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Original Scientific Paper

RELATIONSHIP OF IMMUNOHISTOCHEMICAL EXPRESSION OF CD86/CD163 POSITIVE INTRATUMORAL MACROPHAGES WITH PROGNOSIS OF PANCREATIC DUCTAL ADENOCARCINOMA

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SUMMARY – Recent studies have shown an association of the number of tumor-associated macrophages (TAM) with the prognosis and treatment outcomes of ductal pancreatic adenocarcinoma. This study aimed to examine the correlation between CD86 and CD163 macrophage expression and clinical-pathological characteristics of the disease in patients operated on for pancreatic carcinoma. A retrospective research was conducted in which the archival materials of the Ljudevit Jurak Department of Pathology, Sestre milosrdnice University Hospital Center were used together with all relevant patient clinical data obtained from the Hospital Information System on 76 patients operated on for pancreatic adenocarcinoma. In our study, the number of CD86 macrophages and the CD86/CD163 ratio showed a statistically significant correlation with increasing T and N stages of the disease. The number of CD163 macrophages did not show a statistically significant correlation with the mentioned variables. These results indicated that in our clinical conditions, proinflammatory (M1) macrophages were more expressed in locally advanced tumor stages with worse long-term prognoses. In conclusion, TAMs could be a valid prognostic marker or even a target for therapeutic agents but more studies will be needed to fully comprehend the impact of M1/M2 differentiation.

Keywords: *Pancreatic adenocarcinoma; M1/M2 macrophages; CD68/CD163; Tumor-associated macrophages; Cancer research; Surgical oncology*

Introduction

Pancreatic cancer is a neoplasm that, in most cases (85%), originates from ductal epithelium of the pancreas[1]. In terms of mortality at the world level, it ranks 7th for both men and women[2]. The incidence is higher in developed areas such as the USA, Western Europe, and parts of East Asia[2]. The disease rarely

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occurs before the age of 45; the incidence is highest in the 65-69 age group for men and 75-79 age group for women[2]. Males are affected somewhat more often (1.3:1)[3]. Risk factors for the occurrence of this disease include smoking, obesity, diabetes, diet, alcoholism, as well as genetic factors[4-6]. Surgical resection is the only potentially curative treatment currently available but at the time of diagnosis, only 15%-20% of patients are candidates for pancreatic resection[7]. Five-year survival after R0 resection is 30% in cases where lymph nodes are unaffected and 10% in cases with dissemination to lymph nodes[7]. The most common symptoms of the disease are asthenia, weight loss, dull pain in the abdomen, anorexia, jaundice, nausea, and back pain[8]. In 60%-70% of cases, the tumor is located in the head of the pancreas, 20%-25% in the body/tail, and the rest of cases involve the entire organ[9]. The basis of diagnosis are endoscopic and radiology tests (endoscopic retrograde cholangiopancreatography and computed tomography/magnetic resonance imaging), which are complemented by laboratory findings, including the tumor marker CA 19-9[10-12]. In defining the advanced stage of cancer, the TNM classification according to the American Joint Committee of Cancer is used, which classifies cancers based on the depth of tumor invasion (T), the involvement of regional lymph nodes by the tumor (N), and the presence of distant metastases (M)[13]. When deciding on the treatment method, it is important to follow the criteria that determine unresectable (locally and generally extended) disease, which include the relationship of the tumor to large blood vessels, as well as the presence of distant metastases[14]. Recently, neoadjuvant chemotherapy has been used in an increasing number of centers for oncology treatment of borderline resectable cases, although studies report mixed results on the effectiveness of this approach[15,16]. Tumor biopsy could be used to improve the results of neoadjuvant therapy as it can allow for a more targeted approach through RNA-sequencing[17,18]. After surgery, chemotherapy is administered in almost all cases (a potential exception is only the T1N0 stage in well-differentiated tumors)[19].

Considering the poor prognosis of treatment of this tumor, the search is being made for the key molecules responsible for the formation and spread of malignant cells. In addition to forecasting the outcome of treatment, the goal of research into the expression of these antigens is the body immune response to their presence, which could enable development of tumor-specific vaccines but most research is still in early stages and without conclusive findings[20].

Macrophages are a type of white blood cells that originate from monocytes and are responsible for various immune functions in the human body, and are found in almost all tissues[21]. Macrophages can be differentiated into two lineages, M1 and M2. M1 macrophages have a proinflammatory effect and inhibit proliferation of tumor cells, whereas M2 macrophages have an immunosuppressive effect, promote angiogenesis, and have a protumor effect[22]. The M1 phenotype is associated with the production of proinflammatory cytokines such as interleukin-12 (IL-12), interferon gamma and tumor necrosis factor alpha, antigen presentation, and the ability to eliminate pathogens, whereas M2 is associated with the production of IL-4, IL-10, and tissue remodeling[23]. When identifying these macrophages, markers associated with a certain type are used; M1 macrophages show predominantly CD80, CD86 and human leukocyte antigen expression, whereas M2 macrophages predominantly show CD163, CD204, CD206, and arginase[24,25]. Markers such as CD68 are positive in M1 and M2 macrophages[26]. Most studies on this topic have been carried out on *in vitro* models, and they have shown greater expression of M2 macrophages in aggressive tumor strains[27,28]. However, some studies focusing on breast and colorectal cancer have shown mixed results or even better survival associated with higher expression of M2 macrophages[29,30]. Monitoring the number of TAMs depending on the direction of their differentiation into M1 or M2 macrophages in pancreatic cancer could indicate earlier lymphogenous dissemination and thus modify treatment approach[31]. The assumption of a recent research is that the effectiveness of neoadjuvant and adjuvant chemotherapy could be improved by screening patients[32]. Modulation of M1 and M2 macrophage responses could be the basis for the development of new therapies that would improve treatment efficacy and increase survival in patients with pancreatic cancer[33].

Materials and Methods

Archival material kept at the Ljudevit Jurak Department of Pathology and Cytology, Sestre milosrdnice University Hospital Center (UHC), obtained after resection of the pancreas due to ductal adenocarcinoma in the period from January 1, 2003 until December 31, 2019, was used in the study. Only those patients who met the following conditions were included in the research: all the necessary clinical data and appropriate histopathologic findings and follow-up were available; and no malignant tumor other than pancreatic cancer. Patients with early postoperative mortality (<30 days) were also excluded. Seventy-six patients met all the criteria and were included in the study. Patient data were collected from the Hospital Information System of the Sestre milosrdnice UHC, while data on the date of death were collected from the Cancer Registry of the Croatian Institute of Public Health. The Ethics Board of both the Hospital and the University of Zagreb approved the research.

The material was processed by a standard histologic method that includes tissue fixation in 10% buffered formalin and mounting in paraffin blocks, cutting to a 5-μm thickness, deparaffinization, and staining with the standard hematoxylin and eosin (HE) method. Primary monoclonal antibodies were used for immunohistochemical analysis, i.e., CD86 (ab234401, Abcam, UK) and CD163 (ab156769, Abcam, UK), for M1 and M2 macrophages. Immunohistochemical analysis for the mentioned antibodies was performed with the EnVision FLEX-PTL method as a visualization system on a Dako Autostainer automated machine for immunohistochemical staining (Figs. 1 and 2).

On statistical data processing, the time of taking the sample during the operation was the starting point of the research. The endpoint was overall patient survival. The goal of data analysis was to analyze the relationship between the number of CD86, CD163 and the ratio of CD86/CD163 positive intratumoral macrophages in pancreatic adenocarcinoma samples. The number and ratio of CD86 and CD163 macrophages were compared with histopathologic characteristics of the tumor, i.e., tumor size, depth of invasion (pT stage) of the tumor, status of regional lymph nodes (pN stage) and lymphovascular invasion, and variables of surgical treatment, i.e., complications, reoperation, type of operation, and positive resection margin. Immunohistochemical and histopathologic variables were compared with patient clinical characteristics, i.e., age, gender and experience. Analysis of the normality of data distribution was checked with the Smirnov-Kolmogorov test, and according to

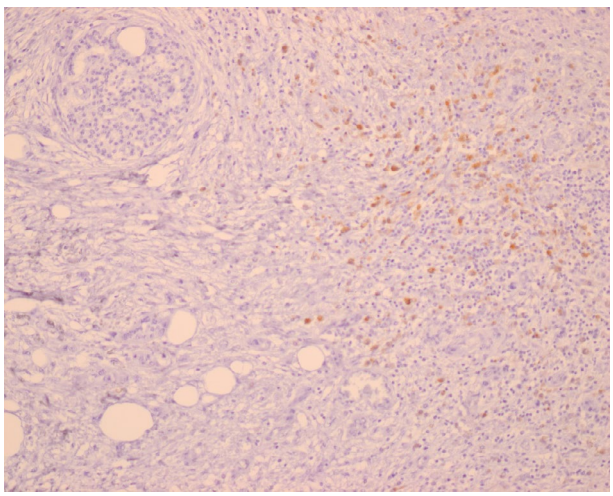

Fig. 1. Positive immunohistochemical reaction of macrophages in the tumor area to CD86 (magnification X200).

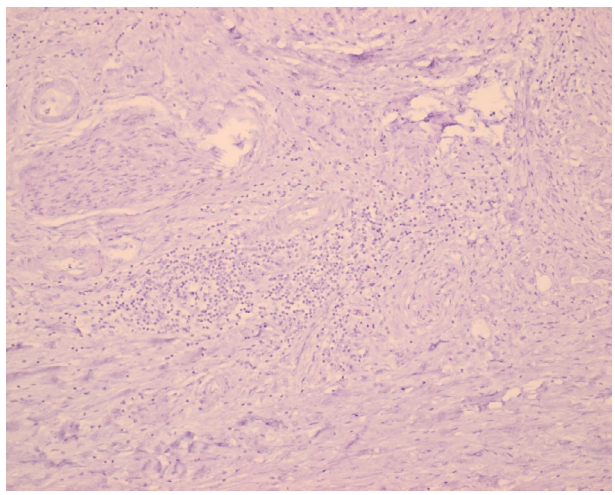

Fig. 2. Negative immunohistochemical reaction of macrophages in the tumor area to CD86 (magnification X200).

the results obtained, parametric and nonparametric tests were used, as well as the corresponding display of continuous values (arithmetic mean and standard deviation (SD), or median). A binary outcome (alive or deceased) is indicated by census. A large number of variables were foreseen and tests were used that can compensate for unfavorable ratios of the number of respondents and variables. In order to reduce the potentially negative influence of a small number of subjects on testing a large number of variables, it is planned to perform a multivariate Cox regression analysis (with time to event as follow-up time in months). Then, for each variable that would show a statistically significant association with survival, the receiver operating characteristic curve (ROC) analysis was performed and a cut-off value was determined using the Youden J factor in order to convert continuous variables into categorical ones, as 0 = below the cut-off value and 1 = above the cut-off value . Using categorical variables arranged in this way, results were presented with the Kaplan Meier survival curve. As a confirmatory method, a principal component analysis with oblimin rotation was performed in order to separate groups of variables that appear together and to confirm the results of the multivariate regression analysis. Variables were compared with each other using Spearman's rho correlation coefficient. All statistical tests were two-tailed. Values of p less than and equal to 0.05 were determined to be statistically significant. Statistical data processing was done using the MedCalc program (Version 11.2.1 © 1993-2010; MedCalc Software bvba Software, Mariakerke, Belgium) and SPSS program (Version 22.0 Released in 2013; IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA).

Results

The study included 76 subjects who were surgically treated for histopathologically confirmed adenocarcinoma of the pancreas at the Department of Surgery, Sestre milosrdnice UHC, from January 1, 2003 until December 31, 2019. The patients were monitored postoperatively and their oncologic status was recorded. At the time of diagnosis, the patients were free of other clinically detectable malignant diseases. There were 32 male and 44 female patients, yielding a 0.73 male-female ratio. Their mean age was 65.14, range 32-86 years, SD 9.97. The mean age of men was 64.6±10.33, median 65.5 years, and of women 65.6±9.67 years, median 67 years.

The mean length of patient follow-up, expressed as arithmetic mean and SD, was 26±22 months. Due to the large SD, the median was a more precise measure of follow-up length and it was 19.33, range 2-129 months.

Statistical analysis of the possible associations of the variables with patient survival marked as time to the critical event was performed using multivariate Cox regression (χ^2=61.433, df=16, p<0.001), which showed the following variables to be statistically significantly associated with the outcome: (a) number of intratumoral macrophages immunohistochemically labeled with CD86 (p=0.025, hazard ratio 5.018); (b) type of operation total pancreatectomy (p=0.025, hazard ratio 5.023); (c) occurrence of complications in the postoperative course (p<0.001, hazard ratio 18.691); and (d) need of reoperation (p=0.002, hazard ratio 9.165)

The ROC analysis of the number of intratumoral macrophages immunohistochemically marked with CD86 and its association with patient survival showed that the cut-off value was expressed with the help of the Youden index (0.2137) where the specificity and sensitivity were the highest values >3 (sensitivity 76.92%, specificity 44.44% with 95% confidence interval (CI) of 46.2%-95.0% for sensitivity and 31.9%-57.5% for specificity). Then, the values of the number of intratumoral macrophages immunohistochemically labeled with CD86 were converted into categorical ones; if the value in the patient sample was >3, it was converted into a categorical variable with a value of 1, and if the value in the patient sample was <3, it was converted into a categorical variable with a value of 0. The relationship between the number of intratumoral macrophages immunohistochemically labeled with CD86 and patient survival was shown using the Kaplan Meier survival curve (Fig. 3) and the hazard function graph (Fig. 4).

Statistical analysis using Spearman's rho correlation coefficient showed that age was not related to other variables, or to CD86 and CD163 values, or to the CD86/CD163 ratio. The number of intratumoral

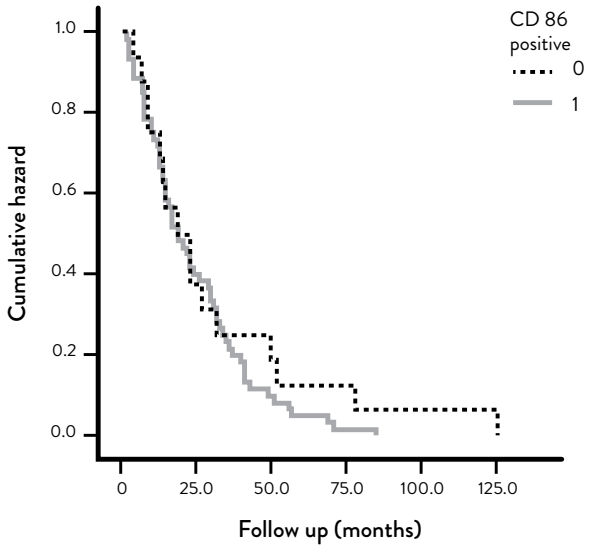

Fig. 3. Correlation between the number of intratumoral macrophages immunohistochemically labeled with CD86 and patient survival (p=0.025).

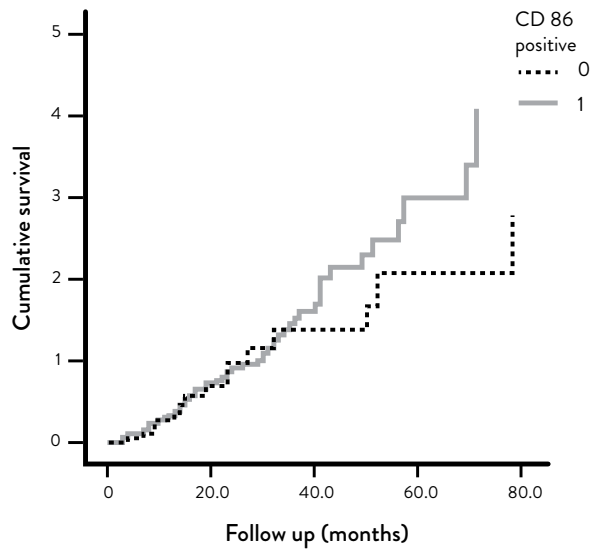

Fig. 4. Correlation between the number of intratumoral macrophages immunohistochemically labeled with CD86 and the increase in hazard in relation to patient survival.

macrophages immunohistochemically labeled with CD86 was significantly positively correlated with the CD86/CD163 ratio (Spearman rho coefficient 0.813, p<0.001), with increasing T category (Spearman rho coefficient 0.223, p=0.05) and N stage of the patients (Spearman rho coefficient 0.229, p=0.046), while the number of intratumoral macrophages immunohistochemically labeled with CD163 did not show significant correlations with other variables.

The CD86/CD163 ratio significantly correlated with the number of CD86 (Spearman rho coefficient 0.813, p<0.001) and with increasing T category (Spearman rho coefficient 0.230, p=0.046). The T category positively correlated with the CD86/CD163 ratio (Spearman rho coefficient 0.230, p=0.046), while the N stage positively correlated with the number of CD86 positive macrophages (Spearman rho coefficient 0.229, p=0.046). Resection margins did not show significant correlations with other variables. A larger scope of the initial operation significantly correlated with lower probability of reoperation (Spearman rho coefficient -0.243, p=0.034), and the occurrence of complications significantly correlated with the probability of reoperation (Spearman rho coefficient 0.906, p<0.001) and with male gender (Spearman rho coefficient -0.255, p=0.026).

Discussion

Ever since the discovery of TAMs, their role has been evaluated and they have come in interest for targeted cancer treatment[34]. While early *in vitro* research polarized TAMs and proclaimed M2 macrophages distinctly pro-tumor[35], other studies have shown a more complex relationship between M1 and M2 types, showing that they can be modulated on a spectrum[36]. Since most studies were done with a variety of malignant diseases, both *in vitro* and *in vivo* (primarily with test animals)[37], we opted for a clinical study with patients suffering from pancreatic adenocarcinoma. We found studies researching the link between TAMs and pancreatic cancer but most were done in a pre-clinical setting[27,38,39], and we decided to try and include a clinical aspect in the research.

Results of clinical parameters (operative results) were as expected, as a more extensive surgical operation indicates a larger or multicentric tumor. This was

indicated by the hazard ratio (5.023) for total pancreatectomy as opposed to Whipple's procedure or distal pancreatectomy. The survival length of the patients was on par with general statistics concerning pancreatic cancer.

In our study, the number of CD86 macrophages and the CD86/CD163 ratio showed a statistically significant correlation with increasing T and N stage of the disease. The number of CD163 macrophages did not show a statistically significant correlation with the mentioned variables. These results indicate that in our clinical conditions, proinflammatory (M1) macrophages were more expressed in locally advanced tumor stages with worse long-term prognosis. The reasons for this can be numerous, and seem to indicate that in certain conditions M1 macrophages can also be regarded as a factor in worse treatment outcomes. We tried to analyze and compare our findings with other authors, since some recent studies could not reach a consensus on the prognostic value of M1 and M2 macrophage activation.

This unconventional correlation was shown in similar clinical studies concerning gliomas, medulloblastomas, and non-small cell lung cancer[40-42]. In a study concerning medulloblastomas, researchers hypothesized that this correlation may be caused by multiple factors, e.g., high M1 macrophage recruitment can assist tumor growth and progression, contrary to its role in other cancers. They also theorize that M1 macrophages are highly recruited to enhance the tumoricidal effect in aggressive group of medulloblastoma (but this mechanism alone was insufficient to fight the particular malignancy); or high M1 recruitment is an epiphenomenon, and these cells are simply recruited by other medulloblastoma initiators and do not directly affect prognosis[40]. Another example of differing results is shown in a research concerning non-small cell lung cancer; M1 macrophage numbers tended to be higher in the stroma of poor prognosis patients compared with good prognosis patients. The M1 macrophages may be in the wrong tissue compartment for the modulation of tumor activity as key immune responses affecting survival in non-small cell lung cancer seem to take place in the tumor islets as opposed to the stroma[41]. In a study featuring gliomas, M1 macrophages were hypothesized to be negatively affecting antibody treatments and consequently reducing survival rates[42].

As mentioned earlier, there are new views about the M1/M2 macrophage relationship that should be taken into account, i.e., the process of macrophage activation is complex and includes monocytes[43] and myeloid-derived dendritic cells[44]. In tissues, this creates a certain macrophage phenotype which cannot always be assorted in a certain group; more information is required about macrophages *in vivo* and at the population level, as well as at the single-cell level[36].

Still, certain studies tend to receive results that indicate M2 macrophages as the more dominant phenotype in tumor cells in patients with shorter survival spans and more aggressive disease strains[45-47]. In a study concerning uveal melanoma positive CD163 (M2), staining was found to be associated with ciliary body involvement and monosomy 3, and the absence of such staining was associated with increased survival[46]. Another study found positive correlation of M2 macrophages and regulatory T lymphocytes which could, together with other suppressor cells such as myeloid-derived suppressor cells, promote an immunosuppressive environment in aggressive prostate cancer[47]. Discrepancies in the results of various studies show that in clinical studies with different types of tumors, more factors than simple M1/M2 polarization are involved. Most studies tend to generally have a relatively low number of participants and have to focus on specific tumor cells, which limits their conclusiveness. These findings would suggest that the immune response in different types of malignant disease is still not entirely clear in clinical, *in vivo* conditions.

Conclusion

Regarding pancreatic cancer, TAMs could still be a valid prognostic marker or even a target for therapeutic agents but more studies will be needed to fully comprehend the impact that M1/M2 differentiation plays in battling the generation and spread of this dangerous disease. Other antigens and their effect (such as MAGE, melanoma-associated antigen) are being studied as well[48]. Nevertheless, surgery will probably remain the mainstay treatment in the near future.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. doi: 10.3322/caac.21590
2. Pourshams A, Sepanlou SG, Ikuta KS, Bisignano C, Safiri S, Roshandel G, *et al*. The global, regional, and national burden of pancreatic cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2019;4(12):934-47. doi: 10.1016/S2468-1253(19)30347-4
3. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: global trends, etiology and risk factors. World J Oncol. 2019;10(1):10. doi: 10.14740/wjon1166
4. Rebours V, Lévy P, Ruszniewski P. An overview of hereditary pancreatitis. Dig Liver Dis. 2012;44(1):8-15. doi: 10.1016/j.dld.2011.08.003
5. Michaud DS, Skinner HG, Wu K, Hu F, Giovannucci E, Willett WC, *et al*. Dietary patterns and pancreatic cancer risk in men and women. J Natl Cancer Inst. 2005;97(7):518-24. doi: 10.1093/jnci/dji094.
6. Weiss FU. Pancreatic cancer risk in hereditary pancreatitis. Front Physiol. 2014;Feb 20;5:70. doi: 10.3389/fphys.2014.00070.
7. Allen PJ, Kuk D, Castillo CF Del, Basturk O, Wolfgang CL, Cameron JL, *et al*. Multi-institutional validation study of the American Joint Commission on Cancer (8th edition) changes for T and N staging in patients with pancreatic adenocarcinoma. Ann Surg. 2017;265(1):185-91. doi: 10.1097/SLA.0000000000001763.
8. Porta M, Fabregat X, Malats N, Guarner L, Carrato A, De Miguel A, *et al*. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. Clin Transl Oncol. 2005;7(5):189-97. doi: 10.1007/BF02712816.
9. Modolell I, Guarner L, Malagelada JR. Vagaries of clinical presentation of pancreatic and biliary tract cancer. Ann Oncol. 1999;10(Suppl 4). doi:10.1093/annonc/10.suppl_4.S82
10. Niederau C, Grendell JH. Diagnosis of pancreatic carcinoma: imaging techniques and tumor markers. Pancreas. 1992;7(1):66-86. doi: 10.1097/00006676-199201000-00011.
11. Freeny PC, Marks WM, Ryan JA, Traverso LW. Pancreatic ductal adenocarcinoma: diagnosis and staging with dynamic CT. Radiology. 1988 Jan;166(1 Pt 1):125-33. doi: 10.1148/radiology.166.1.2827228.
12. Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. J Gastrointest Oncol. 2012;3(2):105-19. doi: 10.3978/j.issn.2078-6891.2011.021.
13. Chun YS, Pawlik TM, Vauthey JN. 8th Edition of the AJCC Cancer Staging Manual: Pancreas and Hepatobiliary Cancers. Ann Surg Oncol. 2018;25(4):845-7. doi: 10.1245/s10434-017-6025-x.
14. Al-Hawary MM, Francis IR, Chari ST, Fishman EK, Hough DM, Lu DS, *et al*. Pancreatic ductal adenocarcinoma radiology reporting template: consensus statement of the Society of Abdominal Radiology and the American Pancreatic Association. Gastroenterology. 2014;146(1). doi: 10.1053/j.gastro.2013.11.004
15. Murphy JE, Wo JY, Ryan DP, Jiang W, Yeap BY, Drapek LC, *et al*. Total neoadjuvant therapy with FOLFIRINOX followed by individualized chemoradiotherapy for borderline resectable pancreatic adenocarcinoma: a phase 2 clinical trial. JAMA Oncol. 2018 Jul 1;4(7):963-9. doi: 10.1001/jamaoncol.2018.0329.
16. Versteijne E, Suker M, Groothuis K, Akkermans-Vogelaar JM, Besselink MG, Bonsing BA, *et al*. Preoperative chemoradiotherapy *versus* immediate surgery for resectable and borderline resectable pancreatic cancer: results of the Dutch randomized phase III PREOPANC trial. J Clin Oncol. 2020;38(16):1763-73. doi: 10.1200/JCO.19.02274.
17. Casolino R, Braconi C, Malleo G, Paiella S, Bassi C, Milella M, *et al*. Reshaping preoperative treatment of pancreatic cancer in the era of precision medicine. Ann Oncol. 2021;32(2):183. doi: 10.1016/j.annonc.2020.11.013.
18. O'Kane GM, Grunwald BT, Jang GH, Masoomian M, Picardo S, Grant RC, *et al*. GATA6 expression distinguishes classical and basal-like subtypes in advanced pancreatic cancer. Clin Cancer Res. 2020;26(18):4901-10. doi: 10.1158/1078-0432.CCR-19-3724
19. Khorana AA, Shapiro M, Mangu PB, Berlin J, Engebretson A, Hong TS, *et al*. Potentially curable pancreatic cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol. 2017;35(20):2324-8. doi: 10.1200/JCO.2017.72.4948.
20. Escudier B, Dorval T, Chaput N, André F, Caby MP, Novault S, *et al*. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. J Transl Med. 2005 Mar 2;3:10. doi: 10.1186/1479-5876-3-10
21. Ovchinnikov DA. Macrophages in the embryo and beyond: much more than just giant phagocytes. Genesis. 2008;46(9):447-62. doi: 10.1002/dvg.20417.

22. Mills CD. M1 and M2 macrophages: oracles of health and disease. Crit Rev Immunol. 2012;32(6):463-88. doi: 10.1615/critrevimmunol.v32.i6.10.
23. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141(1):39-51. doi: 10.1016/j.cell.2010.03.014.
24. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. 2009;27:451-83. doi: 10.1146/annurev.immunol.021908.132532.
25. Takeya M, Komohara Y. Role of tumor-associated macrophages in human malignancies: friend or foe? Pathol Int. 2016;66(9):491-505. doi: 10.1111/pin.12440.
26. Yu M, Guan R, Hong W, Zhou Y, Lin Y, Jin H, *et al*. Prognostic value of tumor-associated macrophages in pancreatic cancer: a meta-analysis. Cancer Manag Res. 2019;11:4041-58. doi: 10.2147/CMAR.S196951
27. Zhang M, Pan X, Fujiwara K, Jurcak N, Muth S, Zhou J, *et al*. Pancreatic cancer cells render tumor-associated macrophages metabolically reprogrammed by a GARP and DNA methylation-mediated mechanism. Signal Transduct Target Ther. 2021;6(1):1-18. doi: 10.1038/s41392-021-00769-z.
28. Liu J, Geng X, Hou J, Wu G. New insights into M1/M2 macrophages: key modulators in cancer progression. Cancer Cell Int. 2021;21(1):1-7. doi: 10.1186/s12935-021-02089-2.
29. Pe KCS, Saetung R, Yodsurang V, Chaotham C, Suppipat K, Chanvorachote P, *et al*. Triple-negative breast cancer influences a mixed M1/M2 macrophage phenotype associated with tumor aggressiveness. PLoS One. 2022;17(8):e0273044. doi: 10.1371/journal.pone.0273044.
30. Zhong X, Chen B, Yang Z. The role of tumor-associated macrophages in colorectal carcinoma progression. Cell Physiol Biochem. 2018;45(1):356-65. doi: 10.1159/000486816.
31. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, *et al*. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res. 2011 May 15;167(2):e211-9. doi: 10.1016/j.jss.2009.05.026.
32. Di Caro G, Cortese N, Castino GF, Grizzi F, Gavazzi F, Ridolfi C, *et al*. Dual prognostic significance of tumour-associated macrophages in human pancreatic adenocarcinoma treated or untreated with chemotherapy. Gut. 2015;65(10):1710-20. doi: 10.1136/gutjnl-2015-309193.
33. Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell. 2015 Apr 13;27(4):462-72. doi: 10.1016/j.ccell.2015.02.015.
34. Li M, He L, Zhu J, Zhang P, Liang S. Targeting tumor-associated macrophages for cancer treatment. Cell Biosci. 2022;12(1):1-13. doi: 10.1186/s13578-022-00823-5.
35. Li C, Xu X, Wei S, Jiang P, Xue L, Wang J. Tumor-associated macrophages: potential therapeutic strategies and future prospects in cancer. J Immunother Cancer. 2021 Jan;9(1):e001341. doi: 10.1136/jitc-2020-001341.
36. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014;6. doi: 10.12703/P6-13
37. Mielgo A, Schmid MC. Impact of tumour associated macrophages in pancreatic cancer. BMB Rep. 2013;46(3):131. doi: 10.5483/bmbrep.2013.46.3.036.
38. Yang S, Liu Q, Liao Q. Tumor-associated macrophages in pancreatic ductal adenocarcinoma: origin, polarization, function, and reprogramming. Front Cell Dev Biol. 2020;8:607209 doi: 10.3389/fcell.2020.607209.
39. Lankadasari MB, Mukhopadhyay P, Mohammed S, Harikumar KB. TAMing pancreatic cancer: combat with a double edged sword. Mol Cancer. 2019;18(1):1-13. doi: 10.1186/s12943-019-0966-6.
40. Lee C, Lee J, Choi SA, Kim SK, Wang KC, Park SH, *et al*. M1 macrophage recruitment correlates with worse outcome in SHH medulloblastomas. BMC Cancer. 2018;18(1):535. doi: 10.1186/s12885-018-4457-8.
41. Ohri CM, Shikotra A, Green RH, Waller DA, Bradding P. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur Respir J. 2009;33(1):118-26. doi: 10.1183/09031936.00065708.
42. Zhou Z, Wen L, Lai M, Shan C, Wang J, Wang R, *et al*. Increased M1 macrophages infiltration correlated with poor survival outcomes and radiation response in gliomas. Dose Response. 2020;18(4):1559325820964991. doi: 10.1177/1559325820964991.
43. Scotton CJ, Martinez FO, Smelt MJ, Sironi M, Locati M, Mantovani A, *et al*. Transcriptional profiling reveals complex regulation of the monocyte IL-1 beta system by IL-13. J Immunol. 2005;174(2):834-45. doi: 10.4049/jimmunol.174.2.834.
44. Mazzoni A, Segal DM. Controlling the Toll road to dendritic cell polarization. J Leukoc Biol. 2004;75(5):721-30. doi: 10.1189/jlb.1003482.
45. Lv C, Li S, Zhao J, Yang P, Yang C. M1 macrophages enhance survival and invasion of oral squamous cell carcinoma by inducing GDF15-mediated ErbB2 phosphorylation. ACS Omega.2022;7(13):11405-14.doi:10.1021/acsomega.2c00571.

46. Bronkhorst IHG, Ly LV, Jordanova ES, Vrolijk J, Versluis M, Luyten GPM, *et al*. Detection of M2-macrophages in uveal melanoma and relation with survival. Invest Ophthalmol Vis Sci. 2011;52(2):643-50. doi: 10.1167/iovs.10-5979.
47. Erlandsson A, Carlsson J, Lundholm M, Fält A, Andersson SO, Andrén O, *et al*. M2 macrophages and regulatory T cells in lethal prostate cancer. Prostate. 2019;79(4):363-9. doi: 10.1002/pros.23742.
48. Cogdill AP, Frederick DT, Cooper ZA, Garber HR, Ferrone CR, Fiedler A, *et al*. Targeting the MAGE A3 antigen in pancreatic cancer. Surgery. 2012 Sep;152(3 Suppl 1):S13-8. doi: 10.1016/j.surg.2012.05.031.

Sažetak

ODNOS IMUNOHISTOKEMIJSKE IZRAŽENOSTI CD86/CD163 POZITIVNIH INTRATUMORSKIH MAKROFAGA S PROGNOZOM DUKTALNOG ADENOKARCINOMA GUŠTERAČE

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Nedavne studije pokazale su povezanost broja makrofaga povezanih s tumorom (TAM) s prognozom i ishodima liječenja duktalnog adenokarcinoma gušterače. Cilj ovog istraživanja bio je ispitati korelaciju između ekspresije CD86 i CD163 makrofaga i kliničko-patoloških karakteristika bolesti u bolesnika operiranih zbog karcinoma gušterače. Provedeno je retrospektivno istraživanje u kojem je korišten arhivski materijal Kliničkog zavoda za patologiju i citologiju Ljudevit Jurak, KBC „Sestre milosrdnice". Svi relevantni klinički podaci dobiveni su iz Bolničkog informacijskog sustava za 76 bolesnika operiranih od adenokarcinoma gušterače. U našem istraživanju broj CD86 makrofaga i omjer CD86/CD163 pokazali su statistički značajnu korelaciju s porastom T i N stadija bolesti. Broj CD163 makrofaga nije pokazao statistički značajnu korelaciju s navedenim varijablama. Ovi rezultati pokazuju da su u našim kliničkim uvjetima proupalni (M1) makrofagi bili izraženiji u lokalno uznapredovalim stadijima tumora s lošijim dugoročnim ishodima. Zaključno, TAM-ovi bi mogli biti valjan prognostički čimbenik ili čak meta za terapijska sredstva, ali bit će potrebno više studija kako bi se u potpunosti razumio utjecaj diferencijacije M1/M2 makrofaga.

Ključne riječi: *Adenokarcinom gušterače; M1/M2 makrofagi; CD68/CD163; Makrofagi povezani s tumorom; Istraživanje raka; Kirurška onkologija*