Prognostic value of microsatellite instability in adjuvant treatment of colorectal cancer

Wartość prognostyczna niestabilności mikrosatelitarnej w leczeniu uzupełniającym raka jelita grubego

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Summary

The dynamics of morbidity and mortality in colorectal cancer (CRC) has changed little in recent years and consistently remains at a high level. The carcinogenesis of this type of tumors includes a large number of genetic disorders and epigenetic changes, which show a number of features specific for this nosology, providing the basis for the first consensus classification of molecular subtypes for colorectal cancer. One of the main mechanisms chosen as crucial for this classification is the microsatellite instability (MSI) caused by defects in the DNA Mismatch Repair System (MMR).

The clinical significance of this CRC molecular profile parameter is hard to overestimate. Over the past three decades, the MSI and MMR have been actively studied for prognosis evaluation, determination of need and selection of appropriate adjuvant chemotherapy scheme for CRC. Despite the significant accumulation of clinical and experimental study data, currently the prognostic value of this parameter is still not well defined with reference to CRC adjuvant treatment strategies, and the released data remain controversial.

The purpose of this analytic review is to analyze the current status of MSI and MMR in the setting of adjuvant treatment of CRC patients from a perspective of evidence-based medicine.

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Abbreviations: **ACPSS** – Australian Clinico-Pathological Staging System, **AJCC** – American Joint Committee on Cancer, **CI** – confidence interval, **CMS** – Consensus molecular subtype, **CRC** – colorectal cancer, **DNA** – deoxyribonucleic acid, **dTMP** – desoxythymidine monophosphate synthesis, **5-FU** – 5-fluorouracil, **IGFRII** – insulin-like growth factor II, **MHC** – Main Histocompatibility Complex, **MLH1** – MutL homolog 1, **MMR** – Mismatch Repair System, **MSI** – microsatellite instability, **NCCN** – National Comprehensive Cancer Network, **PDL** – programmed death ligand 1, **RNA** – ribonucleic acid, RR – relative risk, **SEER** – Surveillance, Epidemiology, and End Results, **TGF-β** – tumor growth factor β, **TS** – thymidylate synthase.

Recent developments of colorectal cancer (CRC) treatment strategies suggest that individualized treatment based on identifying known and newly discovered prognostic factors together with improving target therapy are of major importance. For colorectal cancer, the administration of adjuvant treatment is fundamentally based on generally accepted standards. New perspectives of improving the effectiveness of CRC treatment include the identification of certain molecular, genetic and epigenetic characteristics, which would be potentially capable to highlight the probability of either positive or negative prognosis and also to indicate the effectiveness of different adjuvant chemotherapy regimens (so called "molecular profiling" of malignant tumors) [17]. Despite the theoretical background, hopeful results of experimental and clinical trials, there are no persuading arguments supporting the standardization of adjuvant treatment of CRC based on these parameters. Besides ethical, legal and socio-economic issues, the data regarding long-term treatment outcomes, such as overall and disease-free survival, are crucial for understanding the effectiveness of a given method.

Meanwhile, the first efforts to understand which patients would benefit from adjuvant therapy were concentrated around microsatellite instability (MSI) due to the results of first experimental in vitro trials, which demonstrated the development of drug resistance among microsatellite unstable tumors [2, 27]. On the other hand, earlier clinical trials clearly demonstrated a correlation between MSI-status and the effectiveness of 5-fluorouracil (5-FU) administration [8, 9, 23]. Other series of studies showed no survival benefit for patients with either microsatellite stable or unstable tumors [10, 14]. On the contrary, no impact or even negative effect of 5-FU administration on overall survival was demonstrated for MSI-positive tumors [36, 39]. It remains a question whether MSI or MMR status can have a prognostic or a predictive value during the decision-making processes in planning a treatment combination for CRC. It is also unclear if MSI status could become a basis for future molecular classification of CRC and marker of cytotoxic chemotherapy effectiveness.

It is well known that an information database concerning the mechanisms of cancerogenesis is constantly updated and the amount of related publications is increasing rapidly. The search term "cancerogenesis" yields 890 results, of which 74 articles have been published in 2016 and 3 only in the first months of 2017 [35]. Recently, more attention has been paid to molecular tumor profiling and its alterations during the progression of a neoplastic process [17]. It had been reported that the CRC tumor cells accumulate up to 90 genetic mutations [42]. The molecular pathway of an adenoma – carcinoma sequence is well studied and described, the main triggering proto-oncogene alterations and their combinations were identified [1]. Although CRC screening programs are established and widely used to identify not only advanced tumors, but also a variety of premalignant conditions of the colon and rectum, the rates of mortality caused by CRC remain approximately stable worldwide. According to the SEER database (National cancer institute, USA), in 2015 there were 49,700 cancer-related deaths registered, while in 2010 – 51,500 [4, 42]. It is important to point out that the amount of data regarding the molecular basis of the malignant process was not widely applied in clinical practice, particularly for evaluating the prognosis and prediction of the effect of adjuvant treatment, which was based on standard regimens without any regard to the molecular profile. In most cases, such attempts remained experimental with insufficient evidence for routine clinical practice implementation. Nevertheless, despite the standardization of treatment strategy for each risk group of patients depending on a tumor stage, macro – and microscopic characteristics and the spread of the primary tumor, it was mentioned that a cohort of patients exists, which were unable to benefit from the expected treatment effect in terms of survival rates [18, 39, 43, 45]. After more thorough studies were conducted to explain this circumstance, it became clear that the mentioned characteristics are not sufficient to evaluate a clear prognosis of an adjuvant chemotherapy effectiveness and, consequently, a whole treatment complex. Therefore, it became necessary to identify certain extended criteria by which it would be possible to stratify CRC patients according to those who would potentially benefit from adjuvant treatment and those who would not.

In a series of pre-clinical trials, it was stated that individual combination of molecular alterations for a certain clone of tumor cells is frequently associated with tumor

phenotype, its prognosis and potential treatment outcomes [19, 26]. Later this theory gained acceptance and its further development was associated with the accumulation of strong evidence. Reestimated and standardized, these findings have been represented in a first consensus molecular classification of CRC, established at the end of 2015 [7]. According to this classification, all forms of CRC can be divided into 4 subtypes: CMS1 (Consensus molecular subtype) – immune hypermutated phenotype with microsatellite instability; CMS2 – canonical epithelial subtype with activated Wnt and MYC signaling pathways, enhanced regulatory influence of miRNA 17-29, mostly common for left-sided colon cancers (37%); CMS3 – metabolic subtype, identified in 13%; and CMS4 – mesenchymal subtype with significant TGFβ pathway activation, increased stromal invasion, neoangiogenesis, matrix remodeling, involvement of complement-mediated inflammatory pathways and decreased regulatory influence of miRNA-200, mostly common for primary advanced-stage tumors – 23% [7] (Table 1).

This classification may have a potential impact on choosing a way for adjuvant treatment individualization and may clarify the predictive factors responsible for its effectiveness [32]. However, the data regarding the association with a certain CMS subtype and long-term treatment outcomes of CRC treatment are lacking.

Experts meeting together with the TNM classification revision workgroup in 2015 at the annual AJCC (American Joint Committee on Cancer) have concluded that the main molecular factors of tumor heterogeneity and molecular profile characteristics will be included as new prognostic and diagnostic criteria in the following 8th edition of the TNM classification, which is expected to be published in 2018 [11].

Although there is a wide amount of potentially valuable molecular prognostic factors for CRC with high evidence regarding their significance based on multiple clinical and experimental trials, the highest level of evidence today, sufficient for implementation in clinical practice, is achieved only for MSI and associated KRAS, NRAS and BRAF mutations [41].

Microsatellite instability is an indicator of the mismatch repair system defect (MMR), which occurs due to a hereditary mutation in one of the MMR system genes or as a result of MLH-1 gene promoter methylation. Microsatellites represent repeated sequences, which are composed of mono-, bi – or polynucleotide repeats, diffusely spread throughout the genome (e.g. AAA, CACACA) [34]. They can be encountered in both coding genes and within untranslatable regions. Microsatellite length extension or reduction in the coding genes may lead to either loss or acquirement of new gene function by altering the positioning of a reading frame (frameshift mutation). Presence of microsatellites can also influence the gene expression, altering both the transcription and translation processes. Elongation of microsatellite sequence in non-coding sequences leads to RNA-polymerase "slippage" through the matrix during the transcription with a consequent building of extended mRNA and causes splicing impairment. Triplet microsatellites located inside the introns are capable to initiate the silencing of regulatory genes [21]. Changes in microsatellite length may deactivate those coding genes in which they are located (e.g. TFG-β-RII and IGFRII tumor suppressor--genes) [24, 44].

Microsatellite instability is usually induced by alterations of DNA replication. A mismatch-repair system, called to identify and delete accidentally incorrect paired bases, is composed of MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2 genes [34]. Delta-DNA-polymerase, proliferating cells nuclear antigen, replication proteins A and C, DNA-ligase I, histone proteins and chromatin modifying factors also contribute to the proper functio-

Table 1. Consensus molecular classification of colorectal cancer

CMS type	CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
% of CRC	14	37	13	23
Status	MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
Mutations	BRAF mutations		KRAS mutations	
	Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Prognosis	Worse survival after relapse			Worse relapse-free and overall survival

Based on Guinney J. et al. [7]

ning of the MMR system [20]. During DNA replication, a newly created strand usually contains mismatches. Reparation system identifies non-complimentary connected nucleotides of maternal and newly created strand and binds to those fragments. Afterwards, an endonuclease-active complex is formed, which hydrolyzes a phosphodiester bond and breaks the strand at the site of a replication error. Exonuclease attaches to a liberated end of the strain and starts cleaving nucleotides one at a time in a 3' – 5' direction, deleting the whole non-complementary fragment. Finally, DNA-polymerase rebuilds the gap in a newly created strand and DNA-ligase joins the synthetized fragment with the main strand [25].

Alterations of the reparation system can be a consequence of either a hereditary genetic mutation of MMR-complex proteins or a somatic mutation or an epigenetic gene suppression or by a combination of all these factors, which results in the accumulation of uncomplimentary paired bases, leading to high microsatellite instability. Mutations in MMR genes are observed in all cases of hereditary nonpolyposis colorectal cancer (HNPCC – Lynch syndrome) and in 15% of sporadic CRC as well [40]. In sporadic CRC, a high MSI is mostly a result of MLH-1 gene silencing due to DNA methylation (epigenetic mechanism), while in cases of Lynch syndrome, a hereditary mutation of MMR gene(-s) is clearly observed. Recently, it was stated that there is another pathway of MMR alteration development. Ectopic expression of miRNA-155 and miRNA-21 demonstrated a strong impact on MLH-1 and MSH-2 expression suppressing [49, 50]. Microsatellite and DNA-repair system status is most precisely identified by polymerase-chain reaction method, because it is capable of amplifying the microsatellite repeats, which enables a comparison between their length in the tumor and in normal tissue. If the difference between the length of repeated sequences in two samples is more than 30%, the presence of MSI is stated [53].

Though it is not specific, most tumors with high MSI have a common phenotype, which includes localization in the right colon, low differentiation grade, mucus production, lymphocyte infiltration of the surrounding tumor stroma (mostly with T-Helpers-1 and cytotoxic T-cells); it is mostly identified in females in primary stage II more frequently than in stage III (22% and 12% respectively according to PETACC-3 trial, p<0.0001) [37]. MSI-high tumors are also capable of inducing a systemic immune response, similar to that found in Crohn's disease and showing high sensitivity to anti-PD1/PDL1 monoclonal antibodies (pembrolizumab, nivolumab) [13, 33, 36, 39].

Most authors emphasize three main reasons why the evaluation of MMR status is of major importance:

• prognostic value – patients with MSI-high tumors develop better overall survival rates (RR for mortality 0.31, 95% CI 0.14-0.72; p=0.004) and demonstrate signifi-

cantly lower frequency of lymph node involvement and distant metastases [18, 36];

• predictive effectiveness of 5-FU and irinotecan-based adjuvant treatment. There is strong evidence suggesting that patients with stage II and MSI-low status will not benefit from adjuvant treatment with those drugs (PETACC 3-EORTC 40993-SAKK 60/00) [26, 31, 38, 51, 54];

• MSI/MMR status still remains a main diagnostic criterion for Lynch syndrome.

Many studies demonstrated that MSI-high status has a strong correlation with overall survival of CRC patients [33]. A recent meta-analysis of 7,642 patients with CRC from 32 trials, including 1,277 patients with MSI-high tumors has shown a significant improvement of both overall and disease-free survival comparing to the patients with MSI-low tumors (RR for overall survival comprises 0.65) [33]. MSI-high status was also associated with lower incidence of lymph node invasion and distant metastases, which is why it was stated to be an independent factor of good prognosis [36, 37, 39, 43]. It was also mentioned that only 4% of MSI-high tumors possess a metastatic potential, while this rate among MSI-low and stable tumors comprises 15-17% [3, 12, 16]. However, tumors with MSI-high status and simultaneous V600E BRAF mutation are an exception and are characterized by a poor prognosis [30, 48]. Further studies have demonstrated a similar unfavorable prognosis for both MSI-stable tumors and tumors with MSI-high status combined with BRAF-mutation [22].

Several hypotheses were developed to explain a better prognosis for patients with MSI-high tumors. Tumor cell antigens, similar to those in normal tissues from which they have been developed, are represented on the cell membrane surface bonded to a MHC-I complex and therefore CD-8+ T-cells do not show there cytotoxic activity due to the negative selection in the thymus [15]. However, in the case of MSI, a wide amount of genes is mutated and the proteins they code contain significantly different peptide sequences compared to those in normal cells. Unique protein fragments in MSI-H tumor cells are presented as foreign, leading to high immunogenicity. Intraepithelial CD-8+ T-lymphocytes, detected in CRC tumors with high MSI, show an increased concentration of granzyme B and perforins (apoptosis inductors) compared to those in MSI-stable ones [5]. Significant accumulation of mutations associated with alterations of MMR-genes in MSI-high tumors leads to the synthesis of functionally impaired apoptosis regulating proteins (APAP-1, BAX, BCL-10, caspase-5, FAS, RIZ), growth factors and their receptors, which significantly reduces cell life capacity and results in more active biological elimination [28].

Therefore, a clear understanding of main pathological pathways of replication, reparation, structural and regulatory processes in the genome that are altered when

MSI is present can theoretically serve as an explanation of either the effectiveness or ineffectiveness of the main adjuvant chemotherapy regimens that are used for CRC treatment with regard to the pharmacodynamical and metabolic pathways of drugs.

CRC treatment strategy is based on curative surgery, followed by adjuvant chemotherapy for colon cancer, and radiation or chemoradiation therapy followed by curative surgery in cases of rectal cancer. A key role in the chemotherapy of CRC, starting from the early 1960s, has been played by antimetabolite fluoropyrimidine-based drugs, among which the most widespread is 5-FU. This is an uracil analogue containing fluorine in C-5 position instead of hydrogen. Cytotoxic action of 5-FU is realized by substituting thymine in the DNA or uracil in RNA that leads to a disintegration of a strand. 5-FU turns into fluorodeoxyuridine monophosphate, which forms a stabile complex with thymidylate synthase (TS), which is also responsible for desoxythymidine monophosphate synthesis (dTMP), alterations which lead to further impairment of reparation and synthesis of DNA. Other widely used cytotoxic drugs in CRC adjuvant regimens are irinotecan and oxaliplatinum. Sargent et al. analyzed the effectiveness of adjuvant 5-FU treatment for patients with stage II and III disease and had identified via multivariate analysis that an improvement in disease-free survival was achieved only in a subgroup of MSI-stable patients regardless of the stage [39]. Drug resistance to 5-FU treatment in MSI-high patients can be explained by the excessive incorporation of antimetabolites in DNA containing a large amount of microsatellites instead of thymidilate synthase inactivation as the main target [45]. Overexpression of miRNA-21 also significantly reduces the sensitivity to 5-FU due to an inactivation of MSH2 protein synthesis suppressing mRNA production [49]. On the other hand, the experimental study of Arnold C.N. et al. demonstrated that 5-FU regains its effectiveness after the administration of a demethylation agent 5-azacytadine, which could provide a demethylation of a MLH-1 gene promoter [2]. Other series of clinical and experimental trials demonstrated a higher effectiveness of irinotecan in a subgroup of MSI-high tumors compared to MSI-stable ones. Irinotecan is an analogue of camptothecin and acts as a powerful inhibitor of topoisomerase I and, respectively, alters DNA replication. MSI-high tumors frequently have concomitant MRE11A and hRAD50 genes mutations, responsible for the reparation of DNA strand ruptures. Probably, these mutations alone have a direct impact on irinotecan effectiveness without relation to MSI status [52]. Takahashi et al. in their trial demonstrated that the effectiveness of platinum-based drugs and particularly oxaliplatin in patients with altered MMR functions is related not to MLH1 activity, but to MSH3--gene expression, a product of which is responsible for detecting and repairing of intra – and interstrand connections of DNA. Inactivation of MSH3 (either through mutation or by miRNA) leads to a prolonged interaction of oxaliplatin molecule with DNA strand, which leads to

the impairment of its further replication. These are the main mechanisms of increasing the impact of oxaliplatin on MMR-deficient tumors [6, 46].

Nevertheless, all of the abovementioned factors allow us to connect the pharmacological characteristics of 5-FU, irinotecan and oxaliplatin, with the realization of their biological effect only in regard to the MSI-status of CRC tumor cells. Heterogeneity and "genomic chaos" present in all malignancies are turning this process into a multilevel cascade of molecular pathways, driven by multiple "side" factors, which are impossible to identify as either of primary or secondary importance [17]. Keeping in mind the simultaneous presence of multiple pathways for cytotoxic drugs inactivation, it would be impossible to display the whole picture of molecular interactions in a single malignant cell based only on MSI status.

As for the prognostic significance of MSI status in terms of overall survival and the effectiveness of adjuvant treatment, the Board of NCCN (National Comprehensive Cancer Network) in a recently updated version (4.2017) has stated that identification of MSI status should be mandatory for patients with CRC stage II and IV and as a screening parameter for Lynch syndrome as well [29]. Certain series of publications demonstrated better treatment outcomes for patients with MSI-high status who underwent only curative surgery without adjuvant chemotherapy, claiming that assessment of MSI status could give an opportunity to avoid administering unnecessary chemotherapy to a cohort of patients, who will not receive a survival benefit from it [39]. However, the role of MSI remains controversial today due to the rising confrontation of the results of recently published studies. In a retrospective cohort study of Michelle L Thomas et al., it is stated that adjuvant chemotherapy with 5-FU of CRC patients with ACPS (Australian Clinico-pathological Staging System) stage C provides a significant survival benefit for both MSI-high and MSI-stable subgroups of patients [47].

CONCLUSIONS

Microsatellite instability is a unique biological entity, particularly specific for CRC patients. Although many studies concerning the prognostic effectiveness of MSI in adjuvant treatment of CRC have been conducted during the last three decades, the data suggesting MSI status as a marker of individual prognosis remain obscured. Though we have seen a significant progress in the understanding of microsatellite instability molecular pathways, there is still a lack of data regarding the interaction between the molecular pathways with the pharmacological effects of the major cytotoxic drugs used for adjuvant treatment of CRC. The controversy concerning the impact of MSI status on the effectiveness of adjuvant chemotherapy still exists. Strong evidence suggesting either presence or absence of a certain drug resistance among this cohort of patients are lacking. Keeping in mind the results of multiple clinical, experimental and

population-based studies, a statement claiming an independent prognostic value of MSI with disregard to other molecular predictive factors is unfounded. But at the same time, it is hard to overestimate the diagnostic and prognostic value of MSI status due to a significant amount of evidence suggesting the correlation between CRC tumor phenotype, presence and a grade of microsatellite instability.

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