

ORIGINAL ARTICLE

Development of random forest machine learning model for the detection of changes in liver tissue after exposure to iron oxide nanoparticles

✉ Jovana Paunović Pantić¹, Danijela Vučević¹, Igor Pantić^{1,2,3,4},
Svetlana Valjarević^{1,5}, Tatjana Radosavljević¹

¹ University of Belgrade, Faculty of Medicine, Belgrade, Serbia

² University of Haifa, Haifa, Israel

³ Khalifa University of Science and Technology, College of Medicine and Health Sciences, Abu Dhabi, UAE

⁴ Ben-Gurion University of the Negev, Faculty of Health Sciences, Be'er Sheva, Israel

⁵ Clinical Hospital Center "Zemun", Belgrade, Serbia

Received: 6 October 2023

Revised: 25 October 2023

Accepted: 10 November 2023



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Funding information:

This research was supported by the Science Fund of the Republic of Serbia, grant No. 7739645 SensoFracTW, and the Ministry of Education and Science of the Republic of Serbia grant No. 451-03-66/2024-03/200110, subgrant "Development of artificial intelligence models based on the random forest algorithm for the detection of discrete structural changes in the cell nucleus".

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Competing interests:

The authors have declared that no competing interests exist

✉ Correspondence to:

Jovana Paunović Pantić

University of Belgrade, Faculty of Medicine,

Department of Pathophysiology,

9, Dr Subotica Street, 11000 Belgrade, Serbia

Email: jovana.paunovic@med.bg.ac.rs

Summary

Introduction/Aim: The aim of our study was to create a machine learning model, specifically a random forest model, which uses textual data from liver micrographs to differentiate between normal hepatic tissue and damaged tissue exposed to iron oxide nanoparticles.

Material and Methods: Regions of interest in micrographs of hepatic tissue, obtained from mice treated with iron oxide nanoparticles and controls, were analyzed using the gray-level co-occurrence matrix (GLCM) method. The resulting GLCM features were employed as input data for the training and testing of the random forest model using the "Scikit-learn" library in the Python programming language. Additionally, a conventional decision tree model was developed, based on the classification and regression tree (CART) algorithm.

Results: The random forest model outperformed the alternative CART decision tree approach in terms of classification accuracy, correctly predicting the class for 73.67% of the instances in the validation ROI dataset. The area under the receiver operating characteristic curve was 0.81, indicating relatively good discriminatory power. The F1 score for the model was 0.74, showcasing fairly good precision and recall, though not perfect.

Conclusion: The data obtained from this study may be utilized for further development of artificial intelligence computation systems to identify physiological and pathophysiological changes in hepatic tissue. The results also serve as a starting point for additional research on the automation of histopathological analysis of liver tissue exposed to external toxic agents.

Keywords: artificial Intelligence, machine learning, iron oxide, liver

INTRODUCTION

Rapid development of narrow artificial intelligence (AI) has led to a higher degree of automation in fundamental biomedical research as well as novel and innovative approaches to detection of physiological and pathophysiological phenomena. Nowadays, narrow artificial intelligence strategies based on supervised machine learning are often used for facilitation of signal analysis in various medical fields. Supervised machine learning is focused on presenting the machine (computer) with a series of examples with known input and output (target) data after which the machine learns to associate the data and identifies the complex patterns in data organization. Due to these new associations, subsequently developed machine learning algorithm acquires the ability to independently predict target data from new inputs. Some examples of supervised machine learning algorithms include the ones based on random forests, multilayer perceptrons, support vector machine and naive Bayes. All of these approaches can be used for versatile classification or regression tasks and have numerous potential applications in predicting analytics in medicine and biology (1-4).

Random forest algorithms are especially powerful and robust when used for classifying biological structures, based on experimental data derived from signal analysis. Random forest is an ensemble machine learning method that constructs a variety of decision trees connecting the input and target data. For classification tasks, trees in a Random Forest are typically constructed from bootstrapped samples of the original dataset and the final classification information is determined based on majority voting from these trees (5-9). This approach may provide high accuracy, especially when using data obtained from two-dimensional signals such as digital micrographs in pathology and pathophysiology. Also, such models can be both robust and flexible, they are resilient to overfitting data while at the same time they can handle a mix of categorical and numerical variables. In the past, random forest machine learning models were developed on numerous occasions to classify cells previously exposed to toxic substances and environments, and there are indications that they can be used as additional tools for differentiating normal from pathological structures in various experimental conditions (5, 6, 10).

Our previous research has shown that it is possible to develop a random forest model capable of separating normal from damaged cells based on gray-level co-occurrence matrix (GLCM) features (11). Gray-level co-occurrence matrix is essentially a contemporary and innovative signal analysis mathematical and second-order statistical technique used for extracting information from two-dimensional signals. Features such as angular second moment which is an indicator of textural uniformity and inverse difference moment as an indicator of local homogeneity can indeed serve as useful quantifiers of mi-

croscopic changes that cannot be observed even by most experienced microscopy experts. Previously, it has been shown that these features exhibit significant changes in liver tissue and cells exposed to potentially toxic chemical agents such as iron oxide nanoparticles (12). It has also been suggested that GLCM features could be used as inputs for random forest and other supervised machine learning algorithms to classify and predict discrete morphological and functional changes in the liver following the exposure to iron nanomaterials.

In this paper we have demonstrated that it is indeed possible to create a relatively accurate random forest model that uses GLCM data of liver micrographs for differentiation between normal and pathological tissue. We have shown that the model has relatively high discriminatory power in separating regions of interest of the liver tissue exposed to low dose of iron oxide nanoparticles from intact tissue despite their morphological similarity and a lack of conventional microscopic indicators of tissue damage. The model outperformed the alternative approach based on the classification and regression tree algorithm demonstrating its potential for further application in contemporary pathophysiology and pathology research.

MATERIAL AND METHODS

In this research we used the data obtained from 20 male, healthy C57BL/6 (C57 black 6, B6) mice divided into two groups (N=10). The experimental group received Iron (II,III) oxide nanoparticles (80-100 nm, Hongwu International Group Ltd. HWNANO materials, Guangzhou, Guangdong, CN) for 3 days, each day in a dose of 3 mg/kg. The controls received IP physiological solution for the same period. The study was a part of a PhD thesis research which received approval from both the Ethics commission for the protection and welfare of experimental animals of the University of Belgrade, Faculty of Medicine (approval number 229/2) and the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia - Veterinary Division (approval number 323-07-07783/2020-05). The procedures that followed were in line with the Universal Declaration of Animal Welfare (WSPA, London, 2000), the European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Reasons (1998), and various other domestic and international guidelines promoting responsible and compassionate treatment of experimental animals.

The liver tissue was acquired from all animals and fixated in Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) after which it was embedded in Paraplast, as previously described (13). The tissue sections were stained using the conventional Hematoxylin – Eosin technique. The digital micrographs

of liver tissue were obtained using Pro-MicroScan DEM 200 instrument (Oplenic Optronics, Hangzhou, CN) attached to OPTIC900TH Trinocular Biological Microscope (COLO LabExperts, Novo Mesto, Slovenia). The micrographs were sized 1200 x 1600 pixels with bit depth of 24 and horizontal and vertical resolution equaling 96 dpi (**Figure 1**). Based on the micrographs, a total of 2000 Regions of interest (ROIs) were selected and analyzed: 1000 ROIs of the tissue exposed to IONPs and 1000 ROIs from controls. The ROI selection and GLCM analysis were done in Mazda software, previously created for COST B11 European project “Quantitative Analysis of Magnetic Resonance Image Texture” (1998-2002) and COST B21 European project “Physiological modelling of MR Image formation” by researchers from the Institute of Electronics, Technical University of Lodz (TUL), Poland (14, 15).

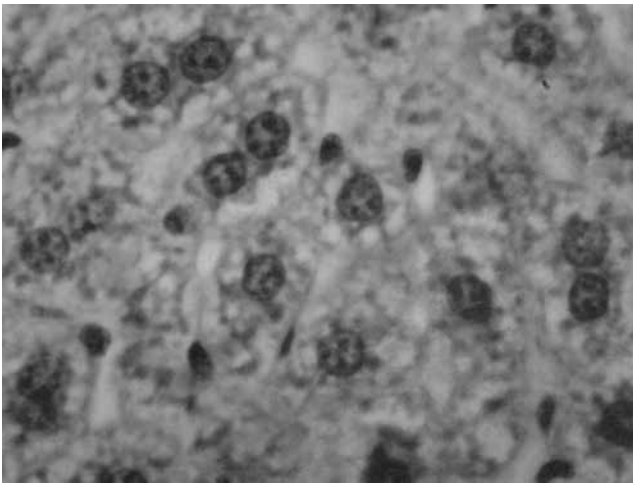


Figure 1. An example of gray-scale digital micrograph of hepatic tissue in 8-bit format suitable for GLCM analysis.

During the GLCM analysis, the resolution units of the grayscale ROI are associated with gray level values after which a complex second-order statistical and mathematical analysis is performed taking into account the value pairs. Similarly to our previous works (11, 16), we calculated the GLCM features of inverse difference moment (IDM), angular second moment (ASM), in line with the following formulae:

$$ASM = \sum_i \sum_j \{p(i, j)\}^2$$

$$IDM = \sum_i \sum_j \frac{1}{1+(i-j)^2} p(i, j)$$

In GLCM algorithm, the $p(i, j)$ represents the (i, j) th entry of the normalized co-occurrence matrix, whereas the mean and the standard deviation respectively of rows x and y are marked as μ and σ . Inverse difference moment and angular second moment correspond to the levels of local textural homogeneity and uniformity, respectively.

GLCM Contrast (CON), GLCM Correlation (COR), and GLCM Sum Variance (SVAR) were calculated as:

$$CON = \sum_i \sum_j (i - j)^k P_d[i, j]^n$$

$$COR = \frac{\sum_i \sum_j (ij)p(i, j) - \mu_x \mu_y}{\sigma_x \sigma_y}$$

$$SVAR = \sum \left[i - \sum ip_{x-y}(i) \right]^2$$

Textural contrast and correlation are the degree of variation of gray level intensities and the linear dependencies of gray values, respectively, while the variance represents the degree of dispersion of the distribution of gray values (17-19).

Initial statistical analysis was done in SPSS software (v.25.0; IBM, Chicago, IL). The Random Forest (RF) classifier, a widely accepted ensemble learning method was used to train and test the model in Scikit-learn library for Python programming language (20). In this model, a multitude of decision trees are constructed based on a random subset of features at each split. We used all 5 GLCM indicators as input data whereas the designated target was the class of the ROI, in terms of its affiliation to the experimental or control group. This approach and Python code were previously developed on yeast cell model as a part of the SensoFracTW project supported by the Science Fund of the Republic of Serbia. Approximately 80% of the data were used for training while the remaining data were used for testing and calculation of classification accuracy. The discriminatory power of the model was determined through receiver operating characteristics analysis. An alternative, decision tree model, based on the classification and regression tree (CART) classifier was also created in Scikit-learn with similar settings.

RESULTS

The average values for GLCM angular second moment were 0.014 ± 0.002 and 0.016 ± 0.003 for the experimental and control ROIs, respectively ($p < 0.01$). The mean values of inverse difference moment were also significantly reduced in the experimental group (0.767 ± 0.012) compared to controls (0.770 ± 0.0123 , $p < 0.01$). On the other hand, the values of textural correlation significantly increased in hepatic tissue exposed to iron oxide nanoparticles (0.9953 ± 0.0008 compared to 0.9951 ± 0.0009 , $p < 0.05$). A similar increase was observed in the values of GLCM sum variance which in the experimental group equaled 284.8 ± 49.6 and in controls 277.1 ± 56.9 ($p < 0.01$). No significant difference was detected in the values of textural contrast (0.65 ± 0.06 in the experimental group, 0.64 ± 0.06 in controls, $p > 0.05$).

The traditional classification and regression tree model was successfully developed in Scikit-learn Python library in Google Collaboratory project platform. The estimated accuracy of the CART model was solid, although

not outstanding 68.67% (Figure 2). The area under the roc curve for this approach equaled 0.75 indicating acceptable discriminatory power in separating ROIs from treated and control hepatic tissue. When feature importance analysis was performed, it was concluded that angular second moment was the most contributing input parameter with the importance value of 0.74.

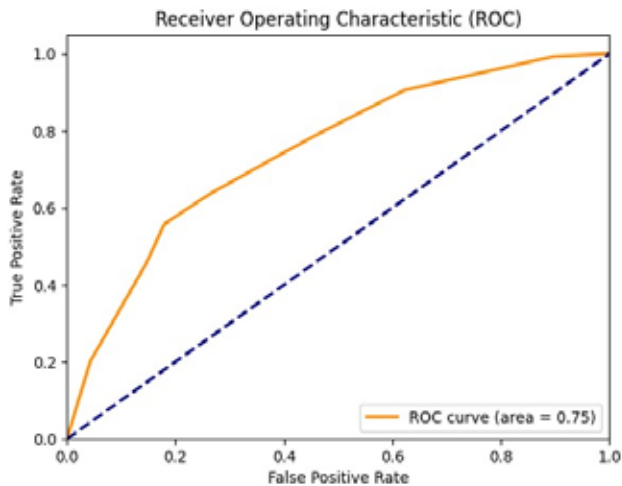


Figure 2. Receiver operating characteristic curve for CART machine learning model.

Random forest model had classification accuracy of 73.67%, after hyperparameter tuning. This accuracy was achieved grid search instantiation with multiple-fold cross-validation. The area under the receiver operating characteristics curve for this approach equaled 0.81 which may be considered as relatively good discriminatory power in terms of separation of ROIs associated with damaged and control tissue (Figure 3). As with the CART model, when feature importance analysis was performed, angular second moment was shown to be the most contributing input parameter although with much lower importance score of 0.42. The F1 score for the model equaled 0.74 meaning that the model had fairly good, but not perfect, precision and recall.

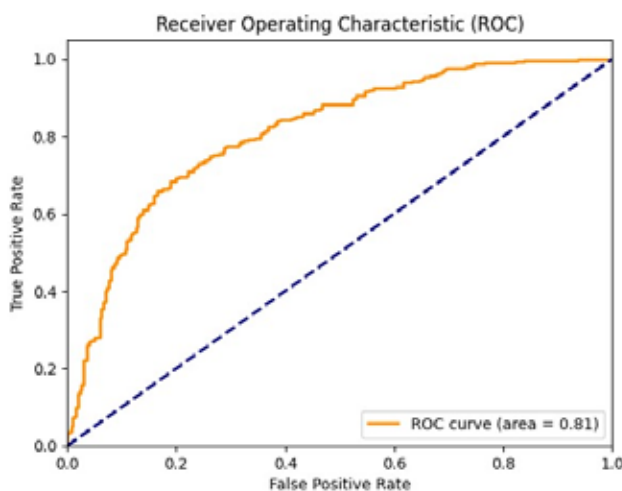


Figure 3. Receiver operating characteristic curve for Random Forest machine learning model

DISCUSSION

In this paper we showed that it was possible to develop a machine learning model based on the random forest algorithm capable of differentiating between ROIs of normal hepatic tissue and the tissue exposed to iron oxide nanoparticles. The application of the textural indicators of the gray level co-occurrence matrix as input data for model training was demonstrated to be a potentially useful approach for the assessment of hepatic structure in these experimental conditions. The random forest model outperformed the alternative classification and regression tree classifier in terms of the classification accuracy and the estimated area under the receiver operating characteristic curve. The results of this study as well as the developed computer code for machine learning models serve as the foundation for further exploration of artificial intelligence strategies in pathophysiology and pathology research.

This is not the first research where gray level co-occurrence matrix features were applied as input data for decision tree model training. Recently, Davidovic et al. (2022) used similar computation approach to detect subtle changes in cell nuclei associated with exposure to sublethal dose of ethanol. On a cell experimental model angular second moment, inverse difference moment, GLCM contrast, GLCM correlation and variance were considered as input for the development of not only random trees but also models based on binomial logistic regression and multilayer perceptron neural network (21, 22). All models showed relatively solid parameters of performance although the perceptron slightly outperformed the alternatives in terms of classification accuracy. Unfortunately, due to significant differences in methodology for GLCM calculation, as well as in techniques for micrograph creation and model training, the results are not comparable to the ones from our current study.

Random forest itself, created in scikit-learn Python library trained using GLCM and other data was demonstrated to be a potentially useful artificial intelligence approach in yet another recently published in vitro research (11). Here, cells were exposed to hyperosmotic environment and discrete changes in nuclear structure were evaluated by calculating the values of inverse different moment, angular second moment, contrast, correlation and variance, similarly to our current study. However, unlike the current study, the authors performed additional calculations of nuclear fractal dimension as an indirect indicator of structural complexity and level of detail. Also, a feature of discrete wavelet transform (wavelet coefficient energy) was calculated to further explain variations in structural heterogeneity of the nuclear structures (11). Finally, all the calculated features were used as input data while the target was the class/status of the cells in regard to the treatment. Random forest achieved a solid classification accuracy of 79.8% which was significantly higher compared to the alternative support vector machine approach.

Additional future work and applications related to our data might include research on further optimization of model hyperparameters which could additionally increase both classification accuracy and discriminatory power. The model should be compared with alternative approaches such as the ones based on multilayer perceptron neural networks, support vector machines and k-nearest neighbors algorithm (3). One should also consider alternative ensemble decision tree models that use sequentially – built trees fitted to residual errors (negative gradient) of the loss function such as gradient boosting trees. These approaches might in some cases be more powerful than random forest, especially when applied to the features of two-dimensional microscopic data. Additionally, all the abovementioned models should be created taking into account data obtained from different experimental protocols that vary in histological staining, magnification, micrograph dimensions and resolution, as well as settings during the micrograph acquisition. The best performing model could be deployed as a part of a computer application using a Python module for serializing and deserializing objects, the best example probably being the well-known “pickle” library. The application could be tested on other datasets obtained from experiments that used alternative iron compounds and could serve as an addition to the current pathohistology and pathophysiology protocols.

The limitation of our research includes several methodological challenges that need to be commented on in this paper. First, the methodology of machine learning, including the applied random forest approach has its own limitations regarding interpretability and reproducibility. Unlike individual decision trees, random forest due to its complexity suffers from the relative lack of interpretability, and inability of a researcher to precisely demonstrate the inner workings of the model. This phenomenon in machine learning is called “black box” and is not specific to random forests (1). Second, in our research, the model was trained on the GLCM data obtained from individual regions of interest of hepatic tissue. This approach where ROIs are considered as statistical units of measurement has its weaknesses in terms of not being certain that the model can be successfully applied in other conditions. For example, the level of classification accuracy in future models designed for differentiation of animals instead of ROIs, may be much lower, even if researchers’ overcome challenges related to dataset size for model training. Finally, GLCM as a computational signal analysis technique may sometimes yield results with significant variability depending on the applied methodology and experimental protocol. For example, variations in staining protocol, micrograph acquisition settings, software platform used for GLCM computations

and many other factors may influence the values of GLCM features. Subsequently, this may lower the reproducibility of the trained machine learning model and render it less applicable in future research.

CONCLUSION

In conclusion, our study demonstrates that it is possible to create a supervised machine learning model based on random forest that uses GLCM data to differentiate hepatic tissue based on its exposure to iron oxide nanoparticles. The random forest model is superior to the alternative CART decision tree approach in terms of its classification accuracy and discriminatory power. The data obtained as a result of this study may be utilized for further development of artificial intelligence computation systems for identification of physiological and pathophysiological changes in hepatic tissue. The results are also a starting point for further research on the automation of histopathological analysis of liver tissue exposed to external toxic agents.

Conflict of interest

None to declare.

Author contributions

Jovana Paunović Pantić and Igor Pantić contributed the conception and design of the paper, the acquisition, analysis, and interpretation of data and preparation of the draft of the manuscript. Danijela Vučević, Tatjana Radosavljević, and Svetlana Valjarević contributed to the conception and design of the paper and preparation of the draft of the manuscript.

Ethical approval

The study was a part of the PhD thesis research which received approval from both the Ethics commission for the protection and welfare of experimental animals of the University of Belgrade, Faculty of Medicine (approval number 229/2) and the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia - Veterinary Division (approval number 323-07-07783/2020-05). The procedures that followed were in line with the Universal Declaration of Animal Welfare (WSPA, London, 2000), the European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Reasons (1998), and various other domestic and international guidelines promoting responsible and compassionate treatment of experimental animals.

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RAZVOJ MODELA MAŠINSKOG UČENJA ZASNOVANOG NA ALGORITMU SLUČAJNIH ŠUMA ZA DETEKCIJU PROMENA U TKIVU JETRE NAKON IZLAGANJA ČESTICAMA OKSIDA GVOŽĐA

Jovana Paunović Pantić¹, Danijela Vučević¹, Igor Pantić^{1,2,3,4}, Svetlana Valjarević⁵, Tatjana Radosavljević¹

Sažetak

Uvod/Cilj: Cilj naše studije je bio razvoj modela mašinskog učenja zasnovanog na algoritmu slučajnih šuma, koji koristeći teksturalne podatke iz digitalnih mikrografa tkiva jetre, ima sposobnost da napravi razliku između normalnog tkiva jetre, i oštećenog tkiva izloženog česticama oksida gvožđa.

Materijal i metode: Regioni interesa u okviru digitalnih mikrografa tkiva jetre, dobijenih od miševa koji su tretirani česticama oksida gvožđa i miševa kontrolne grupe, analizirani su korišćenjem metode matriksa simultanog pojavljivanja sivih vrednosti („GLCM“ metoda). Dobijeni teksturalni parametri su korišćeni kao ulazni podaci za treniranje i testiranje modela slučajnih šuma u okviru biblioteke „Scikit-learn“ u programskom jeziku Python. Dodatno, razvijen je i konvencionalni model mašinskog učenja drva odluke, zasnovan na algoritmu drva klasifikacije i regresije („CART“ algoritam).

Ključne reči: veštačka inteligencija, mašinsko učenje, oksid gvožđa, jetra

Primljen: 06.10.2023. | **Revizija:** 25.10.2023. | **Prihvaćen:** 10.11. 2023

Medicinska istraživanja 2024; 57(1):21-26

Rezultati: Model slučajnih šuma nadmašio je alternativni pristup CART drva odluke u pogledu tačnosti klasifikacije, predviđajući ciljnu klasu uzorka u 73,67% instanci u validacionom skupu podataka. Površina ispod krive karakteristika operatera bila je 0,81, ukazujući na relativno dobru diskriminativnu moć modela. F1 skor modela je iznosio 0,74 što pokazuje dobru, mada ne savršenu preciznost i povrat informacija.

Zaključak: Podaci dobijeni iz ove studije mogu se iskoristiti za dalji razvoj sistema zasnovanih na veštačkoj inteligenciji za identifikaciju fizioloških i patofizioloških promena u tkivu jetre. Rezultati takođe služe kao početna tačka za dodatna istraživanja vezana za automatizaciju histopatološke analize jetrenog tkiva izloženog eksternim toksičnim agensima.