Predictive model of synovial membrane degradation using semi-automated morphometry and artificial neural networks

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Abstract
Gonarthrosis is a degenerative disease that affects mainly older people, but whose incidence has increased significantly in the last decade in population under the age of 65. The main objective of this study was developing a predictive model of synovial membrane degradation in relation to local nerve structures in patients with knee osteoarthritis, based on advanced morphometry and artificial neural networks (ANNs). We present here a pilot test of the method, describing preliminary findings in analyzing a pre-set number of images. We tested the system on a pre-defined set of 50 images from patients suffering of gonarthrosis in different stages. Biological material used for the histological study was synovial membrane fragments. We included 50 anonymized images from 25 consecutive patients. We found significant differences between mean fractal dimensions (FDs) of histological elements of normal and pathological tissues. In the case of immunohistoch emistry, we found statistically relevant differences for mean FDs of all antibodies. We fed the data to the ANN system designed to recognize pathological regions of the examined tissue. We believe that further study will have an important contribution to the development and will bring new local targeted therapies. These could slow or reverse joint damage and pain relief in patients with osteoarthritis.

Keywords: synovial membrane, gonarthrosis, morphometry, artificial neural networks.

Introduction
Gonarthrosis is a degenerative disease that affects mainly older people, but whose incidence has increased significantly in the last decade in population under the age of 65 [1]. This disease has an increased debilitating potential, leading to significant care costs and a significant decrease in quality of life [1–5].

Several risk factors associated with the occurrence of osteoarthritis have been documented so far, such as genetic predisposition [6], aging [4], obesity [7], and perturbations in existing alignment in the affected joints [9]; however, the pathogenesis osteoarthritis remains largely unknown [1, 4, 6, 9–11]. During the disease there are changes in all parts of the joint (cartilage, synovium, bone underlying), ultimately leading to its total compromise [11]. Structural changes in the synovial membrane, in osteoarthritis of the knee, are varied, from the hypertrophy and hyperplasia, in synovial cell growth, the appearance of inflammatory infiltrate and the appearance of neovascularization at this level [12–15].

Morphometric analysis of histological images can be considered a borderline method when using fractal dimension [16–20], combined with fractal box counting methods providing information both on existing structures areas in an image, as well as to the nature of each investigated element [21]. Computer-aided diagnosis systems can provide new possibilities in the diagnosis and staging of tumor. Artificial neural networks (ANNs) are the result of research in medical informatics, representing a form of artificial intelligence [22–25].

Little is known about the role of synovial membrane in the initiation and propagation of pain in adult patients with osteoarthritis and how it can affect synovial membrane degradation. There are studies that demonstrated the role of neuropeptides expressed in the synovial membrane in the initiation and propagation of local pain [26]. It was established that there is a correlation between increased densities of neuropeptides in the synovial membrane in the perception of painful sensation in patients with osteoarthritis compared to patients with other pathology located at this level [27]. The changes in the synovial membrane may be a predictor of the occurrence of pain [28, 29] in patients with osteoarthritis, also may be associated with the degree of alteration of other structures that are part of the joint [27–31].

Aim
The main objective of this study was developing a predictive model of synovial membrane degradation in relation to local nerve structures in patients with knee osteoarthritis, based on advanced morphometry and ANNs. We present here a pilot test of the method, describing preliminary findings in analyzing a pre-set number of images.

Patients and Methods
Patient selection
We tested the system on a pre-defined set of 50 images from patients suffering of gonarthrosis in different stages,
according to the *American College of Rheumatology* (ACR) criteria, whom were investigated with non-invasive imaging techniques and underwent arthroscopic diagnosis in the affected knee. Each patient signed a consent, in which he or she were presented with all the information relating to participation in the study. No personal details were disclosed or taken into account and the study was approved by the local Ethics Committee.

**Histological study**

Biological material used for the histological study was synovial membrane fragments obtained from patients diagnosed with gonarthrosis, according to ACR criteria, as well as from patients with meniscal pathology of the knee. Biological material collected immediately after sampling was placed in 10% neutral formalin fixing solution and held for 48 hours in it. After completion of fixing, synovial membrane fragments were further processed by classical histological technique for inclusion in paraffin that allowed for serial sections of 4–5 μm thickness that can be stained and studied optimally with the optical microscope. To highlight the histological structures, we used routine staining with Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome staining.

**Immunohistochemical study**

Immunohistochemistry is a technique for the identification of cellular and tissue antigens as a result of antigen–antibody interactions. Immunostaining relies on the affinity between the antigen and the antibody, the antibodies binding site is identified either by the direct method of antibody staining or staining using an indirect method, in which the marking is made by the appointed secondary and tertiary antibodies. To highlight immunohistochemical tissue antigens, a two-staged method was used based on a polymer network visualization system (Dako EnVision). In order to start sequences in immunohistochemistry, the samples were first deparaffinized in three successive baths of xylene (15 minute each), and then rehydrated by washing in a decreasing concentration alcohols (100%, 70%, 50%, 15 minutes each). The sections were then brought into distilled water to remove all traces of the alcohol from the histological samples. Finally, the histochemistry, the samples were first deparaffinized in

Designing the prediction model based on complex morphometry techniques and ANNs

To explore the immunolabeled as well as Hematoxylin-stained areas of interest, image segmentation was performed semi-automatically by identifying the corresponding channel of the color brown (#964B00 hexadecimal, RGB triplet 150, 75, 0) and blue color (#0000FF hexadecimal range, the red-green-blue triplet 0 0 255), with a standard deviation of ±10 points hexadecimal color variations for inherent color tones. The program eliminated decor items that did not contain important information for qualitative and quantitative analysis. We obtained fractal dimension (FD) through the box-counting algorithm implemented in an in-house developed software using the MATLAB (MathWorks, Massachusetts, USA) platform.

We entered the data obtained from the complex imaging study into an ANN prediction system, which was created in the Simulink simulation suite using the same MATLAB (MathWorks, Massachusetts, USA) platform. Computer-aided diagnostic systems often rely on ANNs. They are programs designed to reproduce in code the simplified topography of a working human brain; neurons are divided into layers and provide transmission or processing functions. Our system had a basic layout – one neuron for data input, a hidden middle layer for decision and two output neurons – one for pathological and one for normal images. The ANN system recreated the decision-making algorithm of the human brain, transposing it in computer language. Input data was fed to the first neuron, which associated a transfer function and a self-calculated bias and transmitted it to the middle layer, which made a decision of either pathological or normal image, based on previous training and human correction.

**Results**

We included 50 anonymized images from 25 consecutive patients presenting with various stages of arthritis (Table 1). We observed the predominance of female patients (58% vs. 42% male); the majority of patients had rural residence for both genders (37 patients in total, 74%). We observed a higher incidence of stage III gonarthrosis (18 patients, 36%) when compared to other stages (Figure 1).

Table 1 – Demographic data of the studied group and distribution of disease stages according to gender

<table>
<thead>
<tr>
<th>Disease stage</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>I</td>
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<td>III</td>
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<td>11</td>
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<td>IV</td>
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![Graphical representation of the distribution of disease within the studied group.](image-url)

We have then selected coupled images showing normal tissue and pathological regions, from the same patient Figure 2. We used the data resulted from the analysis of these pairs to test our ANN system.
We found significant differences between mean FDs of histological elements of normal and pathological tissues (Kruskal–Wallis test, \( p < 0.001 \)). The mean FD for Hematoxylin was 1.542 for normal tissue and 1.783 for affected areas. Mean FDs for GS trichrome staining were 1.442 and 1.883, respectively.

In the case of immunohistochemistry, we found statistically relevant differences for mean FDs of all antibodies, as follows: CD45RO – 1.521 vs. 1.817; CD68 – 1.339 vs. 1.612; CD31 – 1.11 vs. 1.473; VEGF – 1.269 vs. 1.518 and CD20 – 1.323 vs. 1.615.

We fed the data to the ANN system designed to
recognize pathological regions of the examined tissue. We performed the training phase with 30% of the images, and two human operators crosschecked the data and adjusted the bias values. We obtained excellent results after training the system for 12 epochs, correctly recognizing 45 images of the total 50 provided. The system inconclusively classified three images; it only wrongly classified two as pathological (false positive).

Discussion

The main idea behind our study was developing and testing of a prediction model of synovial membrane degradation in relation to local nerve structures in patients with osteoarthritis, aims to develop new therapies that may slow and/or reverse joint damage and lead to pain relief in patients with osteoarthritis.

Gonarthrosis has a profound economic impact on our society with high health care costs and their potential augmentation, taking into account factors such as an aging population and increased incidence of disease among adults [2, 3]. By 2030, it is estimated that 25% of the adult population in the United States will be affected by osteoarthritis [4, 5]; it is currently responsible for 90% of knee arthroplasties [6].

Current non-invasive therapeutic options with a short-term effect have proven to be ineffective and fail to adequately address the physiological and biochemical mechanisms underlying the emergence and evolution of the disease. The occurrence of pain is the main determinant symptom and is the reason for high addressability [32–35]. The presence of pain and its increased intensity among patients has important repercussions that can lead to symptoms such as absenteeism from work, everyday tasks failure, loneliness and can even reach severe anxiety and depression [36, 37].

A connection between the clinical and functional status of patients with osteoarthritis and how they perceive and tolerate pain occurring at this level possibly exists, involving the synovial membrane changes at different stages of the disease [38–41]. Description of synovial membrane morphology of the area affected by the seemingly unaltered areas in patients with osteoarthritis of the knee, in different stages of the disease was never attempted; microscopic appearance of the synovium in patients with other types of pathology of the knee joint osteoarthritis excluding typical changes, followed by the description of the disruption of structures that are part of the knee joint, and presentation of quantitative and qualitative changes that occur in the vasculature of the affected synovial membrane and in areas with normal macroscopic aspect in patients with osteoarthritis and those with other types of disorders of the knee joint [42, 43].

The appearance and propagation of pain in patients with gonarthritis is due both to local nerve structures malfunction (peripheral nerve structures) and the imbalance occurred in the central nervous structures [44–50]. Among the components of the joint, the synovial membrane, is considered the main component in the appearance of local pain [48–53] due to increased density of nociceptors at this level and the large number of free nerve endings [51, 52] that express on their surface neuropeptide involved in the propagation of painful stimuli [14, 53–56]. In both synovial membrane layers, the neural network is extensive, having a well-documented role in the initiation and propagation of local painful sensation [13, 57, 58].

A prediction model of synovial membrane degradation will provide new information leading to the detection of pathological and pathophysiological mechanisms presently unknown, which is the basis of occurrence and spread of pain in parallel with the degree of degradation of the synovial membrane in patients with osteoarthritis, data that will be extremely useful to orthopedic doctors, rheumatologists and medical rehabilitation doctors, which frequently face this pathology.

Conclusions

Demonstration of a direct link synovial membrane degradation in parallel with the emergence and spread of painful sensation at this level can lead to a new approach to pain therapy in patients with knee osteoarthritis. We believe that further study will have an important contribution to the development and will bring new local targeted therapies. These could slow or reverse joint damage and pain relief in patients with osteoarthritis.

Conflict of interests

The authors declare that they have no conflict of interests.

References


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