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miRNAs Set Expression Profiles in Whole Blood During Prostate Cancer Patients Treatment

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Abstract

Background: Changes in expression profiles of the 5 selected miRNAs were analysed in a group of PC patients before treatment, after hormonotherapy and radiotherapy.

Objective: Whether the expression profiles of the miRNAs may be useful for monitoring prostate cancer treatment.

Methods: The initial study was carried out on 44 advanced prostate cancer patients and 41 healthy volunteers. The target group consisted of 39 PC patients. Blood for miRNA analysis was taken before treatment, after hormonotherapy and radiotherapy. The miRNAs were analysed by real-time PCR, followed by statistical analysis.

Results: For the target group, the statistically significant differences in the expression level were found after radiotherapy: for miR-21 only in the group of patients above the cut-off value designed in the preliminary study (p=0.0369) and miR-100 for the whole group (p=0.0413) and for the above cut-off value group (p=0.0140). The differences between the levels of each miRNA between the high and low expression groups were statistically significant. The designed groups were stable during treatment. Inclusion to the high and low expression group levels did not influence the treatment result.

Conclusion: The miRNAs studied in this work could not serve as biomarkers for the effectiveness of therapy for prostate cancer patients.

Keywords: Prostate cancer; miRNAs; Expression profile; Hormonotherapy; Radiotherapy

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Introduction

Prostate Cancer (PC) is the second-most diagnosed and the sixth-most common cause of death due to cancer in men worldwide [1]. Diagnostics is based on the serum Prostate Specific Antigen (PSA) level, rectal examination and histopathological estimation of a prostate needle biopsy. Although most cases of PC are histopathologically described as adenocarcinoma, their clinical behaviour ranges from slow growing tumours without clinical significance to aggressive, lethal disease. The group of patients with the highest risk of recurrence includes only approximately 20% of all prostate cancer cases, but the results of the treatment in this group have still been unsatisfying. Therefore, new methods of treatment are tested, and these common practices

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have improved. In the Greater Poland Cancer Center, medical experiments comparing two radiation therapies, namely, hypofractionated (HRT) and conventionally fractionated (KRT), have started. Some of the diagnostic approaches included in this medical experiment have also been considered for the better monitoring of the patients. In this paper, we focused on the circulated miRNAs as a non-invasive, specific and sensitive diagnostic tool.

miRNAs are small (18-24 nt), non-coding RNAs involved in the regulation of gene expression at the post-transcriptional level. miRNAs regulate diverse cellular processes and pathways. Their role in cancer's origin and progression has been described, as well as the expression profiles of many tumour types, including PC. The changes in the expression levels of many miRNAs in the tumour tissue are reflected in the body fluids. The circulating miRNAs probably come not only from the circulating tumour cells but also from the blood cells or other tissues modified by the disease [2]. Although, circulating miRNAs fulfil the criteria for good biomarkers, there is still lack of standardized tests to perform patient diagnostics. To create tests allowing for the monitoring of both types of studied radiotherapy, we focused on the circulated miRNAs described as important for the PC course. Our tests consisted of 5 miRNAs: miR-21 [3], miR-100 [4,5], miR-141 [3], miR-143 [6,7] and miR-221 [3].

The aim of this study was to look up whether the miRNAs expression profile may be a useful tool for monitoring prostate cancer treatment.

Materials and Methods

Patients: The initial study to estimate the diagnostic value of the selected miRNAs was carried out on a group of healthy volunteers and prostate cancer patients. The first group consisted of 41 persons, including 32 males and 9 females, that were recruited mainly from students. The second group included 44 advanced PC patients. The clinical data, including the age, age of diagnosis, Gleason score, PSA level, type of therapy and metastasis, were collected **(Table 1).**

The target group consisted of 39 prostate cancer patients with a high risk of progression. Patients were qualified according to the following criteria:

(i) Age 40-80 years;

(ii) Documented prostate cancer (adenocarcinoma) with Gleason score;

(iii) Patients' general condition according to the Eastern Cooperative Oncology Group (ECOG) 0-1;

(iv) No distant metastasis; and

(v) A high risk of progression. A high risk of progression was based on the presence of at least one of the following criteria: cT3, PSA>20 ng/mL, Gleason score >7; alternatively, a high risk of progression was based on the presence of two of the following criteria: cT2c, Gleason score 7, PSA 10-29 ng/mL. Clinical data are shown in **Table 2**.

In the first step, all the patients underwent neoadjuvant

Table 1 Clinical characteristics of the initial prostate cancer group of patients.

Age		Status		Gleason		Treatment		Total
mean +/- SD	69 +/-9	no data	5	no data	6	prostektomy	3	44
mean (range)	69 (52-85)	alive	38	low (6-7)	19	chemioterapy	13	
no data	0	dead	1	high (8-9)	19	radical RT	10	
> 60	8					no data	5	
< 60	36							
PSA first value		**PSA after treatment**		**Metastasis**				
no data	3	no data	7	no data	11			
mean +/- SD	278.21 +/- 646	mean +/- SD	278.17 +/- 424	mean (range)	30m (1 - 127)			
0 – 4.0	3	0 – 4.0	10	mean +/- SD	30m +/- 38			
4.1 - 20	6	4.1 - 20	3	multifocal	17			
> 20.1	32	> 20.1	24	bone	10			
				spine	11			

Table 2 Clinical characteristics of the target PC patients group.

Age		Gleason		Treatment		Total
		no data	1	hormonotherapy	40	40
mean +/- SD	69.97 +/- 6.4	low (5-7)	25	HRT	20	
mean (range)	69.97 (56-82)	high (8-9)	14	KRT	20	
PSA	**First value**	**Next to hormonotherapy**		**Next to HRT**		**Next to KRT**
mean +/- SD	42.05 +/- 39	3.79 +/- 7		0.65 +/- 1.2		0.16 +/- 0.21
mean (range)	42.05 (2.4-180)	3.79 (0.044-36.37)		0.65 (0.003-3.99)		0.16 (0.003-0.592)
0 – 4.0	1	29		20		20
4.1 - 20	6	9		0		0
> 20.1	33	2		0		0

hormonotherapy (4 months), and then the pelvic lymphatic system was irradiated. In the second step, in accordance with randomization results, 19 patients were irradiated by a conventional (KRT branch) dose of 2 Gy/day till the summary dose of 78 Gy was reached. Twenty patients (HRT branch) were irradiated by two fractions of 7 Gy until the total dose of 64 Gy was reached. After radiotherapy, hormonotherapy was continued for 24 months for all patients. During therapy, clinical and laboratory data were collected. For molecular analysis, blood was taken at 3 time points: before treatment (point A), after hormonotherapy (point B) and after radiotherapy (point C). The collected clinical data let us define a group of 25 (64%) responders to hormonotherapy, while two years of observation after therapy identified a group of 33 (84.6%) patients whose radiotherapy was effective.

All patients signed an agreement for blood collection and data processing.

RNA isolation: Total RNA was extracted from the whole blood samples using the TRI Reagent (Sigma) according to the manufacturer's protocol. RNA was quantified using a NanoDrop 2000 spectrophotometer (ThermoScientific) before being aliquoted and stored at -80°C until needed.

Real-time quantification of miRNAs: To quantify the mature miRNAs, the following TaqMan MicroRNA Assays (Applied Biosystems) were used: hsa-miR-21, has-miR-100, has-miR-141, has-miR-143 and has-miR-221. U18 was used as a universally expressed endogenous control (Applied Biosystems). All the reactions were performed on a Light Cycler 480 (Roche). Experiments for each miRNA were repeated a minimum 3 times. The miRNAs expression level was determined by equation 2-deltaCt. For further analysis, all the results were transposed to the logarithmic scale (log10).

Statistical analysis was performed with MedCalc version 10.3.2. (MedCalc Software, Mariakerke, Belgium) and Statistica 10 (StatSoft Inc., Poland). Comparison of the miRNA expression profiles between the prostate cancer patients and the controls was done by Student's t test and the Mann-Whitney test. Receiver operating characteristics (ROC) curves were calculated. An optimal cut-off point was calculated according to the highest accuracy (minimal false negative and false positive results). An area under the ROC curve (AUC) was used to estimate the prognostic value of a particular miRNA. Binary logistic regression with a subsequent cross validation was performed to identify the best discriminating combination of miRNAs and to calculate the percentage overall correct classification. All the tests were performed as two-tailed tests and were considered significant at $p<0.05$. Patients were grouped into similar categories with respect to their miRNA expression (Ward's method). Comparison between the groups was performed by Student's t test. Normality was analysed by the Shapiro-Wilks test. A linear trend test was performed using an ANOVA.

Results

miRNAs as diagnostic markers of prostate cancer

In general, both groups (patients and healthy controls) had low expression of miR-100 and miR-141, while miR-21, miR-143 and miR-221 were highly expressed. The expression profiles of the analysed miRNAs did not differ between the males and females in the control group. Therefore, both males and females may serve as controls. Next, we compared the expression profiles between healthy persons and prostate cancer patients. All the analysed miRNAs demonstrated a higher expression level in the prostate cancer patients. The expression of four of these miRNAs differed significantly in the PC patients: miR-21 ($p<0.0001$), miR-141 ($p=0.0026$), miR-143 ($p<0.0001$) and miR-221 ($p=0.0108$). The results for miR-100 were not statistically significant, which is probably due to high differentiation in the group of cancer patients. Nevertheless, the expression of miR-100 was further analysed. The above data are presented in **Table 3.**

To evaluate the ability of miRNAs to discriminate between controls and PC patients, an ROC analysis was done. This calculation revealed that miR-143 expression was the best single miRNA marker, with an area under the ROC curve (AUC) of 80.3% ($p<0.0001$) and a correct overall classification of 71.8%. The worst result was presented by miR-100 with an AUC=0.551, $p=0.4124$ and an overall classification of 56.5%. On the other hand, it must be noted that the specificity of miR-100 expression was 100%. In analysing all the miRNAs results, the correct overall classification decreased to 67.1% (AUC=79.7%); however, the AUC did not differ significantly ($p=0.839$). The full panel of 5 miRNAs suggested PC in the case of the patients, when the probability under the logistic model exceeded 40%. The model consisted of three miRNAs: miR-100, miR-141 and miR-143 (AUC=83.9%; $p<0.0001$), which resulted in the highest overall correct classification of 78.8% and showed the best diagnostic value. For that panel, prostate cancer was considered when the probability was greater than 59%. Although this model had a higher prognostic value, it did not differ significantly from the whole panel ($p=0.093$). All the data are presented in **Table 4** and **Figure 1.** We did not find a correlation between the analysed miRNA expression levels and the clinical parameters.

miRNAs expression profile during therapy-clustering analysis

The average value of the studied miRNAs expression for the

Table 3 Comparison of the expression levels of the study miRNAs between PC patients and healthy controls.

	Cancer patients n=44			Healthy controls n=41			p-value
	Mean	SD	Median	Mean	SD	Median	
miRNA21	3.76	1.28	3.54	2.75	0.7	2.75	<0.0001
miRNA100	-0.15	1.84	-0.14	-0.52	0.77	-0.7	0.2331
miRNA141	-2.12	1.44	-2.02	-2.93	0.87	-2.85	0.0026
miRNA143	2.54	1.41	2.23	1.42	0.65	1.32	<0.0001
miRNA221	3.66	1.35	3.23	2.88	0.69	2.84	0.0108

Table 4 Sensitivity, specificity and cut-off values designed for the studied miRNAs.

miRNA	AUC (95% CI of AUC)	p-value (H0: Area=0.5)	cut-off point	Sensitivity	Specificity	Overall correct classification (%)
miRNA21	0.764	<0.0001	>3.2300	61.36	78.05	67.06%
miRNA100	0.551	0.4124	>0.8426	25	100	56.47%
miRNA141	0.71	0.0002	>-2.6576	72.73	68.29	70.59%
miRNA143	0.803	<0.0001	>1.9537	70.45	80.49	71.76%
miRNA221	0.661	0.0062	>3.6154	40.91	90.24	58.82%
All 5 miRNAs	0.797	<0.0001	>0.4003	81.82	65.85	67.06%
Combined miRNA100 miRNA141 miRNA143	0.839	<0.0001	>0.5910	70.45	90.24	78.82%

target group at point A (before treatment) was higher compared to the healthy control. Data clustering revealed a group of 16 patients with high miRNA expression and a group of 23 patients with low miRNA expression. In comparing the expression levels between these two groups, statistically significant results were obtained for all the studied miRNAs: miR-21 ($p<0.0001$), miR-100 ($p=0.0175$), miR-141 ($p<0.0001$), miR-143 ($p<0.0001$) and miR-221 ($p<0.0001$). The results are presented in **Figure 2A**. At point B (after hormonotherapy), the average expression level of the studied miRNAs decreased (with exception of miR-141), but without statistical significance. The clustering analysis showed two groups: first group with a high expression level (H), including 17 patients, and the second group with a low expression (L), which included 22 patients. Comparing the expression levels of individual miRNAs between the groups, statistically significant results were obtained for miR-21 ($p<0.0001$), miR-100 ($p=0.0034$), miR-141 ($p<0.0001$), miR-143 ($p<0.0001$) and miR-221 ($p<0.0001$). The data are presented in **Figure 2B**. Finally, at point C (after radiotherapy) the expression level of all the studied miRNAs decreased compared with point A and B (with exception of miR-143); however, the observed changes in expression were not statistically significant. After clustering analysis, two groups were present: H (12 patients) and L (27 patients). Comparison of miRNA expression between the groups revealed statistically significant results as above. Only the p-value for miR-100 was changed ($p=0.0051$), and the results are presented in **Figure 2C**. For the patients from low and high groups at points A, B and C, we observed that only one patient was classified into the H group after hormonotherapy, while the L group increased to 27 patients after radiotherapy (3 HRT and 2 KRT patients). Generally, the composition of H and L groups seemed to be stable during therapy. The kind of radiotherapy did not influence the studied miRNA expression levels.

Patients' response to treatment - clustering

Clinical data let us classify 25 patients as responders after hormonotherapy and 33 patients whose treatment succeed after radiotherapy. From the high expression profile (H group), 58, 8% responded, whereas 65% belonged from the L-group. After radiotherapy, the H-group was reduced to 12 cases, but 10 patients (83%) finished therapy with success, while the same effect was observed in 23 cases from the L group (85%). Finally, due to some individuals being replaced during therapy, we noticed that 13 of the 16 patients (62,5%) with initially high expression profiles responded to the treatment, as well as 20 out of 23 (86,9%) of the low expression group. Although it seems that patients from the L group better responded to the treatment, the total number of 6 non-responders was too small to regard as low expression profiles of the marker, which is a better result of treatment. Therefore, we concluded that belonging to the H or L group did not influence the treatment effect.

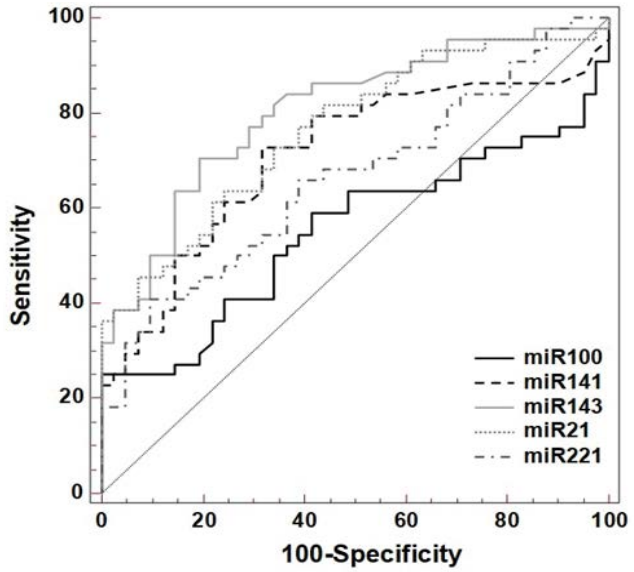

Figure 1 ROC analysis of the studied miRNAs.

Analysis of individual miRNA expression changes during therapy

In this part of the analysis, we examined the expression changes of individual miRNAs at the A, B and C time points. We took a whole group of patients, clusters (high and low expression group) and cut-off values under consideration. The expression level of miR-21 decreased during patient treatment without statistical significance. In both groups, the slope of the expression was observed, but in the H-group, the slope of the p-value was nearly statistically significant ($p=0.053154$). The decline of the miR-100 expression level between point A and C for the whole group was statistically significant ($p=0.0413$), but this effect was not visible in the H and L groups. Although a declining trend was observed, the slope p-value was not statistically significant. The expression level of miR-141 decreased during treatment when whole group

was analysed. In the H-group, miR-141 decreased (slope=-0.2072) while the L-group increased (slope=0.115). We observed expression changes that were not statistically significant. A similar situation was observed for miR-143. In the H group, the expression decreased (slope=-0.2727), while in the L group, expression increased (slope=0.0719). Finally, the expression of miR-221 increased slightly after hormonotherapy, while it decreased after radiotherapy. Similar to the aforementioned results, the expression changes differed depending on the analysed group. In the H group, the expression decrease was statistically significant (slope p=0.0476), while in the L group, it also decreased, but the p-value was not statistically significant. The results are presented in **Figure 3.**

In the last part of the analysis, the patients were grouped on the basis of the cut-off values defined for the studied miRNAs. The miR-21 expression level was above the cut-off value in 30 cases. In this group, the expression of miR-21 decreased between points A and C and it was statistically significant (p=0.0369), but the level of expression was still over the cut-off value. Moreover, the slope was observed (-0.2629), and the p-value was statistically significant (p=0.0019). In the group under the cut-off value, miR-21 expression grew after hormonotherapy, but it decreased after radiotherapy. The observed changes in expression between points A, B and C were not statistically significant. Similarly, the expression level of miR-100 was above the cut-off value in 36 cases; in this group, the decrease in expression (between points

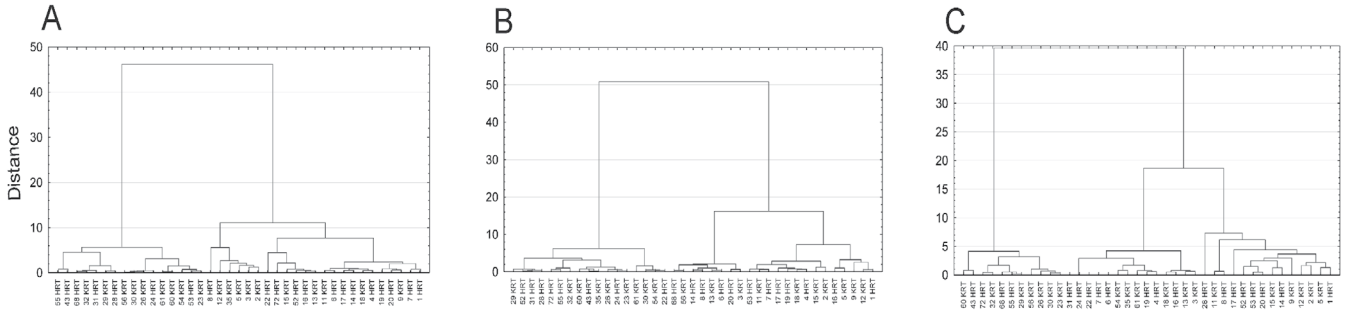

Figure 2 Clustering analysis of the target group of patients based on the miRNA expression profiles. 2A: The average value of the studied miRNA expression for the target group at point A (before treatment) was as follows: 4.1647 for miR-21, 1.8829 for miR-100, 0.5037 for miR-141, 1.7013 for miR-143 and 2.4318 for miR-221. In the first group (high expression, H), the levels of miRNA expression were as follows: 5.4213 for miR-21, 2.2443 for miR-100, 1.7087 for miR-141, 2.7806 for miR-143 and 3.47 for miR-221, whereas in the second group (low expression, L), we obtained the following results: 3.2906 for miR-21, 1.6315 for miR-100, -0.3346 for miR141, 0.9506 for miR-143 and finally 1.7096 for miR-221. In comparing the expression levels between these two groups, statistically significant results were obtained for all the studied miRNAs: for miR-21 (p<0.0001), miR-100 (p= 0.0175), miR-141 (p<0.0001), miR-143 (p<0.0001) and for miR-221 (p<0.0001). 2B. After hormonotherapy, the average expression values were 4.1176 for miR-21, 1.8302 for miR-100, 0.7085 for miR-141, 1.664 for miR-143 and 2.4834 for miR-221. Comparing the expression levels of the two groups of high expression level and low expression level, the differences in the expression levels between the groups for each miRNA were statistically significant for all the studied miRNAs: miR-21 (p<0.0001), miR-100 (p=0.0034), miR-141 (p<0.0001), miR-143 (p<0.0001) and miR-221 (p<0.0001). 2C. After radiotherapy, the average expression levels of the studied miRNAs decreased, giving the following results: 3.8805 for miR-21, 1.509 for miR-100, 0.4693 for miR-141, 1.5624 for miR-143 and 2.2144 for miR-221. Two high and low expression groups were observed, as above. When comparing the expression levels for each miRNA between the groups, statistically significant results were obtained. As shown above, only the p-value for miR-100 was changed (p=0.0051).

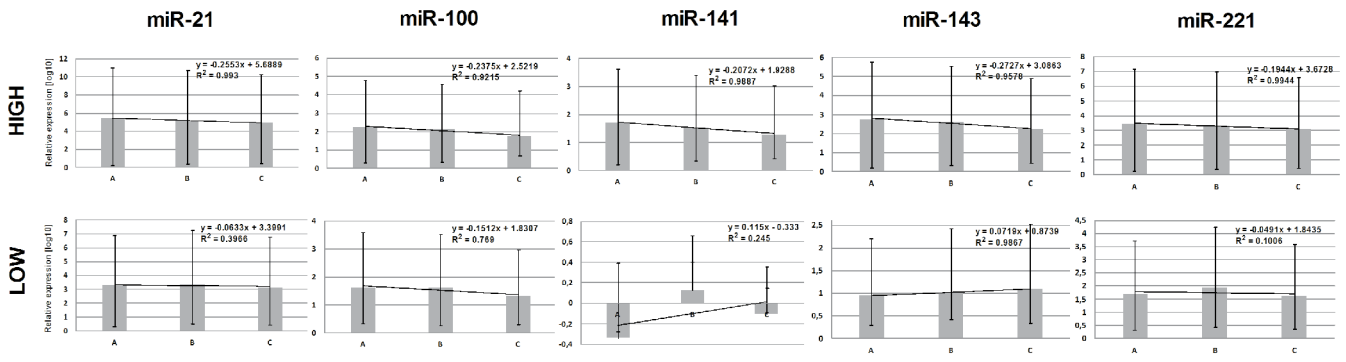

Figure 3 Changes in the expression levels of each studied miRNA in the high and low clusters. A statistically significant decrease was observed for miR-100 (p=0.0413), but this result was obtained for all the groups of patients. Changes in the clusters were not statistically significant.

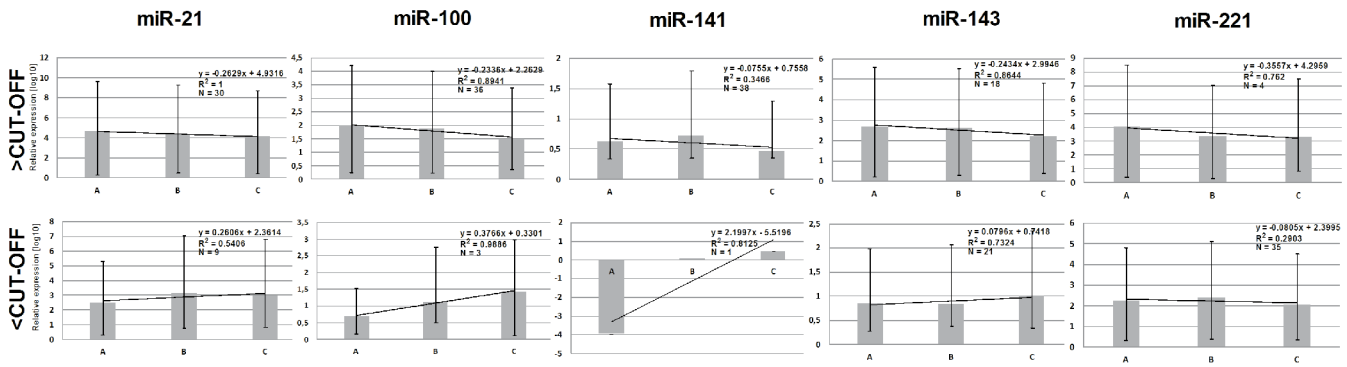

Figure 4 Analysis of the expression changes in the group of patients above and under the cut-off value. Statistically significant results were obtained for miR-21 and miR-100 in the group above the cut-off value (p=0.0369 and p=0.0141 respectively). In this group, a statistically significant slope was observed for miR-21 (p=0.0019).

A and C) was statistically significant (p=0.0141), and the slope was observed (-0.2336). In the remaining group, the expression of miR-100 increased (slope 0.3766), but without a statistically significant result. The expression level above the cut-off value was observed for miR-141 in 38 cases. Therefore, the statistical analysis was not performed. For miR-143, there were 18 cases under the cut-off value. The differences in the expression between points A, B and C were not statistically significant. In this group, the slope was observed. Finally, there were 4 cases under the cut-off for miR-221. The differences in the expression between points A, B and C were not statistically significant as observed in both slope groups. In sum, statistically significant changes in the expression level were observed between points A and C and they were related only to patients with over-expressed miR-21 and miR-100. Similarly, a statistically significant slope was observed only for patients recruited from the H group or who demonstrated an expression level above the cut-off value. The results are presented in **Figure 4.**

Discussion

Currently, serum prostate specific antigen (PSA) is routinely used as a marker in clinical practice for monitoring the response to treatment. Nevertheless, it is not a perfect tool. Many researchers focused on other molecules with disease association, such as the circulating miRNAs. Although miRNAs are promising molecules as biomarkers, to date, there has been no standard diagnostic panel used in clinical practice. The first controversy regards the source of miRNAs. There are many reports showing differences in the miRNA content between the serum, plasma, exosomes and whole blood [3,8-11]. In our study, we used the whole blood for miRNA isolation and an internal control U18. The best results (sensitivity and specificity) were obtained for miR-141 and miR-143. In a similar study carried out on the serum of patients with metastatic prostate cancer, the best result was also obtained for miR-141 (60% sensitivity, 100% specificity) [9]. What is interesting our study indicated the over-expression of both these miRNAs, which are regarded as tumour suppressors. On the other hand, a higher level of miR-141 was also observed [9,12]. Moreover, the level of miR-143 expression let us distinguish two groups of PC patients. In the first group, showing low miR-143 expression is characterized by a higher Gleason grade, with a higher PSA level and bone metastasis [13]; in the second group, over-expressed miR-143 shows a reduced number of bone metastases [14]. These findings indicate that the demonstration of up- or down-regulation in the studied miRNAs depends on the selection of the study group of PC patients. Similarly, there are some articles showing a correlation between miRNA expression and clinical data [10,15,16]. Another author considered that the clinical parameters had a limited impact on the overall abundance of miRNAs, as well as on the changing miRNA expression profiles [17]. In our study, no correlation between miRNA expression and clinical data was found.

In the second part of this study, we analysed the changes in the expression levels of the selected miRNAs at three time points: before treatment, after hormonotherapy and radiotherapy. Androgen Receptor (AR) and its down regulated signalling pathway is crucial for the origin and development of PC. Therefore, PC therapy is often focused on blocking AR activity. Certain miRNAs are known to be AR regulated or connected with the regulation of the AR pathway [18]. Among the miRNAs studied in this work, miR-21 is an example of an androgen receptor-regulated miRNA [19]. It was shown that the inhibition of miR-21 expression abolished androgen-dependent cell proliferation, whereas high expression of this miRNA promoted proliferation [11]. These data suggest that miR-21 can stimulate cell growth via an androgen-dependent pathway, as well as the development of castrate-resistant prostate cancer (CRPC) [19]. Next, miR-141 also belongs to a group of molecules regulated by AR [20]. Moreover, miR-141 can activate the AR signalling pathway through inhibition of the orphan receptor Small Heterodimer Partner (SHP). Finally, miR-221 takes part in androgen dependent cell proliferation same as miR-21. When its over-expressed, androgen independent growth may be observed [21]. These data came from prostate cancer cell lines or tissue analysis. Our research concerned blood sample analysis. Therefore, the expression levels of the studied miRNAs do not exactly reflect expression changes in the cancer tissue. In our research, lower expression of miR-21 was observed after hormonotherapy, but in fact, this observation can be applied only

to individuals from the H group and/or above the cut-off value. In the remaining cases, expression of miR-21 slightly increased. Similar behaviour was observed for miR-141. Its expression decreased in the group above the cut-off value, whereas in the expression in the remaining patients increased. Expression of miR-221 slightly increased after hormonotherapy, irrespective of the analysed group. Regardless of described above differences in expression levels it should be emphasized that these changes were not statistically significant. Moreover, clustering analysis revealed that classification to the H or L group was not important for the hormonotherapy response.

miRNAs are important elements of the cell response to irradiation, regulating many processes such as radiosensitivity, the DNA Damage Response (DDR), the cell cycle, and apoptosis [22,23]. The role of miRNA response to IR (irradiation) in prostate cancer is barely recognized. Moreover, data came mainly from studies on cell culture. These data enabled the specification of up- or down-regulated miRNAs after irradiation and miRNAs connected with radioresistance [24-26]. Particularly, due to the expression of miR-100 analysed in this work, research demonstrating the role of miR-99a and miR-100 in the insensitivity of prostate cancer cells seems to be interesting [27]. High expression of this miRNAs correlates with sensitivity of prostate cancer cells, whereas low expression prevents p53-dependent apoptosis after irradiation, resulting in a worse response [28]. In our study, the decrease in the expression of miR-100 after radiotherapy was statistically significant for the whole group of patients. The same phenomenon was observed for the above cut-off value group. Linking these observations with the treatment results, we noticed that the expression profiles of miR-100 that were determined in the blood samples differed from those observed in the cancer tissue. Most likely, this reflected the changes in the expression levels of different blood cells. This is another explanation for the down-regulation of miR-21 we found. A study carried out on the blood samples of patients after radiotherapy defined a group of 45 miRNAs which were upregulated after irradiation [29]. miR-21 was among them. In our study, there was a statistically significant decrease in the group of patients above the cut-off value. The authors collected blood samples from the radiotherapy patients 4 hours after total body irradiation. Our patients were locally irradiated, and the blood samples were taken a month after they finished radiotherapy. It is known that the expression profile of miRNAs changes in time [30,31]. After a month, we probably observed the result of the therapy, not the direct effect of the radiation. Additionally, the down-regulation of miR-21 probably depended on the initial expression level of this miRNA (cut-off value), but not on the kind of radiotherapy [32-34].

Conclusions

In this work, we analysed the utility of five miRNAs as biomarkers of prostate cancer patient therapy. We found that statistically significant differences in the expression level appeared after radiotherapy, but they concerned only miR-100 and miR-21 in the group of patients above the cut-off value. Generally, the expression profile defined before treatment facilitates the ability to distinguish groups of patients with high and low expression of the studied miRNAs, but the profile showed stability during treatment. Moreover, membership in the high or low expression group did not influence the treatment result. Taken together, our data suggest that the miRNAs investigated in this work could not serve as biomarkers of the effectiveness of prostate cancer patient therapy.

Declaration

Ethics approval and consent to participate

The current study was approved by the Institutional Review Board of University of Medical Sciences in Poznan (No. 317/14), and informed consents were obtained from all patients.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and material

All data are available in manuscript.

Competing Interest

The authors declare that they have no competing interests.

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Author's Contribution

KML was a major contributor in study conception and writing the manuscript. PM. clinical patients management. TK, AT, RB, AK, AJ-R laboratory work, EL-clinical laboratory analysis, WS, JM data interpretation, MM statistical analysis and figure preparation.

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The language correctness was checked by American Journal Experts.

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