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REVIEW

Alternatives for the Intensive Follow-Up After Curative Resection of Colorectal Cancer. Potential Novel Biomarkers for the Recommendations

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Abstract Early diagnosis of recurrence and metastasis of colorectal cancer following surgery of curative intent is of vital importance in terms of survival and quality of life. The consistent implementation of appropriate patient follow-up strategy is therefore essential. Debates over the methodology, evaluation and strategy of follow-up have been known for many years, and continue today. By introducing several follow-up models, the present paper offers different options featuring certain individual, national and international, conceptual and financial aspects. Colorectal cancer is an important public health concern due to its destructive nature and frequency, it is therefore essential to develop new monitoring strategies, involving new biomarkers and extensive clinical validation. Since the recurrence rate is very high in high-risk patients, the improvement of individual patient risk estimates and the utilization of a corresponding follow-up model require broad international co-operation and common practice, along with the determination of optimal levels of evidence.

Keywords Colorectal cancer · Follow-up · New strategy · New biomarkers

Abbreviations

ESMO	European Society for Medical Oncology
ESCRS	American Society of Colon and Rectal Surgeons
NCCN	National Comprehensive Cancer Network
SGG	Schweizerischen Gesellschalt für Gastroenterologie
ASCO	American Society of Clinical Oncology
CRC	Colorectal cancer
JSCCR	Japanese Society for Cancer of the Colon and Rectum
FIT	Fecal immunochemical testing

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Introduction

Colorectal cancer (CRC) is among the leading causes of death in the United States and Europe representing about 14–15 % of all cancers. Other authors have called it the plague of the Western world [1, 2]. There is no doubt that CRC mortality showed a declining trend in both sexes in Europe during the last two decades. A number of European countries are not affected by the decline, such as Romania and the Russian Federation and only some stagnation of undulating character can be detected in Hungary [3]. The decrease was generally 2 %/year between 1997 and 2007, the extent differing by country and age group. In Japan for instance, in the age group of 65–85 years an annual decrease of 1.3 % was recorded for both sexes, during the past 10 years [4]. It is assumed that early diagnosis and advanced treatment modalities also contributed to the achievement of these results, along with effective screening programs in some countries [5]. The European "mortality prediction" forecast further reduction in the standardized mortality rate of colorectal cancer by 2012, though a 7 % decrease is presumably only achievable in certain countries, in others it is not [6].

Review of the international literature reveals varied 5-year survival data with average values of 65 % in the United States, 55 % in Europe, and generally more favorable

numbers in the northern states [2, 7]. Survival data in Germany is very favorable between 2002 and 2006 showing a continuous growth from 60.6 % to 65.0 % [8]. The 5-year survival of CRC patients in the United Kingdom diagnosed between 1999 and 2004 was 50.8 % in females and 49.6 % in males, while the 10-year survival was estimated to be 45 % in 2008 [9, 10].

Unfortunately, the Czech Republic and Hungary display exceptionally bad CRC data with regard to the fact that the average European mortality rate is between 10 and 13/100 000 women and 17–20/100 000 men. By contrast the Czech rates are 17.94/100.000 women and 35.77/100 000 men, and the Hungarian equivalents are 18.20/100 000 and 34.56/100 000 respectively [11]. Data of the Hungarian National Cancer Registry show that the expected 5-year survival rate in both male and female CRC patients is about 40 %, and half of all cancer deaths occur in the first year of diagnosis [12].

The public health significance of CRC is thus outstanding and as a generally resectable tumor, if diagnosed early, treatment may be very successful. An early diagnosis is very often delayed due to the slow reaction of the health care system, the physician or the negligent attitude and lack of patient information [3, 13].

Rapid early detection is vital to the survival of CRC patients since the 5-year survival rate of early cancers (Dukes' A) is almost 90 %, whereas the rate approximates 15 % in late stage (Dukes' D) tumors. At present, however, at the time of diagnosis 20–25 % of patients present with metastases, and further delay results in the appearance of metastases by an additional 20–25 % of patients, greatly reducing the chance of survival [10, 14]. With long-term persistent work we can eliminate the distressing situation (especially in countries with negative epidemiological indicators). The proper construction of effective, well-organized and planned screening program and postoperative follow-up of appropriately resected tumors are the cornerstones of the realization of such aims. The pursuit of early detection requires both screening and patient follow-up, since effective screening can identify early-stage cancer in asymptomatic subjects, and the early treatment of recurrent or metastatic disease can improve life expectancy and survival in regularly followed-up, operated, asymptomatic patients [3, 13, 15, 16].

The present paper addresses the current framework and contradictions of the follow-up strategy after tumor resection with a healing intent as the methodological and strategic debates continue today. There is no doubt that the development of a new strategy is inevitable with new potential biomarkers and tools to estimate the risk for recurrence involved in the follow-up models. There are various indicators in association with neoplastic diseases, including classic tumor markers such as blood in stool and genetic alterations (e.g., gene mutations, copy number variation, single nucleotide polymorphisms) identified by molecular biology methods, and the latter ones as new biomarkers may play a role in the assessment of susceptibility to various diseases and a particular response to treatment and toxicity [17, 18].

Realistic and expected practical value of the new markers is various and their clinical verification is usually delayed. Therefore, selection and fitting of new markers in the follow-up models requires great caution [19–22].

Alternative approaches to follow-up: national and international recommendations

Early diagnosis and effective follow-up of colorectal patients are the cornerstones of successful treatment and favorable survival. Follow-up can be further divided into two groups, according to the stage of the disease and type of treatment. The first group consists of advanced stage colorectal cancers, where monitoring is determined by the observed effectiveness or ineffectiveness of the radiation or drug therapy and aims at increasing survival, symptom control, and quality of life. Carcinoembryonic antigen (CEA) still is the most valuable of the number of markers (and their combinations) found in the literature, and marker levels (and its changes) yield information on both the effectiveness of the treatment and the prognosis of the cancerous process [15].

The second group of patients had already received some kind of treatment (primarily surgical of nature), and their fate is determined by a series of tests in the framework of close monitoring. The primary intervention done on the latter group of patients is usually qualified as curative, and during the reasonably determined control periods, clinicians make decisions about the next step after joint evaluation of the clinical, laboratory, and imaging results [23].

Unfortunately, an optimal follow-up strategy cannot yet be offered after curative surgery for colorectal cancer patients. Although trial results are loaded by many contradictions, it can be concluded that intensive follow-up has a beneficial effect on survival. Studies included in the meta-analysis, however, are extremely varied, in terms of the methods applied, number of patients included, and therefore it is difficult to determine which type of monitoring would be appropriate in clinical practice, with the proper level of evidence [15].

Without being exhaustive, this paper presents some sample follow-up models, which partly overlap. CEA still is the predominant primary follow-up marker. There is no doubt that the measurement of serum CEA levels and parallel liver imaging can achieve significant improvement in survival [23]. An increase in serum CEA level is often the first sign of tumor recurrence, generating suspicion 1.5 to 6.0 months prior to the results of clinical or instrumental examinations. However, it is not suitable for monitoring alone due to the high proportion of false positive [7–16 %] and false negative (approx. 40 %) results.

The follow-up methods and their combinations (clinical, endoscopic, imaging, laboratory) have been considered by many authors in recent years, and an attempt was made to develop an optimal model especially in the early detection of recurrences, with a wide range of results [24–26]. These related primarily to cancers recognized in more advanced stages and CEA was identified as the key marker of each model despite its limitations.

However, methodological and strategic discussions over follow-up continued, and are still one of today's outstanding problems. One of the most prestigious international organizations, the European Society for Medical Oncology (ESMO) announced their intensive follow-up strategy, applied not only to high-risk, more advanced cases, but to the exploration of early-stage patients too, with levels of evidence indicated [23] (Table 1).

A major step forward in the field of evidence based medicine is the desire of American and Canadian surgical societies to establish a monitoring service covering risk factors, diagnosis and patient follow-up as well, with the possibility of public debate [27]. The American Society of Colon and Rectal Surgeons (ESCRS) also strives to establish practical guidance based on the best available evidence, through the work of a special committee. These proposals include international efforts, are of inclusive nature, and are intended to promote appropriate therapeutic decisions [28]. According to their evaluation, within the routine laboratory tests CEA determination received 1C level of evidence, both before and after surgery. Recently, in many cases, instead of colonoscopy (1C) advanced imaging techniques are recommended (CT colonography, PET/CT colonography) with grade 1B level of evidence, and the importance of preoperative imaging techniques is stressed.

Three more remarkable follow-up strategies are presented in Tables 2, 3 and 4, offering choices, and creating a basis for discussion on testing the acceptance and further development of monitoring systems.

The extension of post-operative patient follow-up for early-stage CRC is also recommended by ASCO, this way post-recurrent therapy can be more effective [31]. A large number of follow-up proposals appeared in the literature during the last 10 years, but a more detailed presentation of these would exceed the framework of the present paper. Two trends, however, are recognizable: a more intensive, and a less intensive, potentially cost-saving trend [33–37]. After surgeries of curative intent, scheduled follow-up procedures with appropriate quality-assurance organized according to common principles are included in the recommendations, as well as the less intense, more economical methods [34, 38]. The following four follow-up samples (Tables 5, 6, 7 and 8.) also suggest that the development of a common position (and the extensive clinical proof) is still pending.

Table 2 National Comprehensive Cancer Network (NCCN) guidelines of follow-up for up to 5 years postoperatively [28]

- A review of medical history, physical examination and CEA determination every 3–6 monthly in the first 2 years, then every 6 monthly for the next 3 years.
- CT scan (abdominal, pelvic) annually for 3 years.
- Colonoscopy annually, based on clinical indication.

A different model is widely accepted in Japan. While in Europe and the United States postoperative CEA measurements in stage II and III CRC patients are generally recommended in every 1–3 monthly for at least 3 years, such measurements in Japan are recommended to be done in every 3–6 monthly for 5 years, depending on the condition of the patient. Furthermore, CA 19–9 is also measured as proposed by the Japanese Society for Cancer of the Colon and Rectum (JSCCR), despite its lower efficiency [36, 39]. The Japanese surveillance model is the last one presented in this paper [Table 8].

Beside the illustrated follow-up recommendations (and similar models), attention must be paid to studies which incorporate the immunochemical method of the detection of occult intestinal bleeding (fecal immunochemical testing, FIT) for certain patient groups into their surveillance program, promoting early detection of local recurrence [26].

Acceptance of national and international recommendations

There is no doubt that patient monitoring systems, regarding both theoretical and practical aspects, lack commonality, although credible test data are already available according

Table 1 ESMO follow-up recommendations with levels of evidence [I-V] and grades of recommendation [A–D] [23]

- Intensive follow-up must be performed in colon cancer patients [I. A]
- History, physical examination and CEA determination are advised every 3–6 monthly for 3-years and every 6–12 monthly at years 4 and 5 after surgery [II. B].
- Colonoscopy must be performed at year 1 and thereafter every 3–5 years looking for metachronous adenomas and cancers [III. B].
- CT scan of the chest and abdomen every 6–12 monthly for the first 3 years can be considered in patients who are at higher risk for recurrence [II. B].
- Contrast-enhanced ultrasound could substitute for abdominal CT scan [III. C].
- Other laboratory and radiological examinations are of unproven benefit and must be restricted to patients with suspicious symptoms.

Table 3 Follow-up model of the Swiss Gastroenterology Society (SGG) [29, 30]

- Physical examination and CEA monitoring 3 monthly in the first year, every 6 monthly in the 2nd and 3rd years, then annually in the 4th and 5th year.
- CT [thoracic and abdominal] at 12, 24, 36, 48 and 60 months.
- Colonoscopy in the 12th and 48th month, and once in 5 years in case of long survival.

to which the post-operative patient follow-up is absolutely justified, allowing mortality rate to be significantly reduced [35, 40].

Unfortunately, even comprehensive cancer centers fail to comply with the criteria of guidance, in more than 70 % of cases, which in the case of ignoring the regular follow-up of CEA levels results in significantly reduced cure and survival rates [41]. Surveys of a similar nature have been made in connection with the Swiss national recommendations (Table 3), which were based on the continuously renewed model of patient follow-up proposed by the SGG [29, 30]. The authors found that following surgery, the entire follow-up proposal was completed for 11.6 % of patients, examinations were completely omitted in 13.0 %, and in a group of patients who did not receive adjuvant chemotherapy, CEA determination was carried out in only 27 %. The Swiss study is the first work in which compliance not only to colonoscopy but also to other tests was assessed [30]. Other relevant authoritative publications only deal with colonoscopy, that is considered by them the first-rate follow-up method [40, 42]. It is currently not known, what is behind the overlook or incomplete implementation of recommendations, while in advanced health systems (such as in Switzerland) the statutory health insurance fund guarantees the swift completion of patient follow-up and reimbursement of costs [43]. The most likely of the possible causes is that physicians were not sufficiently aware of the fact that patient follow-up is of critical importance among the potentially life-saving factors, suggesting the need to improve physicians' skills and knowledge in screening and patient follow-up [29, 30]. Physicians' best judgment alone is not enough without the joint responsibility of patient and doctor.

A Dutch study highlights the variety of views on postoperative follow-up [44]. Every surgeon registered in the Netherlands and active in the management of colorectal cancer was questioned on the subject of relevant international and national recommendations, particularly with regard to the national recommendations (response rate was 91 %), which are based on the ASCO position statement [44, 45].

The greatest agreement occurred with regard to the measurement of CEA: the need of 3-monthly measurements in the first year formed the basis of general consensus, while later in the detection period checking levels in every 6-months is considered necessary, along with liver ultrasound examination [even though the latter was not included in the national recommendation]. A French study (Table 7) which took into account the cost and efficiency of the follow-up strategy concluded too that beside CEA determinations abdominal ultrasound should also be integrated into the system as a standard tracking method, despite the fact that it is not included in the ASCO recommendations [38]. Sixty-five percent of the respondents agreed that colonoscopy should be performed at the end of the first year, but this rate substantially decreased to between 18 and 35 % by the question relating to the necessity of colonoscopy at the end of the 5th

Table 4 Follow-up recommendations of the American Society of Clinical Oncology (ASCO) [15, 31, 32]

- Physical examination 3 to 6 monthly years, then 6 monthly for 2 years; CEA monitoring 3 monthly for 3 years
- Thoracic/abdominal/pelvic: CT annually for 3 years
- Colonoscopy in the 3rd year, and if negative, once in every 5 years (recommendations may require modification based on risk groups)

Table 5 An intensive programmed follow-up model from Italy [34]

Between years 1 and 3: 3 times annually
Between years 4 and 5: 2 times annually
Between years 6 and 10: annually
- clinical examination
- CEA
- Fecal occult blood test (FOBT)

Imaging:
- abdominal US (in months 8, 20, 30, 742 and 54)
- chest X-ray (in months 12, 24, 36, 48 and 60)
- colonoscopy, or double contrast radiography (in months 12, 24 and 48)
- rectoscopy (in months 12, 24 and 48)
- CT (in months 4,16, 30, 42 and 54)
- MRI (as needed)

Locoregional recurrences:
-PET, based on adequate indication

Table 6 Intensive radiographic and biomarker surveillance of stage II and III colorectal cancers [33]

High risk
- CEA every 3 months for 2 years, then every 6 months for a total of 3–5 years;
- Total colonoscopy in 1 year, then repeat in 3 years, except previous clinical indication;
- CT scan [thoracic, abdominal, pelvic] : every 6 months for 2 years, then annually for 3–5 years;

Low risk
- only the frequency of CT scans differ: annually for 5 years

Table 7 A standard, cost-effective surveillance model from French authors [38]

- CEA in every 4–6 monthly for 3 years, then annually for 2 years;
- physical in every 3 monthly for 2 years, then every 6 monthly for 3 years;
- abdominal ultrasound in every 4–6 monthly for 3 years, then annually for 2 years;
- thoracic X-ray annually;
- colonoscopy once in every 3 years

year. The acceptance of chest X-rays performed annually shows a similar pattern in the 5-year breakdown, with the exception of the examination performed at 6 months, which showed an acceptance rate of only 18 %. Chest and abdominal CT (67 %) with complementary PET scans are preferred to abdominal ultrasound (11 %) and colonoscopy (4 %) to confirm suspicion of recurrence.

The assessment of follow-up proposals and demands are packed with individual concept, and even differences of opinion within each country hinder the foundation of the uniform, transparent clinical practice. The finding of the Dutch study is encouraging according to which colleagues expressed their need to change practice and develop new monitoring protocols: 92 % of the respondents declared their willingness of participation in such work. The most recent audit covering the western part of the Netherlands (performed between 2006 and 2008) displays a better picture: the former heterogeneity between some of the hospitals decreased and, some standardization efforts were observed partially facilitated by the audit itself [46].

The need for the elaboration of new follow-up models

Invasive surveillance techniques (especially colonoscopy) are associated with certain complications, side effects, and other well-known limitations [47, 48]. A gentler method, computed tomography colonography (CTC or virtual colonoscopy) is recently recommended for screening, but despite its undoubtedly remarkable efficiency, it does not exclude the necessity of colonoscopy [48, 49].

Table 8 Japanese postoperative surveillance program [36]

- CEA and CA 19–9 levels within 30 days preoperatively
- Examinations to be done during the first 5 postoperative years:
  - Physical in every 3 months
  - CEA and CA 19–9 levels in every 3 months
  - Chest X-ray and abdominal CT scans in every 6 months
  - Total colonoscopy at the end of the first and third postoperative years

Beside the more efficient and consistent patient follow-up, further development and specialization of surgical techniques and progress seen in other medical procedures, especially the spread of personalized treatment plans, all have beneficial effects on survival [14, 50].

Special attention shall be paid to CRC patients with hepatic metastases following metastasectomy [51]. Metastastatic liver lesions are present at the time of diagnosis of the primary tumor in 30 % o the patients, and further 20 % of hepatic metastases are formed after surgical removal of primary CRC lesions [52]. There is no doubt that evidence is contradictory regarding the follow-up of patients who underwent resection of hepatic metastases, but there are results according to which the discovery of early recurrences improves the success rate of repeated resection and survival [51, 53]. Follow-up procedures in this case too are very diverse, but all of them include CEA measurements and abdominal imaging studies, usually CT [51].

The role of CEA as the primary marker continues to hold up despite its limitations previously discussed. Recent studies suggest that a particularly strong predictive role is attributed to CEA in rectal cancer cases, considering even the effect on overall survival [54]. It has been proposed that tumor stage alone cannot fully predict prognosis, while the preoperative determination of CEA may be included as an independent prognostic factor. The observed differences in overall survival between patients with normal and elevated preoperative CEA levels, were not only statistically significant but also clinically relevant, and so the differences observed were real. Since 33 % of CRC patients prove to suffer from rectal cancer and currently substantially different treatment strategies apply to colon and rectal tumors, routine preoperative CEA measurements are strongly recommended, also confirmed by the observation that elevated preoperative CEA levels are associated with a doubled risk of mortality [54, 55]. The role of postoperative CEA determinations in surveillance is therefore justified, even in cases of normal initial levels (<5 ng/ml), because 41 % of these patients display higher CEA level when a recurrence occurs. In more than half of the cases (but at least in one third) however, preoperative CEA values do not yield positive results [39, 44]. These patients are vulnerable because a biological indicator of recognition fails, which despite its limitations is still essential in postoperative surveillance.

The renewal of tracking models in the near future cannot be further avoided, and this demand is widely known. The need for renewal and the change of attitude is well illustrated by the Dutch survey cited above, where the very large majority of respondent colleagues expressed their need for the development of new follow-up recommendations, and would also to take on an active role in the realization [44].

The search for new biomarkers and the implementation in clinical practice

The designations *biomarker* and *tumormarker* were often used interchangeably in the last decade, while others separated the two terms from each other, and primarily molecular and genetic markers were referred to as biomarkers.

In 2001 however, the National Institute of Health's Biomarkers Definitions Working Group clarified the definitions: "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes*, *pathogenic processes*, *or pharmacological responses to a therapeutic intervention*" [56]. This definition is now widely ratified and adopted, and includes population screening and clinical markers too in relation to neoplastic diseases [17, 21, 57].

Indicators associated with neoplastic diseases are diverse and include stool blood, "classic" tumor markers, genetic markers (e.g., gene mutations, copy number variation, single nucleotide polymorphisms), and the "new biomarkers" which may play a role in the susceptibility to various diseases and the assessment of toxicity and response to a particular treatment [17, 18].

Over the years, classic tumor markers were widely criticized. Firstly, these markers were not tumor-specific, and on the other hand, their sensitivity was not satisfactory. In addition, false positive test results may be gained under physiological conditions and in the dysfunction of elimination (degradation) processes, but tissue disruption under inflammatory conditions may also increase their level, and therefore they are of limited clinical value [58, 59]. In the light of the foregoing, the search for new biomarkers is essential, that have the potential to change the medical management of patients with cancer in its various stages.

However, despite the fact that a great deal of money was spent on cancer biomarker discovery and validation over the last 15 years and hundreds of articles were published on "revolutionary" new biomarkers in diagnosis and treatment over the past 10 years, clinical implementation remained a mere experiment. The Early Detection Research Network of the National Cancer Institute, USA spent hundreds of millions of dollars to discover new biomarkers during the past 10 years, but hardly any was recommended for clinical use, and less than 1 % of currently published cancer biomarkers can be used in clinical practice [60].

It is possible that the research and validation work is much more difficult than expected, and undervaluation of any of these as well as the weaknesses of the research plans have contributed to the failure of the expected results [18, 57, 60]. Detection and identification of new biomarkers are amongst the most important tasks of cancer research, in favor of diagnostic efficiency, safe assessment of prognosis and more effective medical treatment. From the very large number of candidates it is difficult to recruit markers which show statistically significant correlations with important clinical characteristics of CRC, and the exact mechanism of which can be clarified by further tests [20].

Detection of tumor DNA in different body fluids; novel non-invasive methods of patient follow-up

It is known that malignant tumors bear multiple somatic genetic and epigenetic alterations. The various types of DNA changes described in CRC can be detected in free circulating DNA of these patients, of which the two most widely studied molecular alterations are DNA methylation disorders and point mutations in KRAS oncogene [61]. During postoperative surveillance 17 % of patients were detected to carry oncogenic mutations in their non-cell-bound DNA, and 63 % of these patients indeed had a recurrent disease within a median of 4 months following positive results, which is remarkable from a prognostic point of view [61, 62]. However, recent studies have shown that there is no clear evidence regarding the prognostic role of KRAS mutations in circulating DNA in average risk CRC cases and it had no influence on the overall survival [63].

DNA methylation is known to play an important role in the early stages of tumor development. Circulating methylated SEPT9 DNA is a valuable new biomarker for a minimally or non-invasive early detection method performed from peripheral blood samples, and development and application of a plasma-based SEPT9 test is highly desirable in patient follow-up [64]. According to studies by other authors, methylated DNA was detected in the feces of 75 % of CRC patients; this proportion was 44.4 % in advanced adenoma patients and 10.6 % in case of normal mucosa [65]. These results show that the method should be further investigated in patient monitoring, and there is hope that the new biomarkers will be integrated into the new follow-up model [48, 66].

Current limitations include high trade prices, and that the reimbursement of these methods and reagents approved for clinical use are not recommended by the FDA due to the high costs [48]. A recently developed "new generation" automated stool DNA test method allows a more sensitive measurement technique: sensitivity in CRC and adenoma (> 1 cm) patients is 85 % and 54 %, respectively, with 90 % specificity [67]. Sensitivity does not depend on localization (proximal – distal), but only on size and the method is suitable for the detection of neoplasms located on both sides of the colorectum, albeit it is not feasible to distinguish between adenoma and carcinoma. However, follow-up studies confirmed that stool tests are suitable only to discover local recurrences, while metastases cannot be detected.

Detection of circulating tumor cells

A number of genetic and epigenetic motifs were identified in samples of primary CRC and metastases, the molecular profile of which proved to display difference between the

primary tumor and its metastases. Molecular pathology studies pointed out that, for instance, KRAS and BRAF mutational status of the primary tumor is often different from the metastases, raising the possibility of potential prognostic and therapeutic significance [68, 69]. In the majority of cases, cancer fatalities are related to the metastases of the primary tumor, associated with the presence of circulating tumor cells [70]. Therefore, the discovery of recurrence of a primary tumor alone is insufficient during follow-up; it is also vital to the detect metastases.

A standardized sensitive method (Cell Search System) approved by the FDA for clinical use is suitable to detect and quantify small number of tumor cells from blood [71]. Although profiling of metastatic tumor lesions has not been a part of clinical practice before, protein marker studies may lead to it eventually, on the way toward new therapies. Approval for clinical use however does not imply the recommendation of financial compensation as costs are very high, thus wide-spread introduction is currently not possible, and clinical indications are not yet set down. Testing of a single blood sample costs ca. USD 800, and therefore other authors recommend a technically faster and much cheaper alternative, the Transcription- Reverse Transcription Concerted (TRC) method which assesses the miRNA fraction of circulating tumor cells (CEA) [72]. Large multicentric trials are still lacking for authentic clinical validation. Other methods are also known for the detection of circulating tumor cells in CRC patients, and comparative studies suggest that future follow-up models will consist of a combination of various methods of detection for a better prognostic value [71].

Combination of traditional and new biomarkers in patient follow-up

Out of the large number of new biomarkers of unconfirmed practical use, special attention is paid to research results, which combine traditional and new biomarkers with each other. Since at least two markers of independent biological and biochemical background can provide more information about the cancerous process, future benefits may be greater [73]. Simultaneous determination of circulating free DNA and CEA levels showed that median CEA levels were lower in Duke's stage A – C than in metastatic state, while free DNA concentrations were higher from early stages, and cancer patients had five-times higher free DNA concentrations compared to healthy donors. Tumor specific sensitivity (81.3 %) and specificity (73.3 %) could be increased to 84 % and 88 % respectively by the combination with CEA, that is now considered a potential alternative to the occasional replacement of invasive colonoscopy, and this combination can be a useful diagnostic tool for the detection of early cancers [73]. The combination of methylated free circulating DNA and CEA is considered effective as a prognostic factor by newer studies too [74].

The search for effective, non-invasive methods of a simple follow-up strategy is still in progress. An important direction of this search is the use of multiplex serologic biomarker groups (panels), for example the combined detection of serum autoantibody profiles versus antigen panel "associated with colon cancer" [75]. The determination of antibodies reactive to these antigens combined with serum CEA detection was associated with a significant improvement in sensitivity (65.9 %) in finding early cancers. It became clear that the old biomarkers (along with other traditional methods) are no longer, and the new biomarkers are not yet effective in forming a model for early detection.

MicroRNAs as new potential biomarkers for colorectal cancers

Not only extracellular free DNA, but also RNA molecules can be detected in the serum and other body fluids. Free miRNA in body fluids (such as serum) was first detected in 2008 and proved to be highly resistant against several physicochemical factors (e.g., pH changes, freezing and thawing, long-term storage). These properties impart a high degree of stability including resistance to enzymatic breakdown, resulting in substantial reproducibility of the determination of miRNA levels [76]. Because certain pathological processes are characterized by well defined changes in the specific pattern of miRNAs, there is hope that by the evaluation of the miRNA profiles, tumor recurrence, spread and metastasis trends can be predicted, and may become a valuable biomarker in clinical practice, after evaluation of the extensive investigations of several author groups [76]. Preliminary results indicate that the sensitivity and specificity (89 % and 70 %) of tissue miR-92 were more favorable than the similar parameters of hidden intestinal bleeding and, in another study adenomas were successfully separated from healthy controls by comparing plasma miRNA profiles, with a sensitivity of 73 % and specificity of 79 % [77].

It is questionable however, how much influence the overlapping miRNA profiles detected in different tumor types will have on the detection of asymptomatic tumors and the possible prognostic value, as the detection of cases of high-risk and poor prognosis is the key to a more effective treatment [78]. Tissue miR-21 and miR-155 expression studies reported favorable results in the field too [79]. Furthermore, the findings of a recent study indicate that increased miR-10b expression was significantly associated with the presence of lymphatic invasion and progression, and is an independent prognostic factor for survival. Increased expression of miR-10b

CRC cells also induces resistance to 5-FU, a key chemotherapeutic drug widely used in CRC [80].

Limitations to the clinical utilization of miRNAs: the need for a paradigm shift

Many from the database of these short RNA molecules are known to possess the ability through the change of a special pattern to describe certain pathological processes [81]. Practical use however, is prevented by a number of difficulties: expression profile of miRNAs found in blood can vary due to certain risk factors, timing of blood samples whether taken before or after treatment, and the type of therapeutic intervention can influence its utilization [81, 82]. The effects of biological changes are difficult to be evaluated in the absence of an adopted endogenous miRNA control. Thus for each miRNA tested average expression level should be determined, in order to reduce technical variation [82, 83].

The analysis of circulating miRNAs has only a brief history, however despite the difficulties in evaluation, as their participation in cell transformation is non-debatable, miRNAs are believed to be a promising new group of biomarkers [76]. The deeper understanding of the biological role is essential for these potential markers to become effective diagnostic, prognostic and predictive biomarkers in clinical practice, made suitable for such use by their close relationship between the disruption of regulatory mechanisms and the development of cancer and progression.

The systematic review and meta-analysis of miRNA-related literature (46 publications based on the results of 43 from studies) revealed that primarily overall survival or recurrence rates were assessed in the studies, and that increased miRNA expression is often associated with poor prognosis [84]. The exploration of larger cancer patient groups and consecuitive elimination of incomplete methodological conditions by external validation are required for a more accurate assessment of the clinical value of miRNAs. Since over one hundred circulating miRNAs were detected in healthy individuals, and at least 69 miRNAs were identified only in the sera of colorectal cancer patients, it is preferable to develop a simple blood test to indicate the current stage of the tumor and exacerbation of the pathological condition, as part of an appropriate assessment model [85]. One such model could be suitable for the monitoring of high-risk polyps. Despite these encouraging data to date mRNA-associated methods do not achieve adequate sensitivity to become tumor-specific biomarkers used in clinical practice, but a more accurate assessment would be possible through age-adjusted miRNA profiling from blood samples [83, 85].

The combination of molecular and conventional biomarkers can significantly increase the efficiency of patient follow-up and prognostics. A more effective prognosis and patient follow-up may be achieved by the combination of the detection and quantification of colorectal cancer-specific, blood-based miRNA and CEA measurements [10]. Since miRNA is present in tissues in a fairly stable form, and it is protected from endogenous degradation due to its small size, detection from not only serum but also other body fluids such as stool is promising for the early discovery of colorectal cancers, although there is only limited data available [83, 85].

One of the disadvantages of stool tests is that the detection of the new biomarkers are only suitable for the detection of local recurrence, as mentioned earlier.

Stool may contain a significant number of colonocytes detached from neoplasms, so alterations of miRNA expression associated with the tumor can more easily be detected in stool than in blood. Fecal detection, however, is associated with a number of disadvantages; taken into account esthetic considerations, both types of tests are necessary to be further developed for data collection along with the methodological aspects. Since there is no difference between early and late stage cases in terms of sensitivity miRNA tests may have great significance in case of resectable tumor detection, which is useful in follow-up, thus reducing mortality.

Conclusion and future perspectives

The collection of additional material and methodological work are essential, and the parallel application other markers (older and newer) can increase efficiency. MiRNAs in the test samples (body fluids) are very resistant to external influences, expression profile measurements are highly reproducible and their specific patterns are promising in combinations of new and traditional biomarkers. The independent systems complement each other well, thus more effective models can be created.

Relevant international surveys show that proposals and strategy recommendations are extremely diverse, they are not devoid of certain local (national) character and individual concept, and include different levels of compliance to recommendations, affecting medical practice and approach, and the willingness of patients to participate.

Early detection is known to be the key to successful treatment. However, risk assessment of each patient cannot be ignored either, since the recurrence rate is considerably increased in high-risk patients, and thus more intense monitoring is desirable in this group. Several options are available, taking into consideration certain traditions and financial opportunities.

In order to develop a new strategy and involve novel biomarkers, a wider international cooperation and regular

exchange of information is necessary, with the standardization of terms of clinical validation.

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