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IN VIVO 1H MAGNETIC RESONANCE SPECTROSCOPY OF WOMAN'S NORMALAND CANCEROUS UTERUS

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Abstract

Endometrial cancer is the sixth most common cancer in women worldwide. In last years the diagnoses in endometrial cancer has been increased, being more important as a cause in new cases, than in terms of mortality. Endometrial cancer has a reasonable survival prognosis, if diagnosis is made early. Therefore, new diagnosis methods are tested every day. A series of ^{1}H NMR spectra were recorded in vivo at a short echo time of 35 ms using a 3 T GE magnetic resonance apparatus for two groups of five healthy voluntaries and five patients with endometrial cancer. All NMR spectra were primary analyzed using jMRUI software and visually inspected without a definitive conclusion especially in the case of those belonging to patients with uterine cancer. Next, the ^{1}H NMR spectra were numerically analyzed by three specialists using dedicated software written in Processing and the concentrations of more than 20 metabolites were extracted and averaged. A list of the metabolites with highest concentration (total choline, Alanine, lactate, intramyocellular lipids, N-acetyl aspartate, Glucose) was used together with water content, sum of a macromolecular resonances, sum of all other metabolites and age of volunteers/patients into an Principal Component Analysis (PCA). We found that for this lot of patients and volunteers the third analysis (PC2 versus PC3) is the best in differentiation between healthy women and those with endometrial cancer, and we evaluate the contribution of each metabolite.

Rezumat

Cancerul endometrial este al saselea ca frecventa dintre cancerele diagnosticate la sexul feminin, avand un prognostic bun daca este diagnosticat intr-un stadiu precoce. Pentru acesta sunt testate incontinuu metode noi de diagnostic. In acest studiu au fost analizate datele spectorsopice obtinute prin examinare RMN 3T, cu timp de ecou de 35 ms, la subiecti sanatosi si la pacienti cu cancer endometrial. Datele brute au fost analizate si fitate intr-un prim stadiu folosind jMRUI. Dupa fitare, datele au fost postprocesate folosind un soft special, elaborat cu ajutorul programului Prossesing, curbele obtinute au fost analizate numeric de catre trei specialisti. Un set de 5 metaboliti, cu concentratiile cele mai mari (colina totala, alanina, lactat, lipide intramiocelulare, NAA, glucoza), impreuna cu concetratia apei, suma resonantelor macromoleculelor, respectiv varsta au fost folosite pentru analiza PCA (Principal Component Analysis). Rezulatul acestui studiu este ca analiza PCA (PC2 vs PC3) este unul adecvat pentru diferentierea pacientilor sanatosi de cei cu cancer endometrial.

Cuvite cheie: 1H MR Spectroscopy, uter normal, uter canceros, analiza PCA

1.Introduction

Endometrial cancer is the sixth most common cancer in women worldwide [1]. The diagnoses in endometrial cancer has been increased in recent years, being more important as a cause in new cases, than in terms of mortality [2, 3]. Endometrial cancer has a reasonable prognosis, the survival rate of

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patients reaches 5 years after diagnosis in more than 80% of women [2]. The diagnosis of endometrial cancer is primarily based on clinical symptoms, combined with imaging, cytology and histopathology. The main clinical symptom is abnormal uterine bleeding, which is often ignored in premenopausal women [4].

Ultrasound is the first line examination in women with suspicion of endometrial cancer, being highly accurate in characterizing the uterine cavity, endometrial thickness and its pathology. In premenopausal women and in early stage endometrial cancer ultrasound is limited and unclear in detection of malignancy. The "gold-standard" for diagnosis of endometrial cancer is curettage and histopathological examination, but the accuracy is not always satisfactory because of its inability to detect small cancerous foci [4, 5].

The goal of this paper is to create and evaluate a complex instrument of evaluation, based on ^{1}H Magnetic Resonance Spectroscopy (MRS), post-processing analysis (using jMRUI™ worldwide available software and a homemade dedicated software written in Processing™) and statistical principal component analysis (PCA) to differentiate between normal and cancerous endometrium, using ten diverse parameters.

2.Materials and methods

2.1.Patients

We included in the study group 5 healthy volunteers and 5 patients with endometrial cancer. The healthy volunteers underwent gynecological examination, which included ultrasound and Papanicolau examination. The cases with endometrial cancer had histological diagnosis/ confirmation. The ethics committee of our institute approved this study and informed consent was obtained from all participants.

2.2.MR imaging and spectroscopic protocols

The examination was carried out on a 3T MRI unit (Discovery MR750w, GE), using a body coil transmission and a 16-channel phased-array receiver. Including all protocols the scanning times were between 15 – 30 minutes. The MRS data acquisition took about 4-7 min/sequence. Additional T1 axial and T2 MR images in axial and sagittal planes were added to the examination to exclude intrapelvic pathologies in the healthy group. Volunteers were asked to fast for at least 6 hour prior to the examination. An antispasmodic drug was administrated intravenously at least ten minutes before

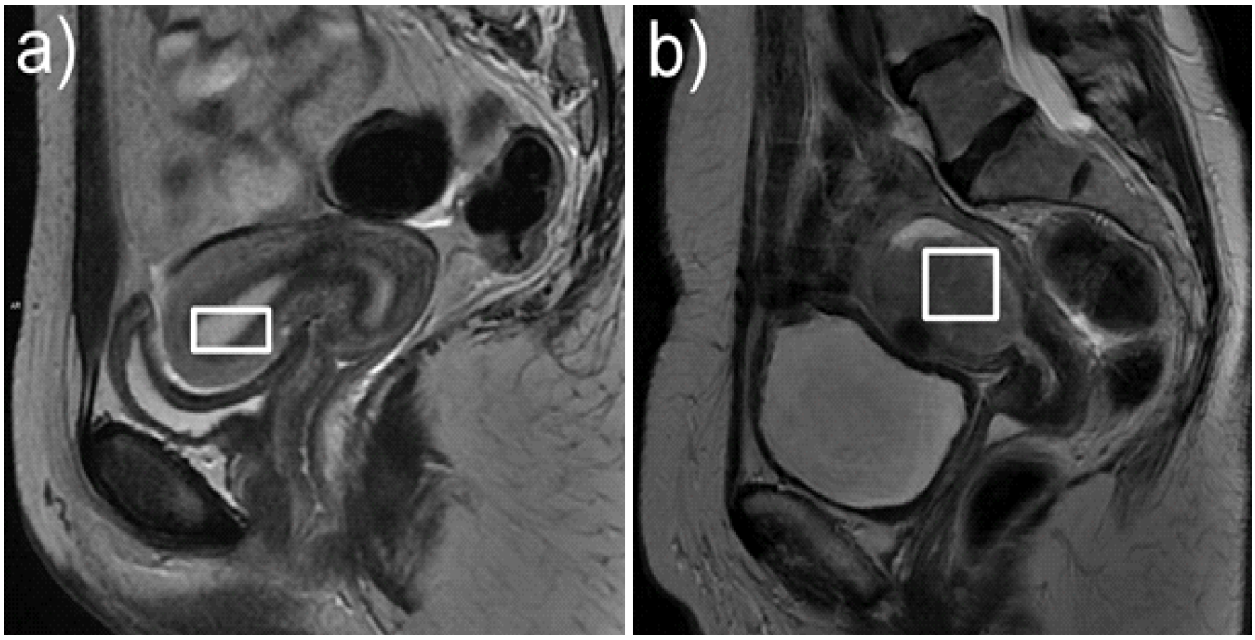

Figure 1. Sagittal T2-weighted image of a) normal uterus, note the layered appearance of the uterine structures and b) endometrial carcinoma, with superficial myometrial invasion (less than 50%);

the examination, to reduce artifacts due to bowel movement or uterine contractions.

The MRS was performed using point resolved spectroscopic sequence (PRESS) with single square spectroscopic volume-of-interest (VOI) placed within the uterine corpus to include the endometrial layer (see Fig. 1). The voxel volume was between 2.1 – 14.6 ml. Outer volume suppression bands were manually placed. Automated shimming method was used for a better homogeneity of the magnetic field to increase the spectral resolution. Repetition time (TR) was set at 1.5 seconds and the echo time (TE) was 35 ms. The number of excitations (NEX) was 8 and the total number of scans was 128 to increase the signal to noise ratio (SNR).

2.3. Principal component analysis

The analysis in principal components a particular case of multivariate analysis, in particular is a standard method used to analyse data characterized by numerous variables or types of variables (many times with different measurement units) usually obtained from more than one group (Afifi *et al* 2012). For our PCA analysis of metabolite content measured from *in vivo* ^{1}H NMR spectra, we implement a numerically program written in Processing and we plot the result using the Microsoft Excel media. For the principal component analysis we produce a data matrix as it is presented in Table 1. As variables, we select the content (percentage concentration) of: bound water, total choline (tCho), Alanine (Ala), Lactate (Lac), intramyocellular lipids (IMCL), N-acetyl aspartate (NAA), Glucose (Glc), macromolecules (MM),the sum of all other metabolites and the age of volunteers/patients.

3. Results

3.1.The ^{1}H Magnetic Resonance Spectra of woman's uterus

One of the modern methods used for *in vivo* investigation of the woman's pelvis is based on the localized NMR spectroscopy of protons known as ^{1}H MRS. Figure 1 presents a sagittal Magnetic Resonance (MR) image of woman's pelvis, non-pathologic (normal) in the top and pathologic (endometrial cancer) bottom. The region of interest (ROI) is located at the level of endometrium, more specific the NMR signal was recorded from a voxel represented in Fig. 1 by white rectangles. The non-pathologic uterus has a trilaminar appearance (Fig. 1a); the zonal anatomy depicted by T2-weighted MRI image shows a homogenous hyperintense endometrium, a continuous low signal intensity junctional zone and the intermediate signal intensity of the outer myometrium. The pathologic images show a sagittal T2-weighted MR image with distention of the endometrial cavity due to the presence of an intermediate-signal-intensity tumor (see fig. 1b).

The ^{1}H NMR spectra recorded for the five volunteers and five patients with endometrial cancer are presented in figure 2. The detailed record and primary data analysis using JMRUI software was

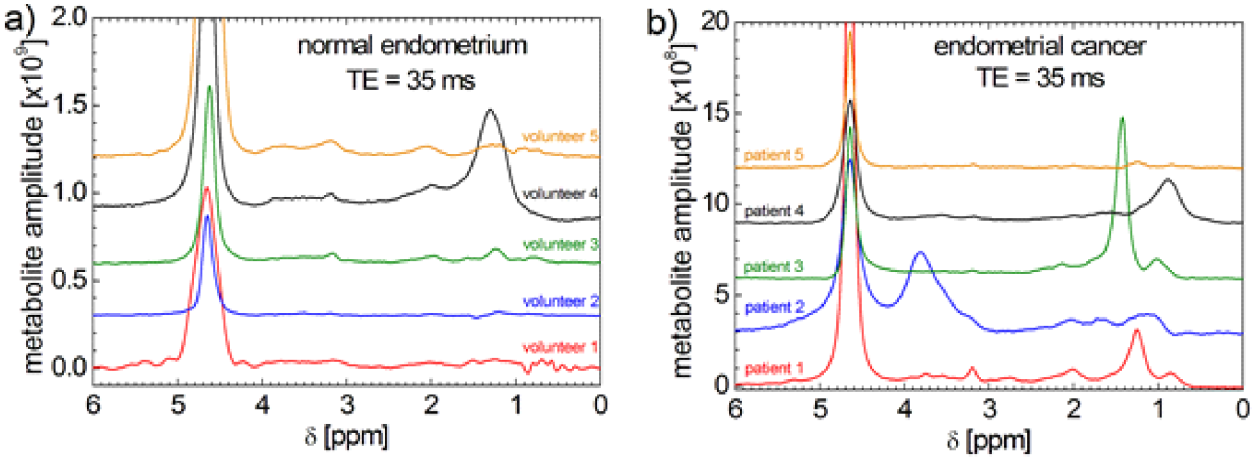

Figure 2. Magnetic Resonance Spectroscopy investigation spectra of a) volunteers with normal uterus and b) patients with endometrial cancer recorded at an echo time TE = 35 ms.

already presented in ref [7]. One can remark that for the normal pelvis the amplitudes of peaks specific to metabolites (spectral region with chemical shift, d between 0 – 4.3 ppm) are much smaller compared to the amplitude of water (the main peak located at ~ 4.65 ppm). Some peaks that exceed the noise limit can be observed localized at ~ 1.24 ppm, 1.3 ppm, ~1.55 ppm, ~1.98 ppm, 3.18 ppm. The spectrum recorded for the volunteer 4 present a large peak associated with lactate (Lac), intramyocellular lipids (IMCL) and M2 from macromolecular.

(MM) resonances [7 - 9]. Contrary, *in vivo* ^{1}H NMR spectra recorded for patients with endometrial cancer presents large amplitudes of peaks associated with diverse metabolites. Just for the patient 5 the recorded ^{1}H NMR spectrum showed small peaks amplitudes in the metabolite specific region (0 – 4.3 ppm)

3.2. Principal component analysis for normal and cancerous uterus

At a visual inspection, the ^{1}H NMR spectra recorded *in vivo* for the patients with endometrial cancer is hard to be interpreted. This is mainly due to the fact that the relative amplitudes (compared to the amplitude of water peak) of specific metabolites are not even closed for this group of patients. Thus, by *in vivo* recorded ^{1}H NMR spectrum the patient 5 should have a normal endometrium and the volunteer 4 should be considered with uterine cancer. Better results can be achieved considering the concentrations of all relevant metabolites and any other parameters as input parameters for a principal component analysis (PCA). For that each spectrum was analyzed using a home-made software following the procedure largely described in reference [7]. The averaged concentrations as of bound water, tCho (total choline), Ala (Alanine), Lac (lactate), IMCL (intramyocellular lipids), NAA (N-acetyl aspartate), Glc (Glucose) and macromolecular resonances (MM) are presented in Table 1 together with the age of volunteers/patients as an additional parameter. In Table 1 are presented also the voxels dimensions (see Fig. 1), parameter used to normalize the *in vivo* ^{1}H NMR spectra shown in Fig. 2. For a better fit of each spectra additional metabolites has to be included to extend the main list presented in the Table 1. The sum of concentrations of all supplementary metabolites is listed in Table 1 as others. The amplitude/concentration finding process implies a fitting procedure, which requires some initial values. The metabolite analysis from *in vivo* spectra is a special type of spectroscopy in the sense that the relevant information can be found at noise level compared to a main peak (in this case the water peak). Therefore, in order to increase the confidence in the results, the initial guess of

Table 1. The list of participating volunteers and patients and the main characteristics: age, voxel dimension for *in vivo* spectroscopy and the average concentration of metabolites (bound water, tCho—total choline, Ala—Alanine, Lac—lactate, IMCL—intramyocellular lipids, NAA—N-acetyl aspartate, Glc–Glucose, MM—macromolecules) measured at 3T with TE =35 ms and evaluated by three specialists.

Volunteers/ Patients	Age	Voxel dimensions [mm×mm×mm]	concentration [%] at TE = 35 ms								
			Water	tCho	Ala	Lac	IMCL	NAA	Glc	MM	O
Volunteer 1	46	20.0×20.0×20.0	73.08	4.35	1.00	1.73	0.26	1.42	0.70	1.19	1(
Volunteer 2	34	19.7×16.9×19.8	76.38	1.25	2.01	0.68	0.67	1.17	3.03	2.52	1:
Volunteer 3	43	18.8×17.5×8.8	49.76	4.04	0.82	1.69	1.45	1.62	2.55	8.84	2(
Volunteer 4	37	18.5×48.1×13.5	41.50	1.96	1.52	12.59	12.90	6.00	2.38	11.02	1(
Volunteer 5	29	22.2×35.1×18.8	77.07	1.78	0.97	0.24	0.85	1.98	1.93	2.48	1
Patient 1	70	23.3×25.2×19.7	64.74	3.28	1.76	2.11	3.89	2.27	1.36	7.06	1:
Patient 2	72	20.0×20.0×20.0	34.14	1.20	0.13	1.75	0.88	1.32	5.91	1.86	5:
Patient 3	53	14.5×12.9×22.0	24.38	2.06	4.69	0.76	0.97	3.83	0.30	35.17	2:
Patient 4	63	21.8×16.8×17.1	37.01	1.78	2.30	1.31	0.29	3.44	1.98	18.42	3:
Patient 5	68	20.0×9.8×10.9	75.85	2.25	0.00	1.38	0.06	1.42	1.67	3.05	1-

The fitting errors are less than 5 %.

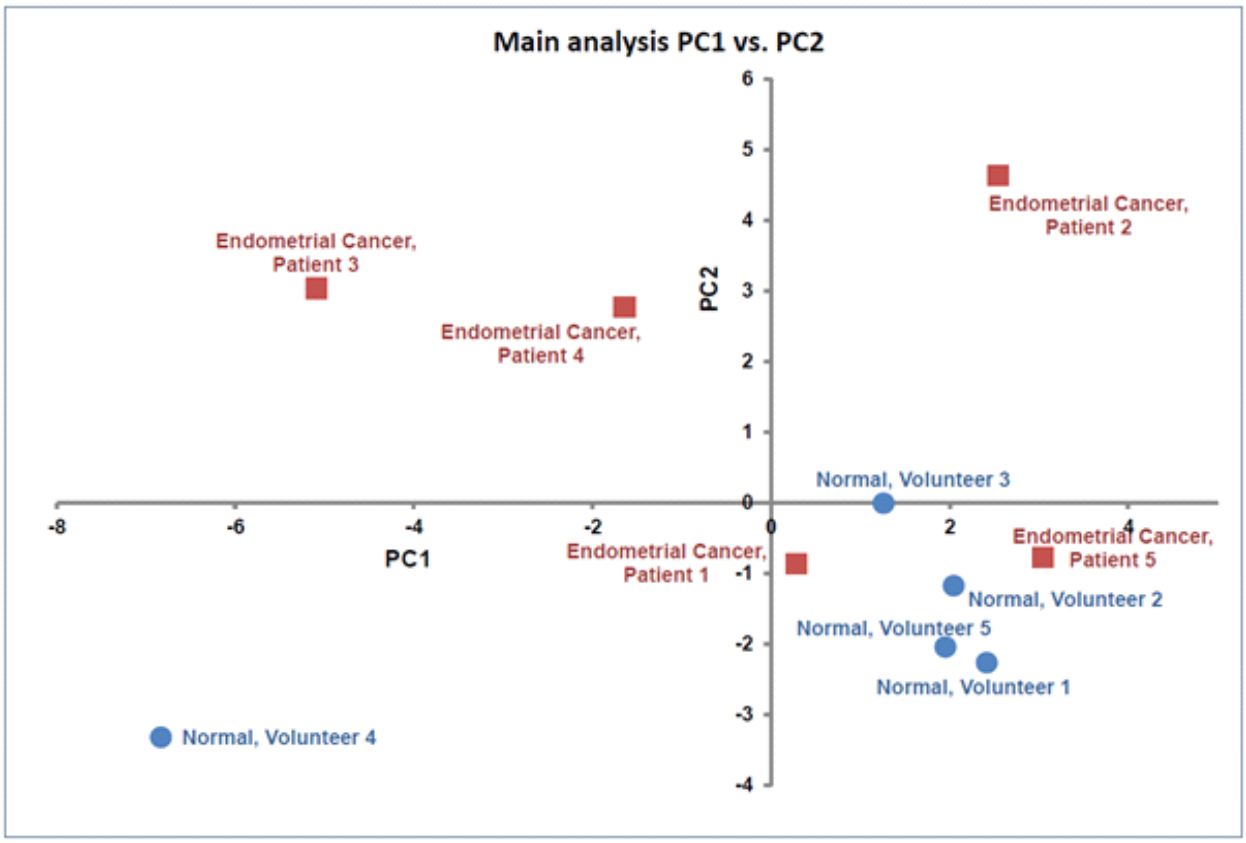

Figure 3. The main analysis (PC1 versus PC2) of age and principal metabolite concentrations recorded from *in vivo* ^{1}H MR Spectroscopy analysis of normal and cancerous uterus.

metabolites amplitudes (the concentration of metabolites is calculated at the end of fitting procedure) was introduced separately by three specialists. At the end, the resulted concentrations of the extended list of metabolites were averaged and the most relevant (higher concentration) metabolites are listed in Table 1. With the exception of voxel volume, which is subjective parameter set by radiologist technicians, all parameters from Table 1 were used for a PCA analysis. We get a number of 10 principal components, which is the number of volunteers and patients but also the number of parameters. We found that it is necessary a number of 4 components to reach a cumulative proportion (to explain the dispersity of obtained data) larger than 90 % (92.4). If we consider the first five principal components (PC), one can explain the dispersity of data in proportion of 98.1 %. But in the same time the first principal component (PC1) can explain the data variation only in proportion of 34.4 % and cumulated with second component (PC2) the proportion has a value of 60.6 % and cumulated with PC3 the cumulative proportion reaches to an accepted value of 82.0 %.

4. Discussions

The metabolites data (average concentration) and age of volunteers/patients represented in first two principal components are presented in figure 3, where the blue circles represents the volunteers with normal uterus and red squares represents the patients with endometrial cancer. One can see that the dots associated with the volunteers (with the exception of volunteer 4) are well grouped at PC1 around 2 and negative PC2. This indicates that the initial data (recorded ^{1}H NMR spectra and list of metabolites used) is relevant for the healthy volunteers. The data points associated with three of patients (2, 3 and 4) are well separated from data associated with healthy volunteers, being located in PC2 dimension between 2.7 and 3.7. The points for patients 1 and 5 are closely to the region of 4 data points associated with healthy volunteers. Moreover, the large dispersion of points in the plot of principal components can be explained by the large variation of the *in vivo* recorded ^{1}H NMR spectra (see Fig. 2b).

Isolated at large and negative PC1 and negative PC2 one can observe the data point

Table 2. The loading values (contribution) of input parameters in PCA to each principal component from PC1 to PC10.

parameter	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Age	0.1548	0.6120	0.0858	-0.5310	-0.5555	0.0600	0.0041	0.0011	0.0052	-5.95E-29
Water	0.6602	-0.6924	-0.1451	0.1534	-0.1983	0.0211	0.0180	-0.0057	0.0093	5.37E-15
tCho	0.1657	-0.3560	-0.3866	-0.7531	0.3517	0.0709	-0.0009	-0.0119	0.0169	2.94E-16
Ala	-0.6661	0.2545	-0.6046	0.2488	0.0027	0.2427	0.0703	0.0174	0.0039	3.55E-16
Lac	-0.6656	-0.4215	0.5764	-0.1842	-0.0045	-0.0051	0.0135	0.1140	0.0021	9.57E-16
IMCL	-0.6986	-0.4524	0.5133	-0.1347	-0.0356	0.1323	-0.0361	-0.0682	-0.0316	1.03E-15
NAA	-0.9627	-0.1194	0.1510	-0.0296	-0.0725	-0.1439	0.0698	-0.0597	0.0315	4.08E-16
Glc	0.3000	0.3934	0.8203	0.1901	0.1492	0.1446	-0.0384	-0.0132	0.0366	4.09E-16
MM	-0.7439	0.4436	-0.4809	0.0223	0.0113	-0.0533	-0.1216	0.0106	0.0084	2.81E-15
Others	0.1010	0.9052	0.2729	-0.1404	0.2617	-0.0567	0.0646	-0.0030	-0.0215	3.58E-15

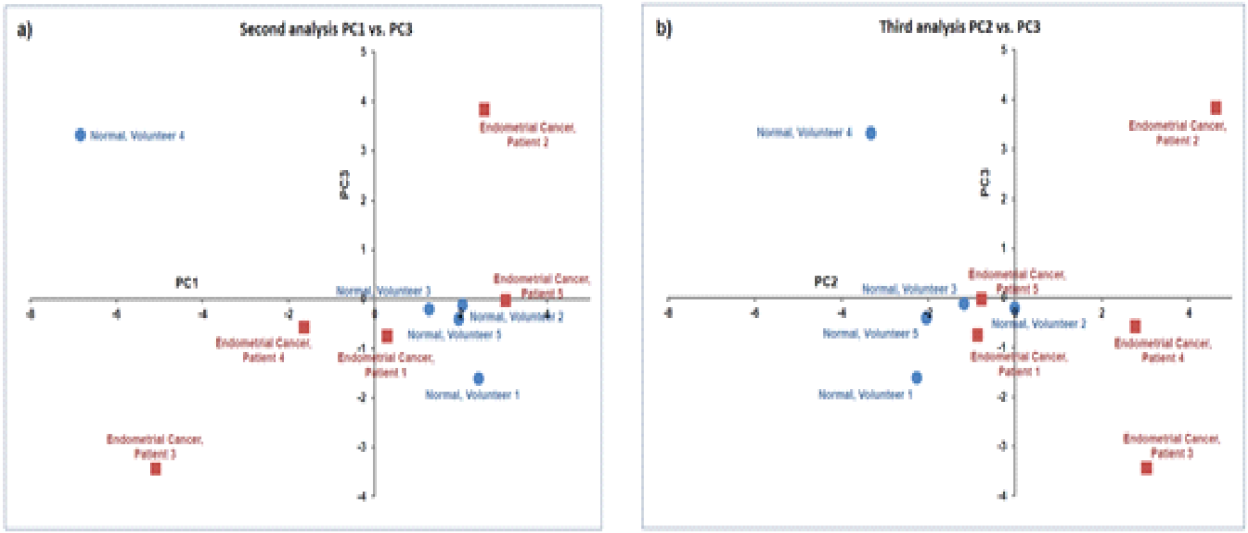

Figure 4. The second (PC2 versus PC3) (a) and third (PC2 versus PC3) (b) analysis in principal components of metabolites concentrations recorded from *in vivo* ^{1}H MR Spectroscopy of normal and cancerous uterus.

representing the volunteer 4. This is not a surprise since the *in vivo* ^{1}H NMR spectrum recorded for this volunteer (see the black line in Fig. 1a) is obviously different than the other spectra of the rest of volunteers. In the same time the location of this point, far away from the points assigned to patients with endometrial cancer, shows that the volunteer 4 doesn't exhibit this disease. In this point, the MR image showing the selected voxel for *in vivo* MR spectroscopy was reconsidered. It was observed that, in this case, the voxel dimensions (see Table 1 – volunteer 4) were larger than usual. Thus, in one dimension of rectangular shape, the sensitive volume (the spectroscopy voxel) was extended outside the uterus, contaminating the data from fatty tissue in the periuterine area. This is due to the relative position of internal organ in respect to the gradient coils of the MRI apparatus, and many times cannot be avoided. Therefore, in the following discussions we will neglect the point represented by volunteer 4 and we will consider the representation of normal uterus by the group of four blue circled grouped on the bottom-right side of PC1-PC2 plot.

The most important dispersion of data points, which usually leads also to separation function of important categories, is given by the first principal

components, PC1. In order to assess the importance of each parameter to values of PC1 one has to take a look to the loading values presented in Table 2. Disregarding the sign, we see that the most important contribution to this dispersion is given by NAA metabolite (~0.96 in absolute value), followed by the sum of concentrations of macromolecules (~0.74). It is known that NAA, usually found in brain, was found also in ovaries [10]. Moreover, NAA is considered to be a specific metabolic signature for serous carcinoma. The overall survival time was higher for patients with ovarian cancer having a lower NAA level [11, 12]. Important contributions have also the concentrations of intramyocellular lipids (IMCL with ~0.70), Alanine (Ala with ~0.67), Lactate (Lac with ~0.67) and water (~ 0.66). For the first principal component (PC1) the rest of metabolites have less importance (~0.1) and age of volunteers/ patients. Taking into consideration not only the separation of data (between healthy volunteers and patients) but the grouping tendency one can conclude that the list of considered metabolites is well chosen to describe the healthy volunteers but has to be extended to get the key element which describes the particularities of endometrial cancer.

An apparent better separation of data can be obtained along second principal component (PC2). To this separation the largest contribution can be found from other types of metabolites (~0.91) followed by (see Table 2) water content (~0.69) and age of patients (~0.61). A less important contribution is obtained from NAA metabolite (~0.12). In this point the analysis can continue with further contributions like the component PC3.

In figure 4 are presented the scatter plots of PC1 versus PC3 (left) and PC2 versus PC3 (right). In the second analysis (Fig. 4a) the representation of the four volunteers (1, 2, 3 and 5) remains grouped while the representation of data for patients is spread along main diagonal. More interesting representation can be observed from third analysis (Fig. 4b) where, with a small overlapping, the healthy voluntaries are located on the left side of graph (PC2 <0) and the patients are located on the right side (PC2 > -0.9). This separation is given by PC2 but in this case (Fig 4b) the effect is more pronounced compare to the first analysis (Fig. 3) due to the fact that the dispersion influenced by PC3 is much smaller than the dispersion induced by PC1, therefore in this case, volunteer 4 seems to belong to the group of healthy volunteers.

The main contribution to PC3 is given by Glucose (Glc with ~0.82), followed by Alanine (Ala with ~ 0.60), Lactate (Lac with ~0.57), intramyocellular lipids (IMCL with ~ 0.51) and macromolecules (MM with ~ 0.48). Less important contribution to this component is the age of volunteers/ patients (~0.09) and water content (~0.14). Also, as in the case of first principal component (PC1), the other metabolites have an insignificant contribution to PC3 values.

5. Conclusions

We use the *in vivo* ^{1}H NMR spectroscopy on two groups of five healthy voluntaries and five patients diagnosed with endometrial cancer to evaluate the ability of this method to discriminate between normal and cancerous uterus. With one exception the ^{1}H NMR spectra of normal volunteers presents similar features while all spectra recorded for patients with endometrial cancer looks different. We found out that a simplest visual inspection of such spectra can lead to misinterpretation; therefore a statistical analysis in principal components (PCA) of data was applied.

As result, we show that the list of chosen metabolites and age of patient is well suited for the heathy volunteers. Otherwise, even if the list leads to an acceptable data separation between the healthy volunteers and patients diagnosed with endometrial cancer this list has to be extended to other metabolites as was shown by the largest loading value (~0.91) in second principal component (PC2). In terms of separation between healthy volunteers and patients with endometrial cancer and point grouping inside the same lot the third analysis (PC2 versus PC3) is the best for this studied lot and chosen metabolites. Finally, one can say that in vivo ^{1}H NMR spectroscopy combined with PCA analysis can be a valuable tool in the assessment of state of uterine health but the studied lot has to be enlarged and more metabolites has to be evaluated.

References

[1] Endometrial cancer statistics | World Cancer Research Fund. (n.d.). Retrieved October 31, 2018, from https://www.wcrf.org/dietandcancer/cancer-trends/endometrial-cancer-statistics.

[2] Endometrial Cancer Incidence Rising Worldwide - National Cancer Institute. (n.d.). Retrieved October 31, 2018, from https://www.cancer.gov/ news-events/cancer-currents-blog/ 2017/endometrial-cancer-incidence-rising

[3] Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., … Bray, F. (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer, 136(5), E359–E386. https://doi.org/10.1002/ijc.29210

[4] Shi, K., Wang, Q., Su, Y., Xuan, X., Liu, Y., Chen, W., … Lash, G. E. (2018). Identification and functional analyses of differentially expressed metabolites in early stage endometrial carcinoma. Cancer Science, 109(4), 1032–1043. https://doi.org/10.1111/cas.13532

[5] Zhang, J., Cai, S., Li, C., Sun, X., Han, X., Yang, C., … Zong, Y. (2014). Can magnetic resonance spectroscopy differentiate endometrial cancer? European Radiology, 24(10), 2552–2560. https://doi.org/10.1007/s00330-014-3237-3

[6] Afifi A, May S, Clark VA 2012 *Practical Multivariate Analysis* (5th edition, CRC Press, Taylor and Francis Group) p 357-379.

[7] R. E. Dávid, R. Fechete, S. Sfrângeu, D. Moldovan, R. I. Chelcea, I. A. Morar, F. Stamatian, T. Kovacs, P. Popoi. *In Vivo* ^{1}H Nuclear Magnetic Resonance Spectroscopy and Relaxometry Maps of the Human Female Pelvis. *Analitical Letters*, (2018) doi.org/10.1080/00032719. 2018.1437169.

[8] De Graaf, R. 2007. *In Vivo* NMR Spectroscopy. Chichester, UK: J. Wiley and Sons.

[9] Stagg, C. and D. Rothman. 2014. Magnetic Resonance Spectroscopy. Tools for Neuroscience Research and Emerging Clinical Applications. Oxford. UK: Elsevier.

[10] Zand, B., Previs, R. A., Zacharias, N. M., Rupaimoole, R., Mitamura, T., Nagaraja, A. S., ... Sood, A. K. (2016). Role of Increased n-acetylaspartate Levels in Cancer. Journal of the National Cancer Institute, 108(6), https://doi.org/10.1093/jnci/ djv426

[11] Fong, M. Y., McDunn, J., & Kakar, S. S. (2011). Identification of Metabolites in the Normal Ovary and Their Transformation in Primary and Metastatic Ovarian Cancer. PLoS ONE, 6(5), e19963. https://doi.org/ 10.1371/journal.pone.0019963.

[12] Ben Sellem, D., Elbayed, K., Neuville, A., Moussallieh, F.-M., Lang-Averous, G., Piotto, M., ... Namer, I. J. (2011). Metabolomic Characterization of Ovarian Epithelial Carcinomas by HRMAS-NMR Spectroscopy. Journal of Oncology, 2011, 174019. https://doi.org/ 10.1155/2011/174019