 doi:10.20944/preprints202210.0266.v1

Article

Passive Microwave Radiometry (MWR) and Mirna for Breast Cancer Diagnostics

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Abstracts: Breast cancer prevention is very important for a woman's health worldwide. We have demonstrated a correlation between mammography and ultrasound with diagnoses using passive microwave radiometry (MWR) and a miRNA oncopanel. While mammography screening dynamics could be completed in 3-6 months, MWR will provide us with a prediction in a matter of weeks or even days with the potential for complementary miRNA diagnostics. An early breast cancer diagnosis may be accomplished using either one of these novel techniques alone or in conjunction with more established techniques.

Keywords: Breast cancer; early diagnostics; passive microwave radiometry (MWR), MicroRNA (miRNA)

1. Introduction

The main goal of screening studies is to detect breast cancer at the earliest possible stages. Modern diagnostics methods include mammography (MMG), ultrasound, computer tomography (CT), and magnetic resonance imaging (MRI) can detect tumours of the smallest sizes from 3 mm.

The classification of breast pathology is Breast Imaging Reporting and Database System score. It's a scoring system to describe mammogram results (BI-RADS) [1]. Clinicians and radiologists can use the same language. It was shown [2] that the main number of deaths is associated with aggressive, fast-growing breast cancers. Of particular importance is the transit BI-RADS-3 category.

Clinical recommendations state that a follow-up mammogram should be done within 3-6 months. Most of the time, BI-RADS-3 changes into BI-RADS-2, and this is considered a benign process.

Unfortunately, after passive observation, 2-4% of cases progressed to BI-RADS-4a, which suggests a cancer risk of up to 10%. So, aggressive breast cancers could be detected, but only after 3-6 months. So, a lot of patients with BR-4a breast cancer are not receiving treatment in time, which leads to an increase in mortality.

It would be advantageous to develop novel diagnostic techniques that would enable us to recognise potentially harmful BR-3 tumours and foresee the development of aggressive BI-RADS-4 cancer. It would be possible to reduce breast cancer-related deaths.

2. MATERIALS AND METHODS

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The purpose of the study is to use two new screening methods, namely Passive Microwave Radiometry (MWR) and micro-RNA for a more accurate assessment of BI-RADS-3 cases, which will allow the identification of the risks of developing aggressive tumours.

We examined 230 patients aged 35-55 years (mean age 43±2.4 years). The patients underwent mammography (FujiFilm Amulet, Japan), which was assessed on the BI-RADS score. Scans were taken in standard projections - craniolateral and mediolateral projections.

In addition, we have used ultrasound (MindRay DC-60Exp, China), Microwave Radiometry (MMWR2020 (former RTM-01-RES), Medical Microwave Radiometry LTD, UK) [3, 4] and micro-RNA oncopanel (Oncounite, Skolkovo) [5]. The follow-up period was 12 months.

Using MWR2020 device internal and skin temperature measurements have been done in 22 points (left and right breast, control points shown in Fig 1. The results were visualised in Fig. 2 and stored in csv data format for further analysis. We have used Vidyukov *et al.* [6] two empirical coefficients to stratify patients, and assessed the risk of developing breast cancer into three groups. The average internal and skin temperature of breast tissue, the difference between the maximum and average skin and the internal temperatures for each gland were calculated. The obtained differences are summarized accordingly for the right and left breasts. The largest value of Qmax was chosen. Next, the maximum value of the temperature difference between separate symmetrical points of the right and left mammary glands (Kint and Kskin=R) were determined. If Qmax > 2.0 and R > 2.5 a malignant tumour is possible. If the indicated values are valid for one criterion (Qmax or R), both malignant and benign tumours are possible. If the values of these criteria are below the specified thresholds Qmax > 2.0 and R > 2.5, a benign tumour is diagnosed.

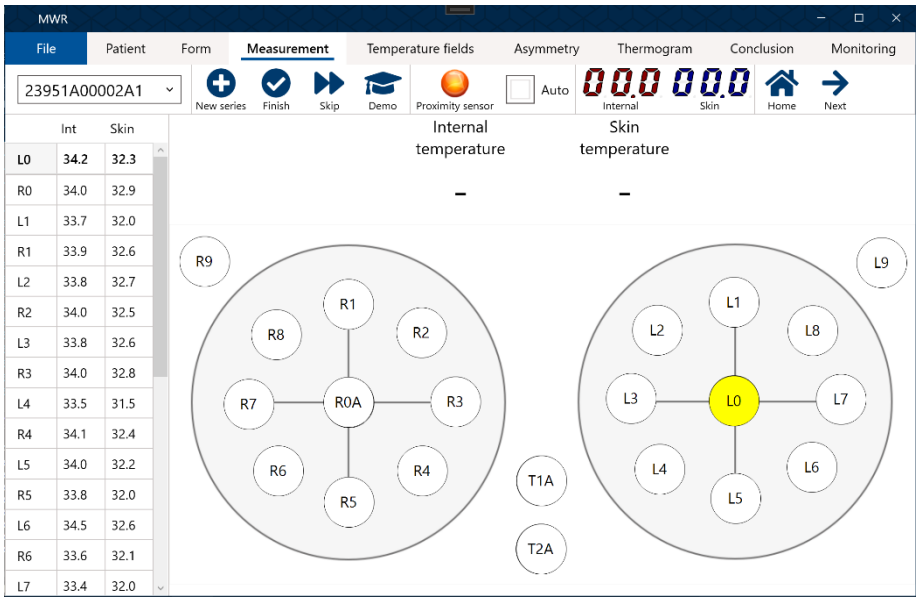

Figure 1. Interface MWR2020 software. Measurement points are shown.

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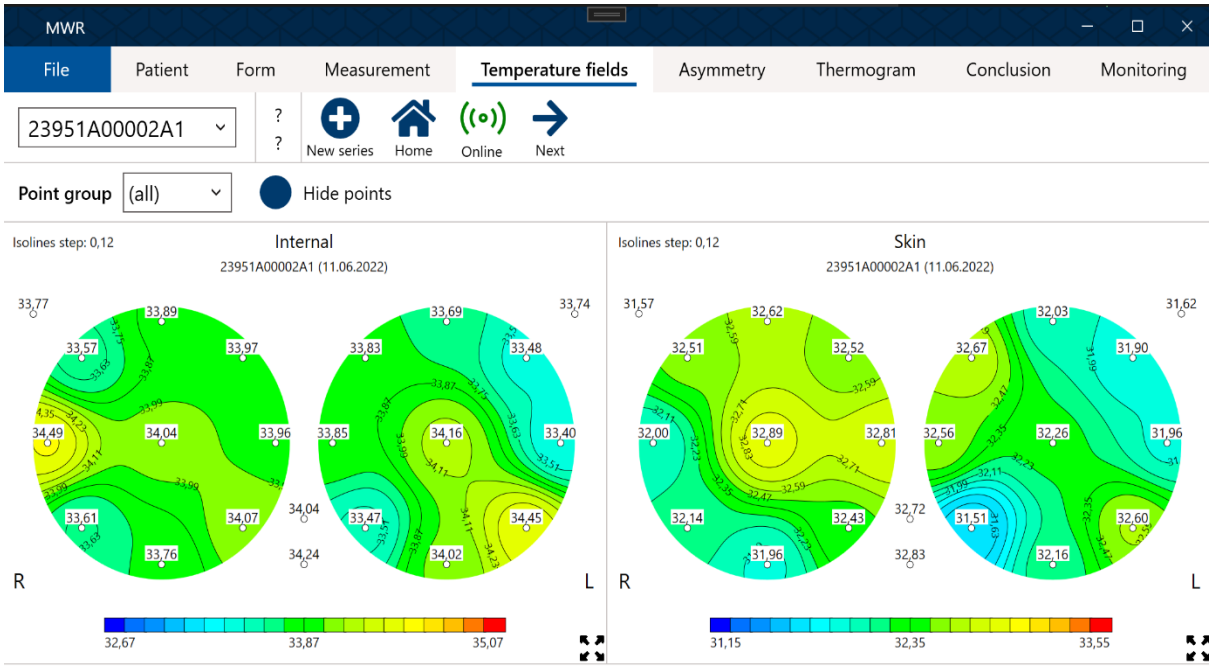

Figure 2. Interface of MWR2020 software. Measurements of Internal temperature are shown in healthy patient.

A novel miRNA panel was used [5] to assess the risk and dynamics of breast cancer development. It includes measurements of eight miRNA samples

1. Hsa-miR-199a-3p is responsible for metastasis. Stimulation of angiogenesis through overexpression of ApoE - an increase in the tumour

2. Hsa-miR-222-3p - suppressor, increased proliferation, as well as differentiation and blockade of apoptosis in cells - increased at risk.

3. Hsa-let-7a-5p-increased levels of integrin B-3 - associated with TNF - control of proliferation - reduced risk of disease

4. Micro-RNA-196a-2 - protective factor. Reduced growth and proliferation, control of migration and invasion - reduces the risk of tumour formation

5. Hsa-miR-106a-5p - induces apoptosis, reduces proliferation - reduced in tumours

6. Hsa-miR-21-5p is an activator of PI-3K Akt pathway - cell proliferation and survival - increase at risk

7. Hsa-miR-21-137 is responsible for proliferation, apoptosis - reduced in disease

8. Hsa-miR-155 - decrease in taurine levels, increase the effect of oxidative stress - increased with taurine deficiency.

Isolation of micro-RNA is carried out according to the following method: 5 μM of proteinase K is added to the sample, inhibiting at 56°C for 1 hour, then Exigon columns (Exigon, Belgium) are used to purify micro-RNA. The sample is applied to the column, after which centrifugation is carried out at 2,000 g in a centrifuge Eppendorf 5104 (, then the column is washed 3 times with washing buffer, after each washing, centrifugation is carried out at 2000 g in a 5104 centrifuge, after which a reverse transcriptase reaction is carried out, for this, 8 μl of the sample is added to 8 μl of the sample and incubated for 1 hour at a temperature 60°C after which the reaction is stopped for 5 minutes. Then PCR (7500 Applied Biosystems, USA) is carried out with real-time detection on the instrument with primers for the following miRNAs: hsa-miR-199a, hsa-miR-214-3p, miR-25, miR-26a, hsa-let-7a -5p, miR-99a, miR-184, miR-24-3p, miRNA-195, hsa-miR-21=5p, hsa-miR-195.

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The results were analyzed using GenEx qPCR software (MultiD Analyses AB, Germany). Concentration is presented as log base 2 miRNA (copies/µl).

Table 1. miRNA expression at the initial investigation in Patient P. Changes in the quantitative indicator (copies/µl) between norm and pathology in 1.5-2 times is a slight increase (risk factor=1), by 2-5 – increase (risk factor=2), by 5 or more - a significant increase (risk factor=3). The threshold for each miRNA is determined by precedent statistics from the literature.

N	Marker	Norm	Pathology	Fold chainge
1	Hsa-miR-155	28294	202238	7.1
2	Hsa -miR-196a-3p	104809	324654	3.1
3	Hsa-miR-222-3p	159928	88908	0.6
4	Hsa-let-7a-5p	13327568	2665531	0.2
5	Micro-196a-2	1665957	333191	0.2
6	Hsa-miR-106a-5p	380633	76126	0.2
7	Has -miR-21-5p	22209402	111047010	5.0
8	Hsa-miR-137	555235050	111047010	0.2

3. Results

Mammography was performed two times during one year. According to mammography, an increase in density (>5) was noted in 3 patients and they were transferred to the BI-RADS-4a category. We have found a positive correlation between the increase in BI-RADS and increase in Qmax and R coefficients Fig 3, and the significant increase in oncological micro-RNA. In those patients where there was no increase in radiological density, there were no negative dynamics according to MWR and no negative dynamics were noted when analyzing the micro-RNA oncopanel.

When conducting morphological studies of punctures in patients with an increase in BI-RADS, it was found that high MWR coefficients (Qmax > 2.0 and R > 2.5) and microRNA changes (>5) indicated a risk of developing obligate precancer, where tumour never progress into types of lesions other than cancer. These results were confirmed by histological parameters Ca in situ in 2 cases

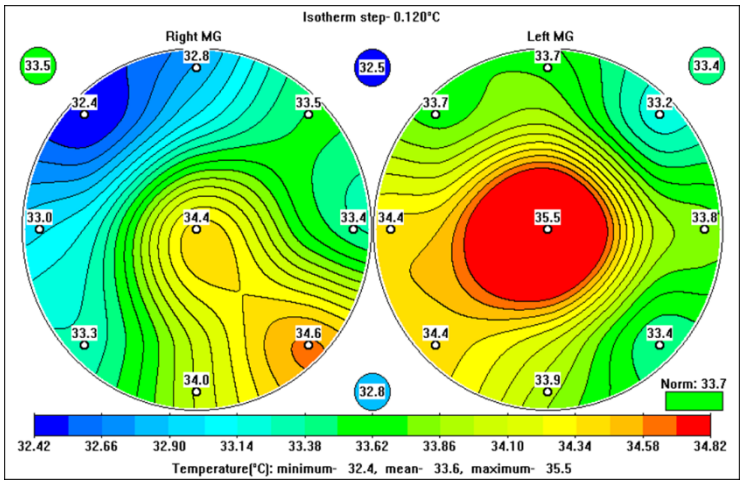

Figure 3. Internal temperature fields in the left (right) and right (left) mammary glands. Possible breast cancer.

Clinical example

Patient P., born in 1957, complained of scanty discharge from the nipple of the right breast. MMG has shown a picture of pronounced adenosis, with high radiographic den-

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sity 5. When conducting an ultrasound of the mammary glands without nodular formations, many microcysts with a diameter of 4-5 mm were found. Using MWR examination of the mammary glands it was found that the Qmax was elevated 1.7 (the threshold is 2), and R was slightly elevated 1.9 (the threshold is up 2.5). To clarify the diagnosis, the miRNA oncopanel was used. As a result of the analysis, the risk of developing breast cancer was slightly increased and equal 3 (1+1+1), which is typical for a low risk of developing breast cancer (Table 1)

Table 2. miRNA expression at the initial investigation in patient P.

N	Marker	Expression
1	Hsa-miR-155	Norm
2	Hsa -miR-196a-3p	Norm
3	Hsa-miR-222-3p	Slight increase
4	Hsa-let-7a-5p	Slight increase
5	Micro-196a-2	Norm
6	Hsa-miR-106a-5p	Slight increase
7	Has -miR-21-5p	Norm
8	Hsa-miR-137	Norm

After six months, the patient went for a follow-up examination with complaints about the appearance of a nodular formation in the right mammary gland of about 2 cm, and an increase in axillary lymph nodes on the right. MMG determined a nodular polycyclic formation 2 cm in diameter in the right mammary gland, and the presence of grouped microcalcifications. Ultrasound of the mammary glands showed quadrant hypoechoic formation of irregular shape 2 cm in the right mammary gland and enlarged nodes up to 2.5 cm in the right axillary region.

MWR study showed that the Qmax =2.45, and the R= 3.2, which corresponds to the probable development of breast cancer over 85%. The dynamic study of miRNAs gave the following results shown in Table 3

Table 3. miRNA expression in patient P after 6 months.

N	Marker	Expression
1	Hsa-miR-155	Norm
2	Hsa-miR-196a-3p	Norm
3	Hsa-miR-222-3p	Pronounced increase-3
4	Hsa- let-7a-5p	Pronounced increase-3
5	Micro-RNA-196a-2	Norm
6	Hsa-miR-106a-5p	Slight increase-1
7	Hsa – miR-21-5p	Norm
8	Hsa- miR-137	norm

The risk factor was 7 (3+3+1), which is typical for a high risk of developing breast cancer. Histological examination confirmed the presence of invasive carcinoma.

4. Discussion

We propose a new scientific approach based on different biophysical and molecular biology principles combined - MWR and the determination of micro-RNA, which could successfully supplement existing methods - mammography and ultrasound.

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In several clinical examples [7] using two coefficients [6], results were showing that the data obtained using MWR were ahead of the results of mammography by 1.5-2 years.

Recently, artificial Intelligence approaches have been applied to MWR data for early-stage breast cancer predictions to approve diagnostics accuracy.

The MWR data from >400 patients were classified by clinicians as either low- or high risk of breast cancer, deep neural networks achieved the best performance with accuracy >0.8 [8, 9]

Later, significant progress has been done in the prediction of cancer using a Weight Agnostic Neural Network (WANN) on the curated dataset of 4912 patients with an accuracy of 0.921. The results are an indication of the potential of MWR utilizing a neural network-based diagnostic tool for cancer detection. [10]

At the same time, with further study of this technique, data were obtained showing the limitations of the MWR in some pathologies of the mammary gland. First, with the slow growth of a malignant neoplasm, heat dissipation is observed, which is masked by the heat generated by the surrounding tissues. Secondly, a large malignant neoplasm of the mammary gland (4-5 cm) can be limited to a fibrous capsule, which, like a Dewar's vessel, does not allow heat to go beyond the border of the tumour. These problems can be largely solved with more frequent measurements. Other methods of thermometry could be explored for cancer detection [11, 12]

If the mammography screening could be performed in 3-6 months, then MWR could be used in weeks or even in days.

Another novel way to identify the pathology of breast tissue is to analyze the ratio of precancerous and neoplastic diseases [13]. Genetic mutations in precancerous and neoplastic pathologies are of the same nature. Thus, the following question is formed - what contributes to the qualitative transition from precancerous pathology to the neoplastic process?

The first option is to determine mutations in precancerous neoplasms. This path is quite promising with relation to the administration of targeted therapy, but so far this method is limited by financial problems

The next promising way to solve this problem is the analysis of intercellular interactions, i.e. the result of the work of mutant genes. It has been proven that exosomes and, more specifically, their qualitative composition play a leading role in this process. The main factor that transmits information is the quantitative and qualitative composition of miRNAs.

Micro-RNAs are "global switches of the genome" that regulate multiple metabolic pathways and the formation of protein products. It has been proven that a number of microRNAs have an oncogenic effect [14], which include microRNAs -21,155,196a-2, 27a, 9,199a-3p, 222-3p, let-7a-5p, 137,106a-5p. Micro-RNA 21 is one of the most well-known and studied micro-RNAs in different types of tumours. Its expression is sharply increased in breast cancer, which is associated with tumour growth, metastases, and an unfavourable prognosis for the course of the disease [15]. Overexpression of miRNA -155 is manifested by a decrease in the level of taurine and an increase in the influence of the level of oxidative stress and is often found in breast tumour tissue and negatively affects the survival and chemosensitivity (through the FOXO3a gene) of tumour cells, while reduced expression of mir-155 can enhance cellular chemosensitivity and apoptosis.

The levels of some miRNAs have been studied in benign pathology. Expression of MiR-21 causes the blocking of genes associated with apoptosis [16]. MiR-221/222 is classified as an oncogenic micro-RNA, overexpression of which in different types of tumours leads to increased cell proliferation, inhibition of apoptosis, and induction of angiogenesis. The suppression and low expression level of MiR 221/222 in breast tumours correlate with the positive status of oestrogen receptors and a more favourable prognosis of the disease.

The activity of MiR-155 [17] is necessary to maintain the normal functioning of cells; an increase in the expression of this miRNA has been noted in autoimmune diseases and

various forms of cancer. In addition, this miRNA is associated with the oestrogen-positive status of the tumour and can potentially serve as a diagnostic marker.

According to [18] MiR-205 induces apoptosis and inhibits the growth and invasion of tumour cells and is also a tumorigenesis suppressor. V.V. Kozlov studied several miRNAs in patients diagnosed with breast fibroadenoma. Studied: miRNA 21, 155, 221, 222. The author noted a tenfold increase in microRNAs that induce apoptosis and cell growth. The study of the role of miRNA 137,199 and others made it possible to conduct a more informative analysis of the body's resistance to tumour aggression [19-21]

5. Conclusion

The combined use of classical methods for early breast screening of the mammary glands - mammography and ultrasound with novel approaches MWR and micro-RNAs panel makes it possible to discriminate between pathologies, which in the foreseeable future will most likely turn into cancer.

All methods separately give a slight high probability of cancer development, but the combination of all methods, if used, could give us an indication of the high risks of malignancy.

This makes it possible to prescribe individual onco-prophylactic complexes that will contribute to the regression of obligate precancer, and, in the future, it is possible to achieve a decrease in cases of breast cancer.

Conflicts of Interest : No

Ethics: Protocol N3 14.09. 2022 Ethics committee of Russian Academy of Medical-Social Rehabilitation

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