MMR-Colon Study: First Attempt at Quantifying MMR Protein Expression as a Prognostic Factor in Colorectal Cancer

Bertolina Carlotta[1], Erra Stefania[2], Mazzucco Riccardo[3], Giacchero Fabio[3,4], Biasion Sara[1], Maconi Antonio[1], Bertolotti Marinella[1]

(1) Research Training Innovation Infrastructure, Research and Innovation Department (DAIRI), Azienda Ospedaliero-Universitaria SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy
(2) Surgical Pathology laboratory, Santa Rita Clinic, Monza Policlinic, Italy
(3) Research and Innovation Department (DAIRI), Azienda Sanitaria Locale di Alessandria, Alessandria, Italy
(4) Specialization School of Medical Statistics and Biometry, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy

CORRESPONDING AUTHOR: *Fabio Giacchero, fabio.giacchero01@universitadipavia.it*

INTRODUCTION

Colorectal cancer (CRC) represents one of the most important public health issues worldwide and the third most diagnosed cancer globally. International Agency for Research on Cancer (IARC) data reported a global incidence of more than 1.9 million in 2022, and at least 900,000 deaths, representing the second leading cause of cancer death[1]. Molecular events CRC include genetic and epigenetic anomalies, such as DNA Mismatch Repair (MMR) system instability. The loss of MMR repair activity results in microsatellite instability[2]. Mismatch repair system deficit (dMMR) is observed in about 15% of all CRCs[3]. While in an undamaged and efficient repair system, MMR proteins identify and repair DNA mismatches that occur during replication, when microsatellites are unstable mismatches that result from DNA replication cannot be repaired [4]. It is of fundamental importance to distinguish MSI CRC from CRC with microsatellite stability (MSS), both from a prognostic point of view, as the former show a better overall survival (OS) and disease- free survival (DFS), but also predictively as MSI tumors respond poorly to adjuvant treatment with 5- fluorouracil (5-FU) in the early stages [5].

OBJECTIVE

Evaluate whether an increase or decrease in the expression of MMR proteins is quantitatively associated with morphological patterns and the impact this has on survival.

METHODS

All patients with CRC diagnosed from 2020 to 2021 carried out at the Surgical Pathology Unit of S. Spirito Hospital in Casale Monferrato (Alessandria, Italy) were included. dMMR immunohistochemical assessment was performed with ROCHE's automated VENTANA BenchMark Ultra platform and the following antibodies: VENTANA anti-MLH1, VENTANA anti-PMS2, VENTANA anti-MSH6. IHC-marked cells for all four MMR proteins are evaluated for presence or loss of expression based on the signal occurrence or absence released by the diaminobenzidine chromogen (DAB) of the detection system. MMR protein expression quantification, mainly MLH1 and MSH6, was performed by optical microscopy observation. MMR quantification was assessed in an independent evaluation by two observers. Any glandolar structure was considered as expressed when more than half of the cells were stained by IHC assay. Further to the independent evaluation of the observers, samples without quantification agreement were jointly assessed and re-estimated to reach a quantitative evaluation mutually agreeable to the investigators, with a 5% variation range between both observations. Specific quantification was then provided for all samples, with a lowest of 5% and a highest of 100%. The resulting expression was lastly subdivided into two scores, high and low, according to the median value that was calculated on the whole sample. Univariate and multivariate logistic regression models were used to find determinants of low or no expression of MSH6 and MLH1 separately. The estimated Odds Ratio (OR) and 95% confidence interval (CI) were considered to this aim. Variable selection for multivariable logistic models was performed with a forward stepwise process. In order to assess the effect of MMR expression on overall survival (OS), Kaplan-Meier

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curves with log-rank test were used. Hazard Ratio (HR) and 95% CI, estimated by semi-parametric proportional hazard Cox regression models, were then also used. Variable selection for the multivariate Cox regression model was performed by considering those variables resulted statistically significant in the logistic regression models and those considered clinically meaningful. For survival analyses, the last episode recorded in the hospital repository was considered as the last follow-up date for patients with a lifetime status.

RESULTS

In our study 73 patients were included. Male gender was the most represented (63.0%), the median age of patients was 74 years [IQR 66-80], with a range of 52-89 years. Most patients were affected by CRC with the following morphological features: histologic grade G2 (68.5%), high tumor budding (71.2%) and infiltrating growth margin (75.3%). The not otherwise specified (NOS) histotype was the most frequently diagnosed (82.2%). Multivariate analysis showed that high-grade budding is associated with increased odds of low MSH6 expression (OR = 11.20; 95% CI: 2.34-53.72), as well as, for MLH1 more aggressive histotype (OR=4.81; 95% CI: 1.25-18.51) and perineural invasion (OR=3.61; 95% CI: 1.20-10.87). Neither MSH6 nor MLH1 expression resulted to have an effect on survival.

CONCLUSIONS

MMR expression quantification in CRCs could be a valuable tool for prognostic patient stratification, as patients with low expression may show a response more similar to that of unstable tumors. Although some aggressive features are associated with lower MMR expression, this does not seems to have an effect on survival. Given the high variability in the resulting estimates, further studies on larger sample are needed to confirm the relationships found.

BIBLIOGRAPHY

1. Bray F., Laversanne M., Sung H. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May- Jun;74(3):229-263.
2. Tariq K., Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. Cancer Biol Med. 2016 Mar;13(1):120-35.
3. Li J., Wu C., Hu H. et al. Remodeling of the immune and stromal cell compartment by PD-1 blockade in mismatch repair-deficient colorectal cancer. Cancer Cell. 2023 Jun 12;41(6):1152- 1169.
4. Fishel R. Mismatch repair. J Biol Chem. 2015 Oct 30;290(44):26395-403.
5. Vatrano S., Pettinato A., Randazzo V. et al. Diagnostic test assessment. Validation study of an alternative system to detect microsatellite instability in colorectal carcinoma. Pathologica. 2020 Dec;112(4):178-183.

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