Jurnal Medical Aradean (Arad Medical Journal)
Vol. XX, issue 1, 2017, pp. 5-11

THE PROGNOSTIC ROLE OF MAST CELLS ACCORDING WITH THE HISTOLOGICAL GROWTH PATTERN IN LIVER METASTASIS OF TUMORS WITH DIGESTIVE ORIGINS

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ABSTRACT. Despite the significant progress regarding molecular classifications for colon, pancreatic and gastric cancer, the therapeutic methods (arterial, chimio and radioembolization), the life expectancy of patients with existing liver metastases did not improve significantly. The aim was to analyze the interrelation between the vascularization, mast cells and the histological growth pattern of hepatic metastasis with digestive origins. 25 patients were evaluated by ways of the immunohistochemical technique. The distribution of the liver metastasis growth pattern was as follows: colon (all 3 types), pancreatic (replacement), gastric (pushing type). A significant correlation between mast cell density in the peritumoral area and vascular microdensity in the metastatic domain of all histological growth patterns was found. Significant correlation between positive mast cells tryptase density and degree of differentiation for the desmoplastic type was noticed. The mast cells may have a prognostic role in this growth pattern and the mast cell inhibitor therapy may be useful.
KEYWORDS: mast cells, liver metastasis, histological growth pattern

INTRODUCTION

One of the most frequent places for metastasis from tumors originating in gastric, colorectal or the pancreas is the liver. Terayama and colleagues (1) and Vermeulen and colleagues (2) found different histological grow patterns in the tumor-liver interface of solid tumors. They described the following patterns: desmoplastic or encapsulated, pushing or expansive, replacement and mixed. At the moment of diagnosis almost 25% of pacients which had colorectal carcinoma also had liver metastasis and overall summing up with another 50%. In the case of gastric carcinoma diagnosis, liver metastasis occurred in 4-14% of pacients and in 50% of patients with pancreatic tumors. Research has proven the existence of a molecular classification for colon cancer, four molecular types of pancreatic cancer and a molecular development for gastric cancer. The use of arterial embolization therapy, chimioembolization and radioembolization has shown to have a favorable outcome for liver metastasis with originating tumors in the colon but unfortunately there is not enough data for other types of tumors. Also there is not enough precise data to clarify the correlation between the type of embolization and life expectancy. With progressing research in patients with primary gastro-intestinal tumors and liver metastasis there is yet a therapy to be discovered which expands the life expectancy semnificantly.

Mast cells derive from hematopoietic stem cells CD34+. These mast cells have a direct role in tumor invasion and progression and also an indirect role in habilitating the tumor's cell environment and modulating the immune system's response to the cancerous cells. (3)

With respect to gastric tumors which have a poor degree of differentiation, the antitumoral response mediated by the mast cells was absent, without a clear explanation being found for this phenomenon (4). Other studies have showed that in gastric cancers which have a poor degree of differentiation that the vascular micro density and the density of the mast cells were risen in comparison to previous stages (4). The correlations obtained by Ammendola (5) and colab. between angiogenesis and mast cell density in gastric tumors with bone metastasis was that in bone tissue the development of metastasis was favored by MCT+ mast cells which favored angiogenesis.

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It was also proven that an inflammatory infiltrate found adjacent to the tumor and containing a great number of mast cells represents an unfavorable prognostic in patients which are operated for liver metastasis with primary tumors of the colon. The number of mast cells found in the liver metastasis has been considered to be a predictive factor for the kind of therapy that should be applied and also for the overall prognostic of the patient with primary colorectal cancer (6). Giusca and colab. (7) have observed that hepatic metastasis originating from colon tumors presented MCT+ mast cells inside the primary tumors which can be significantly correlated with the tumor's grading and also lymph node invasion.

Very few data is available in literature pertaining to mast cells and angiogenesis in primary pancreatic tumors. The mast cells induce migration, proliferation and invasion of the malignant pancreatic cells. It was demonstrated that a reverse relationship exsits between the number of tumor cells, mast cell infiltration and the survival of patients (4).

Having the above starting points we propose to analyze the relationship between the vascularization and stromal cells but more significantly the mast cells and the histological growth patterns of hepatic metastasis with primary tumors of the stomach, colon and pancreas.

MATERIAL AND METHODS

The present study included 25 cases of patients evaluated at the Timisoara County Hospital between 2009- 2016. The hepatic metastasis had the following origins: 15 cases of colorectal adenocarcinoma, 7 cases of pancreatic adenocarcinoma and 3 cases of gastric adenocarcinoma. A signed consent form from each patient which was included in this study was obtained.

All procedures were performed according with references to the principles of the Declaration of Helsinki and were approved by the Institutional Review Board of „Victor Babeş" University of Medicine and Pharmacy Timişoara, Romania. The patients included in this study underwent surgical procedures by way of excisional tumorectomy of the liver metastasis. Metastatic fragments of 10x10x3 mm were washed in saline solution, fixed in 10% buffered formalin for 24 hours and paraffin embedded. 5 µm serial sections were performed and mounted on silanized slides. One slide from each case was stained using the haematoxylin and eosin method for histopathological evaluation and also for case selection for immunohistochemical procedures.

The heat-induced epitope retrieval was performed with Bond Epitope Retrieval Solution 2, a ready-to-use, pH 9.0 solution (Leica Biosystems, Newcastle Ltd, Newcastle UponTyne NE 12 8EW, U K.) for 20 minutes. Endogenous peroxidase blocking was realized with 3% hydrogen peroxide for 5 minutes. The following primary antibodies were used: CD34 (monoclonal, clone QBEnd/10 and mast cell tryptase (monoclonal, clone10D11). Both antibodies, ready to use, were from Leica Biosystems and Newcastle UponTyne, U.K. The incubation time was 30 minutes. The Bond Polymer Refine Detection System was used for visualizing the results. As chromogen 3, 3 diamino-benzidine dyhidrochloride was applied for 10 minutes and hemotoxylin for 5 minutes as a counterstain. The overall immunohistochemical procedure was done using Leica Bond- Max (Leica Biosystems, Newcastle uponTyne, UK) autostainer.

Immunoreactivity was estimated to be positive in endothelial cells and mast cells which had cytoplasmic expression. Counting of mast cells and blood vessels was based on the procedure published by Weidner N (8), at magnification ×200. From the tumor area three fields with maximum density of blood vessels were counted and the mean was the final result. Statistical analysis was performed by means of the commercially available SPSS17.0 used to observe the relationship between the micro vascular density (MVD) and histological growth pattern with $p<0.05$ and being considered as significant. Microscopic observation and image processing was performed with Axiocam 506 color, Zeiss, Jena, Germany.

RESULTS

The liver metastasis, which derived from the colon and had a desmoplazic growth pattern were characterized by a large number of mast cells found in the portal spaces of the remaining liver. There were noted values between 18 and 36 mast cells per portal space (fig 1). Also we observed enlarged dimensions of the mast cells and predominantly degranulated cells found in weakened stages of differentiation.

Inside the metastasis area we found a lowered number of mast cells in comparison to the area adjacent to the tumor, these numbers varying from 2.73 to 16. A higher concentration of mast cells was found inside the

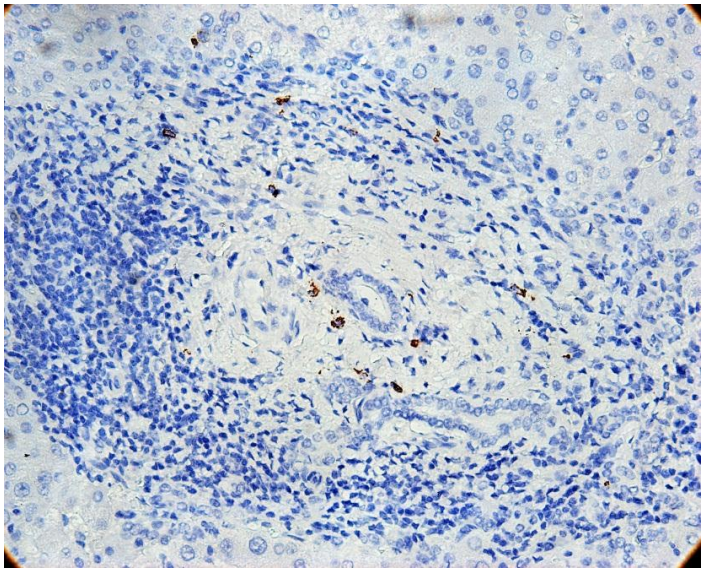

Figure 1-Liver metastasis originating from adenocarcinoma of the colon, desmoplastic growth pattern, immunoreaction for tryptase of the mast cell, located in the portal space, ob. X40 metastasis area which was correlated to the degree of differentiation. In one of the cases which had a degree of differentiation of G1 we found no mast cells inside the metastasis area and the second G1 cases we found a mean of 1.33. The cases, which has a moderate degree of differentiation the mean value was found to be 2. Those cases which had a degree of differentiation of G3 had the highest amount of mast cells in the metastasis area, having a mean value of 9.33 (fig. 2).

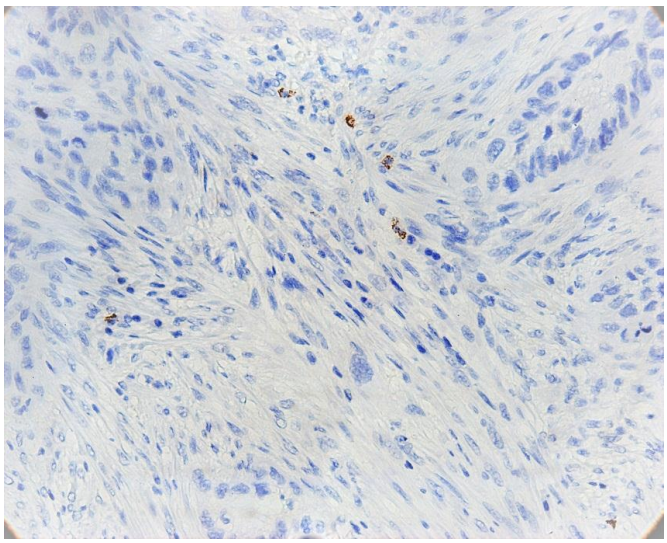

Figure 2-Liver metastasis originating from adenocarcinoma of the colon, poor degree of differentiation desmoplastic growth pattern, mast cells present in the metastasis, immunoreaction for tryptase of the mast cell, ob. X40

The transition zone between the remaining liver and the metastasis was found to be the site where the most mast cells were found.

The replacement growth pattern of the liver metastasis which originated from the colon, were characterized by a high density of mast cells found in places adjacent to the area of metastasis with a mean value of 20 in comparison to the area of metastasis which had a mean value of 3.33. Above all, we observed numerous mast cells in the portal spaces, with a mean value of 30. (fig. 3)

The compression growth pattern showed the least number of mast cells, not only at the transition zone between the remaining liver and the metastasis but also inside the metastasis area. The same number of reduced mast cells was also found in the portal spaces, with a mean value which did not exceed 5 mast cells/portal space (fig. 4). The study showed a mean value of 5 pertaining to the density of mast cells in

spaces adjacent to the metastasis area and a mean value of 1.99 inside the metastasis area.

The cases in which the liver metastasis originated from the pancreas were characterized by a replacement growth pattern. The density of the mast cells inside the area of metastasis had a mean value of 2.85 (Fig. 5). In the spaces adjacent to the area of metastasis the mean value of the density of the

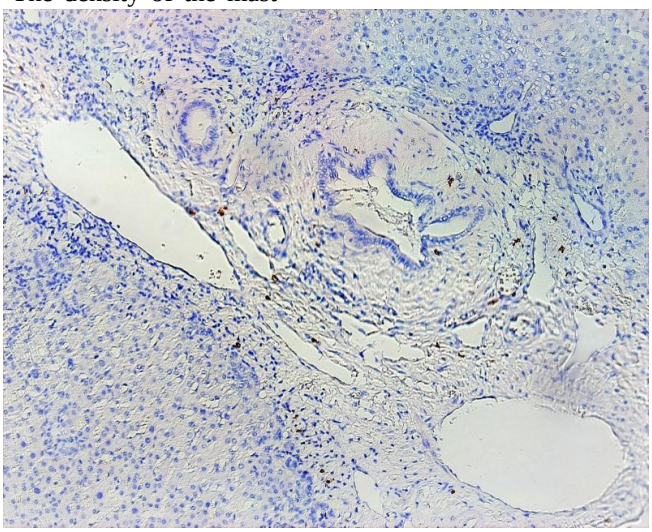

Figure 3-Liver metastasis originating from adenocarcinoma of the colon, poor degree of differentiation, replacement growth pattern, mast cells present in the portal space, immunoreaction for tryptase of the mast cell, ob. X20

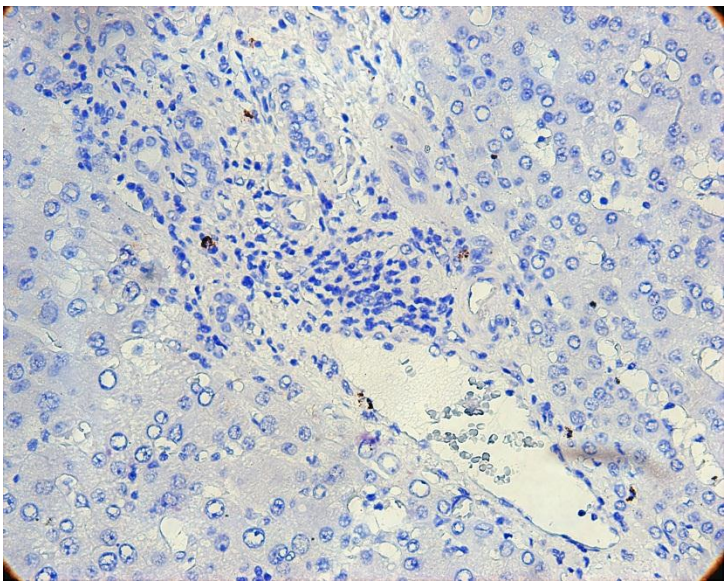

Figure 4-Liver metastasis originating from adenocarcinoma of the colon, moderate degree of differentiation, compression growth pattern, very few mast cells present in the portal space, immunoreaction for tryptase of the mast cell, ob. X40

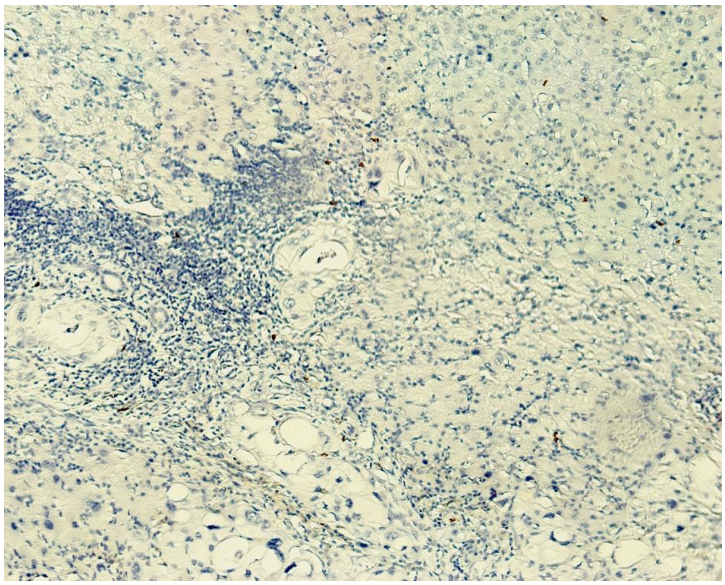

Figure 5-Liver metastasis originating from adenocarcinoma of the pancreas, moderate degree of differentiation, replacement growth pattern, immunoreaction for tryptase of the mast cell, ob. X20

mast cells was 10.8. Greater values of the mean density of the mast cells was found in cases with a poor degree of differentiation in comparison to cases which had a moderate degree of differentiation.

The liver metastasis which had a gastric origin presented a compression growth pattern and had a moderate degree of differentiation. These cases had a mean value of mast cells inside the metastasis area of 2.66. The spaces adjacent to the metastasis area had a mean value of 2.66 mast cells and a reduced number of mast cells was found in the portal spaces.

We found no correlations between the density of the mast cells in the metastasis area and the vascular micro density in liver metastasis with pancreatic origin, p=0,309 and colonic origin, p= 0.555. Also no significant correlations were observed between the density of the mast cells and the vascular micro density inside the metastasis area for histological growth patterns: compression p=0,0113; replacement p= 0,845; desmoplastic p= 0,915. The value of p for the total density of the mast cells and the vascular micro density inside the metastasis area was 0,860.

A significant correlation was observed between the density of the mast cells outside the metastasis area and the vascular micro density inside the area of metastasis, p= 0.022.

There were no significant correlations found between density of the mast cells inside the area of metastasis per total and the degree of differentiation p= 0,061, for the replacement growth pattern p= 0.328, for the compression growth pattern p= 0,772.

Another significant correlation with p= 0,043 was found for the desmoplastic histological growth pattern, between the density of the mast cells inside the metastasis area and the degree of differentiation.

There was no significant correlation found between the mast cell density inside the metastasis area, the mast cell density outside the metastasis area and the age of the patient, p=0.644 and p=0,268.

Also, no correlation was proven between the mast cell density inside the metastasis area and its vicinity in comparison to the gender of the patients, p=0,685 respectively p=0.845.

Last but not least, no correlation was concluded between the degree of histological growth and the mast cell density in the area of metastasis and its vicinity, p=0,467 and p=0,814.

DISCUSSION

It has been demonstrated that an increased peritumoral infiltration with a high number of mast cells is an unfavourable prognostic factor in patients with hepatic metastasis with a tumor originating in the colon. The number of mast cells in metastatic lesions was considered important as a predictive factor for the prognosis and therapy of patients with colorectal cancer and liver metastases (6). In our study, we noticed the existence of a significant correlation between the mast cell density in the vicinity of the metastatic area and intratumoral angiogenesis, a possible indicator of tumor aggressiveness and a predicted prognosis.

Giuşcă et al. (7) observed that hepatic metastasis of colonic origin showed MCT + intratumoral mast cells significantly correlated with tumor grade and lymph node invasion. In our study, the mast cell density within the metastasis area evaluated by immunohistochemical expression of mastocytopathic tryptase (MCT) for tumors originating in the colon was significantly correlated with the degree of differentiation for hepatic metastases with a

desmoplastic growth pattern, and not for models of compression and replacement type. Also, the total number of mast cells within the metastasis did not correlate with the degree of differentiation.

Van den Eynden (9) et al. have demonstrated that liver metastasis of colorectal carcinomas with replacement growth model are non-angiogenic compared to compression-type growth-enhancing liver metastases that are heavily angiogenic, by way of hypoxic mechanism. The compression growth model was the only independent prediction factor at 2 years. This model was characterized by aggressive tumor behavior, being the only independent prognostic factor for a survival of 2 years. In our study we noticed that the colorectal metastasis compression growth pattern was associated with the lowest values of CD34 positive vessels in the vicinity of the portal spaces, between hepatic sinusoids and elevated vascular micro density in the metastatic domain. Mast cell density has shown minimal values for this growth model, both in the vicinity of the portal spaces and in the metastasis area. The values of mast cell density in the metastasis area were significantly increased for colorectal tumors compared to gastric tumors within the same growth model. These observations may suggest the possibility of reduced involvement of mast cells in the angiogenic mechanisms of this growth model.

The significant correlation observed in our study between mast cell density and the degree of differentiation in colorectal liver metastases and desmoplastic growth pattern characterized by the presence of a conjugate tissue band at the invasion area may draw attention to the fact that micro medium rich in collagen directs epithelial cells towards a mezenchimal phenotype in which cells can proliferate and migrate in an inappropriate manner. Chronic inflammation favors fibrosis that induces increased pressure in liver sinuses and induces increased fibrosis. A middle inflammatory micro medium also affects liver function and possibly chemoresponsiveness of tumor cells by altering its metabolism. Theoretically, an increased proliferative fraction increases chemoresponsiveness, while an inflammatory medium can promote therapeutic resistance (10). Peritumoral MCT + and CD117 + mast cells were significantly correlated with overall survival. No significant correlations were found between MCT and CD 117 positive mast cells and clinical-pathological parameters and survival for gastric metastasis (7).

The correlations obtained by Ammendola (5) et al. between angiogenesis and mast cell density in bone metastasis with a gastric entry point support the idea that bone metastasis is favored by MCT + mast cells, which accentuate angiogenesis. In bone tissue, mast cells can be activated by stem cell factors, the c-kit receptor ligand and other growth factors: vascular endothelial growth factor-VEGF, fibroblast growth factor-FGF, thymidine phosphorylase (TP) and by tumoral gastric cells (11-15). The mast cells contain several factors with proangiogenic properties: mast cell tryptase, VEGF, FGF, IL-8, TNF alpha (16, 17). Tryptase is the most abundant of these and has a mitogenic effect demonstrated on endothelial cells and gastric tumors. It is one of the most potent mediators of angiogenesis released by mast cells and may play an angiogenic role through several mechanisms (18).

Blair et al. (18) demonstrated the direct binding of mastocytase tryptase to micro vascularization in endothelial cells grown in matrigel, resulting in a significant increase in capillaries inhibited by specific tryptase inhibitors. Dose-induced endothelial cell proliferation was also observed. The same aspects have been observed by Ribatti et al. (19), using an experimental model in vivo, on the corioalantoid membrane of the embryonated chick egg. Mastocytosis is an activated protease 2 receptor agonist present on the surface of endothelial cells and gastric tumors (20,15). Activation of this receptor favors cell proliferation and IL-6 release, with a stimulating role in angiogenesis. All of these observations support the involvement of positive tryptase mast cells in the angiogenesis of bone metastases with a gastric starting point but did not correlate with the main clinical-pathological parameters. These observations support the importance of using inhibitors of mast cell tryptase in the treatment of patients with primary gastric tumors and bone metastases.

In liver metastases of gastric origin and pushing growth pattern, in our study we noticed a small number of positive MCT mast cells both in the metastatic area and in the vicinity of the portal spaces, suggesting either their low involvement in the aggressiveness of this growth model or the involvement of other types of mast cells.

The replacement growth pattern was characterized in our study by the second value for mast cell density in the metastatic area following by the corresponding value of the desmoplastic model, both for metastasis of colorectal origin and those of pancreatic origin.

Data from the literature have demonstrated the reduction of the growth and survival of pancreatic and endothelial cells in the in vivo experimental model in the corioalantoid membrane of the embryo egg, when a mast cell degranulation inhibitor, Cromolyn (21), is applied.

CONCLUSIONS

The positive mast cell tryptase may have a prognostic role in the desmoplastic growth pattern of liver metastasis with colonic origin. The mast cell inhibitors therapy may be useful in liver metastases with this type of histological growth pattern.

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