameters of the model. If the comparison is necessary, transformation given by Eq. 5 can fix this issue. Scatter plot of parameters μ and σ (see Fig. 4) indicates that information about the structure of the point-pattern is carried by both parameters.

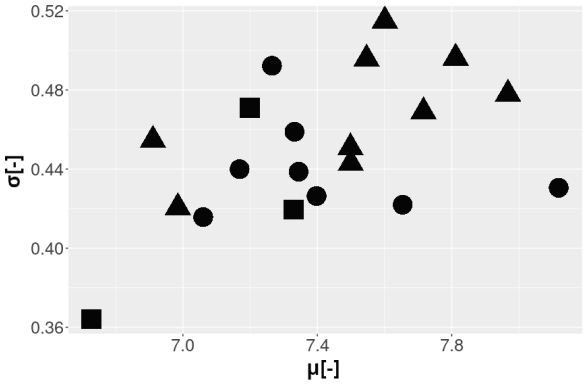

Fig. 4: Scatter plot of parameters μ and σ calculated from areas of Voronoi cells. Boxes indicate the left ventricle, diamonds indicate the right ventricle, and discs indicate the interventricular septum.

Parameters μ and σ fully describe the model and they are appropriate for comparison of two or more sets of samples in order to search for differences. For biological interpretation, parameters of the file of areas A_i can be more appropriate. For simplification, we can now assume that main part of microvessels are capillaries.

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Mean area of Voronoi cells M_1 is given by Eq. 3. Higher M_1 indicates bigger areas supported by one vessel and vice versa. If the area of supply is big, one can expect lack of nourishment with metabolic consequences [6]. Moreover, the inverted value of M_1 is the *MVD* which is often used in the research [4].

Standard deviation of areas of Voronoi cells S is not the same as σ, it can be expressed as follows [10]:

$$S = \sqrt{(\exp(\sigma^2) - 1) \cdot (\sigma^2 + 2\mu)}. \quad (6)$$

Higher value of S indicates more pronounced irregularities of areas of supply related to one capillary, higher number of too big or too small areas. These irregularities can be caused, e.g., by an angiogenesis (production of new capillaries) or by presence of areas of poor blood supply like scars.

Skewness K is a measure of asymmetry of the distribution. In case of the log-normal distribution, K is not constant, but it depends on both parameters of the model [10]:

$$K = \left(2 + \exp(\sigma^2)\right) \cdot \sqrt{\exp(\sigma^2) - 1} \quad (7)$$

Negative value of K indicates more pronounced left tail of the distribution and vice versa. Therefore, negative value of K means that there are more common areas A_i of which size is below the mean M_1, whereas positive value of K means that there are more common areas A_i of which size is over the mean M_1.

For areas of Voronoi cells in our set in square micrometers, values of S are in the range from *23.66* to *503.86* and values of K are in the range from *0.9661* to *2.3951*. For demonstration, Fig. 5 and Fig. 6 show capillary pattern of sample with the lowest and the highest values of S and K respectively. These images are generated from raw data with centerpoints of capillaries.

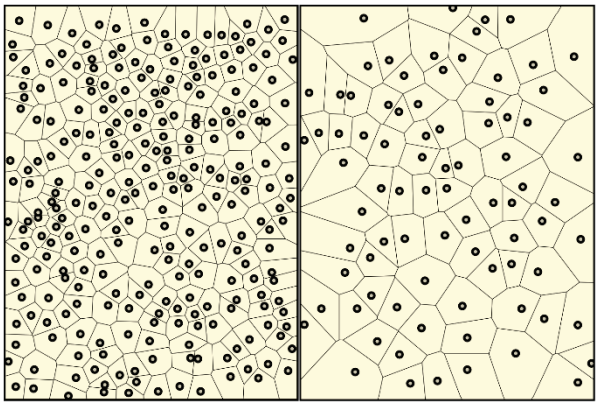

Fig. 5: Influence of S on the capillary pattern. Circles are in sites of capillaries, lines are borders of Voronoi cells. Point pattern and its Voronoi segmentation of samples with the lowest (left, S = 23.33) and highest (right, S = 503.86) values of S in our set. The scale bar is the same for both images. Pattern with smaller S shows smaller variability in size of Voronoi areas. Parameter S depends on the size of the cell as well.

Of course, measures M_1 (see Eq. 3), S (see Eq. 6), K (see Eq. 7) and other measures can be calculated directly from the raw datafile, but it is not so useful. They do not contain more information than parameters μ and σ. These additional measures are rather the aid for biological interpretation of the result of the analysis.

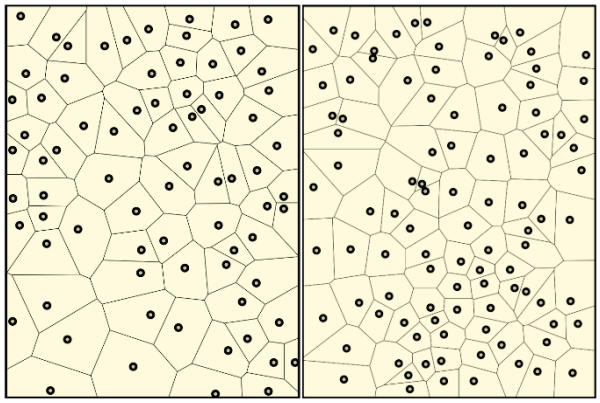

Fig. 6: Influence of K on the capillary pattern. Circles are in sites of capillaries, lines are borders of Voronoi cells. Point pattern and its Voronoi segmentation of samples with the lowest (left, K = 0.9661) and highest (right, K = 2.3951) values of K in our set. The scale bar is the same for both images. Pattern with higher K shows higher number of smaller Voronoi cells. In means that there are more nests of crowded capillaries on the section.

Conclusion

Areas of Voronoi segmentation of the point-pattern given by the pattern of microvessels on histological section of myocardium give rise two parameters μ and σ described by Eq. 1 and Eq. 2, respectively. These parameters can be easily transformed to the classical morphological parameter *MVD*, but there are some systemic differences which prevent direct comparison. This observation means that connection of results based on simple calculation of *MVD* and results based on Voronoi segmentation and subsequent indirect calculation of *MVD* needs some caution. Eq. 5 shows linear model of relationship between two ways of calculation of *MVD* from the same point pattern, but this model is probably sensitive on data and not general rule for transformation.

By other hand, the above-mentioned parameters contain more information about the point-pattern than the *MVD* only. Parameters μ and σ can be transformed by simple calculations to common statistical measures of the set of areas of Voronoi cells which can have more obvious biological meaning. For example, for biological understanding of blood supply are important parameters related to mean area belonging to one capillary, i.e. parameter M_1, variation of size of these

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areas, i.e. parameter *S*, or tendency to having crowded or sparse capillaries in the tissue, i.e. parameter *K*.

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