New strategy is needed for the follow up of the patients with colon- and rectum cancer; microRNS (miRNS) as the potentialbiomarkers of the early detection.

Beneficial effects of Patient's organisation on survival

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PhD thesis

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1. INTRODUCTION

1.1. Primary epidemiological characteristics of colonic – and rectum cancer (CRC)

According to international statistics, CRC is one of the leading cause of death both in the USA and Europe (14-15 % of all tumors). They have already been mentioned in the literature as the disaster of the western (welfare) society before. Undoubtedly, however, that European CRC mortality shows a downward tendency in the last two decades primarily in the western European countries to which the effective screening programs also contribute.

Unfortunately, in the European mortality statistics, Hungary and the Czech Republic is with terrible data represented. In our country annually the number of the new cases reported (for both gender) is approximately 9.000. The number of death is yearly is 5000, stagnant.

The five-year survival data in the international literature are very varied, in the USA 65%, in Europe 55%. Higher or lower data exists also. Data from the northern countries sow a favorable picture.

According to the data of the Hungarian National Cancer Registry 5 year survival of colon and rectum carcinoma (both gender) is 40%. Half of all cancer death happens already in the first year. The public health importance of CRC is therefore outstanding. The delay (or the early detection) is vital in the aspect of the CRC patient's survival.

1.2. The justification of patient tracking after the healing surgery, main aspects of the necessity of paradigm shift

After the healing surgery, the fundamental aspects of the patient follow up are the detection of the recurrent tumor as soon as possible, the recognition of its metastases, the detection of an accidental second primer tumor, as well as the reassuring and the improvement of the quality of life of the patient.

Unfortunately, there is no consensus in the literature regarding the optimal follow up. Furthermore the selection and association of the strategic elements to ensure intensive and less intensive follow up show significant difference.

However, the intense demand and the waiting for the early detection, for the extensive examination of the suitable molecular tests of the „sub-clinical" recurrence and in general for the headway of the non-invasive follow-up models cannot be disputed — neither for better expectation concerning improved survival.

However, it cannot be disputed that there would be a significant demand for extensive molecular testing suitable for early detection, for the subclinical recurrence or usually for the expansion of non-invasive follow-up methods Neither for better expectation concerning improved survival.

1.3. Main objectives of the dissertation

1. In the present work, we would like to focus primarily on the frameworks and contradictions of the follow-up strategies related to the removal of the healing tumor because the methodological and strategic disputes continue still nowadays. It is indubitable that new follow up strategies development is inevitable with possible involvement of new biomarkers into the follow-up models linked to the risk assessment of recurrence.

2. Concerning the research work of the dissertation, we want to deal with the practical value and combinations option of a new biomarker group the miRNA. We are looking for the place of this new marker-family in an effective follow-up strategy with model value.

3. We want to help in the selection of the high-value factors from the possibly large number marker-group, in recognition of new connections which is only through the works of several Institutions possible.

4. With the application of invasive devices and interventions, non-invasive methods should also be offered for the detection of the asymptomatic „subclinical" cases in the hope of longer survival.

5. We also want to investigate the advantageous co-effect on the survival of some patient organizations looking for new interfaces between the modern laboratory-investigations and this patient organization in the public health frame.

2. MICRO-RNA AS NEW TUMOR-BIOMARKERS

2.1. The potential biomarkers of CRC

Next to the colonoscopic and imaging investigations, a non-invasive laboratory test with high sensitivity and specificity is also entirely justifiable, which has a not only early diagnostic but an also prognostic value and so contribute to the improvement of the therapeutic protocols.

This research group has drawn the attention, to the relationship between the miRNA and the malignant disease which found a relationship between the under-regulation of miRNA-15 and miRNA-16 and the chronic lymphocytic leukemia.

Later they also recognized, that the miRNS are in body fluids for example both in plasma and in serum also stable. They were able to detect the presence of miRNA - referring to the malignant process - in mouse-models and also in human blood samples. This started a new biomarker-research process.

2.2. MiRNS patterns- the early detection and the prognostics

In recent years more and more circulating miRNAs were detected as the „candidate" of the clinical practice which can contribute to the reliable separation of the precancerous conditions (primarily advanced adenoma), the CRC and the normal controls.

In the definition of the prognostic judgment, the probabilities of the after-treatment relapse appearance play a significant role.

The reduction of the postoperative miRNA expression was detected in several patterns, but the patients were only seldom followed.

Some test however confirmed, that the plasma miRNA profiles – after surgery – can change within days. However, the question remains open which cell- processes are involved in the expression change.

3. MATERIALS AND METHODS

3.1. Patient-selection

A total of 43 patients with hysto-pathologically confirmed CRC (27 with colorectal cancer, 16 with rectal cancer) and 12 control patients - without any complaints or other diseases - were investigated (Table 1). All patients were treated at the National Institute of Oncology between 2012-2014. All participants gave written consent.

3.2. The course of the miRNS tests and its technical methodological features

RNA isolation: The miRNA were isolated from serum with TRI Reagent (Sigma-Aldrich Inc., Budapest, Hungary) according to the manufacturer's instruction. The RNA quality was checked by nano-drop absorption photometry, and only RNS fractions with A 2.0 at 260/280 nm was used for reserve transcription.

miRNA expression was determined in a Roche LC480 system. PCR primers for miR-155, miR-21, miR-221, miR-30a, miR-34a, and miR29b were designed using a „primer finder database" (www.applied-science.roche.com) and were synthesized at TIB Molbiol, ADR Logistics system (Roche Warehouse, Budapest, Hungary). The data were normalized using 5SrRNA and U6snRNA (as endogenous reference). Relative miRNA expression levels were compared using two methods.

Statistical analyses were performed using IBM SPSS Version 21 (Armonk, New York USA).

Mann-Whitney U-test was used to compare continuous and categorical variable based on data analysis.

4. RESULTS

4.1. Control persons and patients with tumor

The expression of miR-155 was significantly higher in sera of control individuals than that of patients with colonic (p<0,001) or rectal (p<0,001) cancer.

Furthermore, miR-155 expression was significantly higher in rectal cancer patients (p=0,006) than in colonic cancer. The miR-21 was only in one colon cancer case significantly (p=0,005) higher compared to rectum cancer cases.

In the other rectum cancer cases, the serum miR-21 serum expression was higher compared to the controls (p<0,001) and to the colon cancer patients (p<0,001).

For miR-29b, miR-30a and miR-34a the differences were similar, namely significant differences in control-colon cancer as well as control-rectum cancer. The control group values were higher, then that of the tumor group values (Table 2).

Table 1. The selected characteristics of the study participants

Control group	6 men	6 women
	Age: 59,00 (CI:50,48-67,52)	
Colon Cancer (ICD-10: C18.9)	15 men	12 women
	Age: 60,81 (CI:54,53-67,10)	
	1 TNM I. 9 TNM II. 4 TNM III. 1 TNM IV.	4 TNM II. 2 TNM III. 6 TNM IV.
Rectum Cancer (ICD-10: C20)	9 férfi	7 nő
	Kor: 60,81 (CI:54,53-67,10)	
	3 TNM II. 5 TNM III. 1 TNM IV.	2 TNM II. 5 TNM III.

4.2. Comparison of control and tumor cases according to TNM stage

Expression in patients with TNM I cancer was not significantly different from that of the controls for any of the studied serum miRNAs.

Expression of miR-155, miR-34a, and miR29a in TNM II, III and IV were significantly lower than those of the controls. Expression if miR-221 was elevated only in TNM III compared to the controls. It is remarkable that the miR-30a expression was significantly increased in TNM II (p=0,01) and TNM III (p=0,001) compared to the control group.

Table 2. Statistical analysis of the microRNA analyse

Mann-Whitney Test	Relations between variables					
	miR-155	miR-21	miR-221	miR-30a	miR-34a	miR-29b
Group-variables: ICD						
Control; colon cancer	0,000*	0,442	0,543	0,002*	0,000*	0,000*
Control; rectum cancer	0,000*	0,057	0,000*	0,000*	0,000*	0,002*
Colon cancer, rectum cancer	0,006*	0,005*	0,000*	0,183	0,705	0,342
Group variables: stadium						
Control ;TNM I.	0,114	0,114	0,752	0,113	0,114	-
Control; TNM II.	0,000*	0,981	0,362	0,010*	0,000*	0,001*
Control; TNM III.	0,000*	0,437	0,007*	0,001*	0,000*	0,001*
Control; TNM IV.	0,000*	0,929	0,929	0,065	0,001*	0,011*

5. DISCUSSION

5.1 Strategic contradictions of the patient follow up

Regrettably, there are no optimal strategy nowadays which can be recommended for the follow up of the CRC patients undergone healing surgery. Although the test results are burdened with many contradictions, according to the collected data, we can conclude, that the intensive follows up has a positive effect on survival.

5.2. Classical and new biomarkers – as indicators

It became clear, that the old biomarkers (together with other traditional methods) no longer, and the new biomarkers make not yet up exclusive useful follow-up models for the early detection or patient follow up.

Because the characteristic changes of the unique patterns of the miRNA can characterize specific pathological processes. There is hope that during the evaluation of the miRNA profiles the tendency of the tumor recurrence, of spread and metastasis formation are predictable, this can be a valuable biomarker of the clinical practice after more extensive investigations and their evaluation of several author-group.

The circulating microRNA can – already before the clinical symptoms – signal the presence of the tumor and leads to longer survival. While tumor sampling occasionally becomes unnecessary.

5.3. Comparison of miRNA expressions, differential diagnostics, better survival options

The main aim of the present study was the comparison of the expression of selected microRNA from the sera of colon cancer - rectum cancer - and healthy patients.

We found microRNAs whose expression was significantly different from the control group, in colon- and rectum cancer patients.

Our results demonstrated that the miR-30a expression was slightly but significantly higher in the blood of 2. and 3. stadium colon- and rectum cancer patients than in the controls. This contradicts the literary data, since several reports state, that miR-30a inhibits metastasis formation and limits the expression in colon cancer patients.

Generally, it can be stated, that – except in the case of miR-30a – we received the same results as earlier others. Further, we found, that in the blood of rectum cancer patients the expression of miR-155, miR-21, and miR221 was significantly higher compared to the colon cancer patients. Our results can contribute to the application of the microRNA methods better setting the diagnoses and planning the therapy.

5.4. Study observations and new results of for use in clinical practice

1. New values of our results are the observation of the significantly higher miR-155, miR-21 and miR221 expression in the rectum cancer patients compared to the colon cancer patients. Further, in the case of advanced patients, the miR-221 expression was more pronounced. This observation has not only in the early detection and therapy but also in the prediction of the clinical course importance. Whereas during tracking the detection of a new tumor with other location (for example colon cancer – rectum cancer or inversely) therapy-alteration may be necessary. Namely 33 % of the CRC patients have rectum cancer. The present therapy strategies are significantly different. Therefore the differentiation is justifiable.

2. The miR-30a expression is slightly, but significantly higher in the blood of 2.and 3 stadium colon and rectum cancer patients than in the controls. These results refute the previous opinion that the above biomarker blocks the metastasis formation and its expression is decreased in the colon- and rectum cancer patients. Further extended examinations might result in new data about tumorgenesis too.

3. We confirmed earlier information that the blood level of miR-34a is significantly lower in patients with a colon- and rectum cancer, than in the healthy population. By our study miR-34a values mathematically significantly lower in the 2 and three stadium CRC patients versus the control group. In parallel, there-29b measurement trend matches the miR-34a value development.

4. Since the miR-155, miR-21 and miR-221 expressions values „move" in the opposite direction as the blood level of miR-34a and miR 29b our parallel examination offer more information about the process change during the follow-up.

6. ENVIRONMENTAL EFFECTS, LIFE STILE, PSYCHIC FACTORS

6.1. Chance for better survival

The appropriate frames of patient follow up must be found, which is not exclusively „professional" but also depends on the patient and its environment. An effective strategy consists not only of recommendations and suggestions but also is on different degrees in

compliance with them. This affects the medical practice and approach, as well as the willingness of the patient to cooperate.

We consider it possible, that a modern laboratory-method and its theoretical background as well as the background of the patient with it psychic effects can be linked in the interest of a more successful follow-up strategy. For this, we investigated the chances of the members of an excellent organized patient organization. Our goal was to establish a „noninvasive" follow up system to the benefit of the operating framework of the organization. We did not find any similar study in the literature.

6.2. Patient selection method

6.2.1. The group of the respondents – association frames

The Hungarian ILCO Association was organized from such people, who have decisively due to cancer stoma on the abdominal wall. It is a self-help group with the goal „to support the rehabilitation of patients with a stoma, to represent their interests and to help their reintegration in the society." At present, it has 3200 registered members. It is the member of the International Organisation of the Stoma-Associations. The selection was performed by the members of its different clubs.

6.2.2. Model of the data collection

The questionnaires were distributed between the members operated with colon- and rectum cancer with the following questions

1. man, woman
2. year of birth
3. date of operation (date of stoma)?
4. place of control (name of the Institute, city)?
5. intervals of control?
6. last control date?

Answers were collected from 1650 members of 23 clubs. This covers the whole country largely. 1340 patient sent back the questionnaire (81.2%) from which 1071 (64.9%) were evaluable mainly because of uncertain answers to question 5. (haphazard, occasionally, in case of complaints, etc.)

6.2.3. Statistical analysis

The 5 and ten years of survival data of the examined population have been compared to the data of the whole domestic colon- and rectum cancer population. Our data source was the 2010-2015 database of the National Cancer Registry and the Biostatistics Center. Analysis of the latter was performed based on Kaplan- Meier curves. The 5 and ten-year survival rates were SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary NC the US) software determined.

We compared the survival rates of the collection and the whole patient-material with chi-square tests. To this, we used PAST version 1,86B software where we accepted under 0,5 p-value as a significant difference.

7. RESULTS - THE EVOLUTION OF THE PARTICIPATIONS- AND SURVIVAL DATA

From the data of Figure 1. is evident, that the participation of men is higher than that of women, but the average age is barely different. In the case of survival, significant differences can be observed for the benefit of female patients. (5 years survival: 68.64% and 75.05%, 10 years survival: 44.71% and 52.65%).

Figure 1. Data collection among those living with stoma
(with the help of ILCO Club Somogy county, 2010)
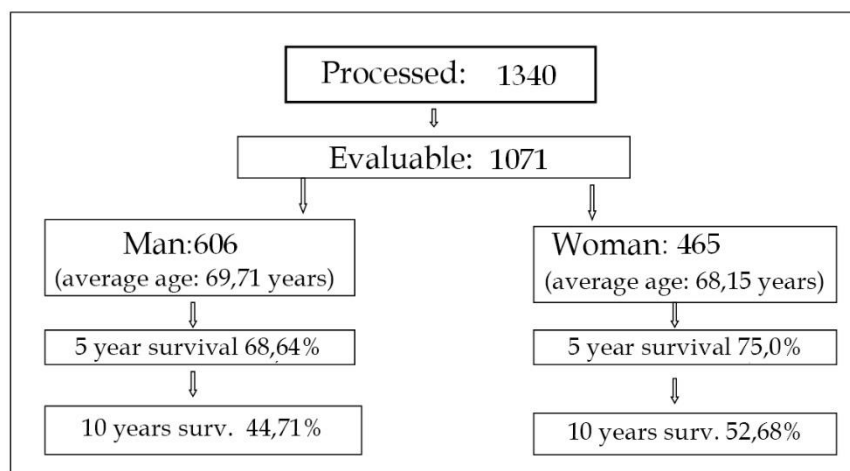
Figure 2. 5 and 10 years survival: The questionaries and NRR
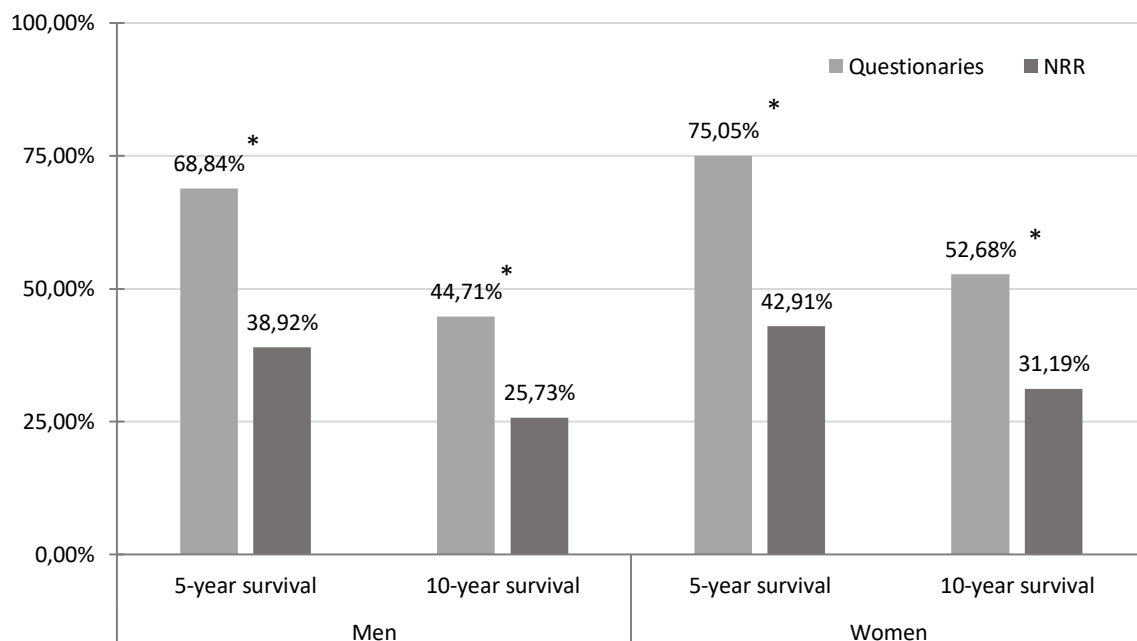
Analyzed and compared the collected (survey) survival data and the whole survival data of the National Cancer Registry it can be stated, that the survival rate of ILCO patients (Figure 2are significantly better compared to the National Cancer Registry survival data between 2001 and 2015. (* p=0,05)

8. DISCUSSION

8.1. Healing surgery – patient follow up – quality of life

Clinical investigations related to the quality of life became important in the last years. Its mayor factor is the feedback of the target groups. Namely some of the patients „give feedback" if they are asked. However, these feedbacks are very varied and reflect in high number dissatisfaction. Maybe on an ethnic basis or depending on insurance systems, psychosocial factors, economic status.

8.2. Target group selection – civil bases

Whereas the development of a valid domestic strategy request not only professional but also public health and social frameworks, in the patient follow up we targeted the ILCO clubs. This resulted in a higher rate of answers as from patients not visiting any organization. Knowing the operation of the Association, on the one hand, it has several doctor-patrons; on the other, the health literacy of the members are much higher than that of nonclub member patients. Therefore it is the more massive and realistic involvement.

8.3. Comparative study of the survival data

Possible causes of differences

In terms of survival rates by gender there is already a significant difference: on one hand in the survival rate data of the nationally registered colon- and rectum cancer patients of the answering members, on the other hand in the relation of the male and female patients of the Association - this is a marked difference for women.

The reason for this is not precisely known. The current age shift can undoubtedly play a role. However, this is in itself not an explanation for the differences because there is hardly any difference in the male and female average age (69,71 years; 68,15 years).

However, it deserves absolutely attention that the conscious lifestyle of the woman is a well-known phenomenon.

Given the above, of a large number of evaluable patient responses from our survey is deductible that the survival data from the ILCO clubs exceed even the best international results. This refers to the integrative, cohesive and helping role of the community.

A small community can create well-being, cohesive force, solidarity, what we want to maintain and which may be essential for our survival.

Presumably, the frames of the social NGOs, with the help of modern new laboratory tests, could promote the creation of a more active patient follow up and survival strategy by reducing the destructive power of a cancer disease group which is in the view of public health very significant.

9. NEW, IN CLINICAL PRACTICE USABLE OBSERVATIONS AND RESULTS OF THE DISSERTATION BASED ON THE FEDERAL SURVEY DATA

1. To our request, a large number (1071) of patient responses arrived, which can be considered as a large sample.

2. This data highlights not only the functioning of the Patient organization, but they can provide a basis for comparison of all colon- and rectum cancer patients in the country. From the National Cancer Registry.

3. Comparing the data within the Association with the public health data, the difference is rather significant for the benefit of the Association.

4. The survival data gained from the ILCO clubs exceed even the best international results, which refers to the integrative, cohesive and helping role of the community.

5. During the statistical evaluation of the two databases, the differences were significant for the benefit of the Association's indicators.

ABBREVIATIONS

BTA: antigen associated with bladder tumors

CRC: colorectal cancer

FDP: fibrin degradations protein

FISC: fluorescence in situ hybridization

IHC: immunochemistry

NMP22: nuclear matrix protein 22

FDA: Food and Drug Administration

TRC-method: Transcription – Reverse Transcription Concerted

CRA: Colorectal adenoma

NRR: National Cancer Registry

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