Tryptase-positive and CD117 Positive Mast Cells Correlate with Survival in Patients with Liver Metastasis

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Abstract. *The prognostic value of mast cells (MCs) in patients with liver metastases is a relatively new topic. The present study comparatively assessed tryptase-positive (MCT^+) and $CD117^+$ MCs in liver metastases from various sites and correlated their expression with clinicopathological prognostic factors and survival. Our data pointed to differences in MCT and CD117 expression in liver metastases that seem to be related to the origin of the primary tumor. For colon cancer metastases, intra-tumor MCT^+ MCs were significantly correlated with tumor grade and nodal status, while peritumoral MCT^+ MCs and peritumoral $CD117^+$ MCs were significantly correlated with overall survival. No significant correlations between MCT^+ and $CD117^+$ MC number and clinicopathological parameters or survival were found for gastric cancer metastases. To the best of our knowledge, this is the first report regarding MC involvement in liver metastases from different malignant tumors correlated with clinicopathological parameters and overall survival. Different mast cell phenotype together with their specific correlation with tumor grade, nodal status and survival suggest their involvement in the metastatic process in a specific manner related to tumor origin. Mast cells from liver metastases remain a questionable issue regarding their origin, pathogenic role and their ability to be potential targets for adjuvant therapy.*

The molecular and functional heterogeneity of mast cells (MCs) has led to several controversies regarding their role in malignant disease. These cells are extensively studied mainly in relation to tumor angiogenesis (1-3) and, to a lesser extent, to tumor lymphangiogenesis (4, 5), but their function in these processes is not yet fully-understood.

The heterogeneity regarding mediators released in the presence of tumor not only influences endothelial cells but also other components of the tumor microenvironment, from malignant cells to other connective tissue cells. It seems that their different origin and molecular markers make them behave in a different manner in accordance with the particularities of normal or malignant tissues. The differences between the distribution of MCs in normal tissues possibly influence their number and role in malignant tissues. In a healthy condition, a high number of MCs characterize to breast and colon, while a low number is present in brain and thymus, probably due to their particular functions (6). These variations are maintained in the development of malignant tissues, thus the mainstream publications report a well-represented MC population in some tumor types, and a low one for others (7).

The most studied MC markers in tumor are MC tryptase (MCT), followed by CD117 and MC chymase.

Although many studies focused on the role of MCs in primary tumor (8-10), few data refer to MC contribution to the pathogenesis of nodal or distant corresponding metastases (11, 12).

In this context, the involvement of MCs in liver metastases (LM) is a relatively new topic – to the best of our knowledge fewer than 20 articles regarding this issue have been published at the moment. Consequently, the MC profile in different types of LM is incompletely characterized (13, 14), and information on the prognostic role of MCs or their influence on survival rate in patients with LM with different origins is scarce (15, 16).

Based on the above mentioned evidence, our study aimed to comparatively assess tryptase-positive and $CD117^+$ mast cells in LM originating from different sites and to correlate their expression with clinicopathological prognostic factors and survival.

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Materials and Methods

Patient data. Seventy-one patients diagnosed with LM at the Department of Pathology, St. Spiridon University Hospital, Iaşi, Romania, between January 2009 and December 2011, were enrolled in our study: 39 cases with colorectal cancer origin, 17 cases with gastric cancer origin, and 15 cases with pancreatic cancer origin. From a total of 71 patients, 40 patients had metachronous metastases, and had received conventional chemotherapy and radiotherapy specific for each type of primary tumor. Patients were followed-up between January 1st 2009 and January 31 2013; at the end of the follow-up period, 15 patients were still alive. All patients' deaths were related to cancer. The overall survival ranged between 1 and 46 months.

Tissue preparation and processing. Tissue specimens, collected by open surgery, were fixed in 10% buffered formalin for 48 hours followed by paraffin embedding. Three-micrometer-thick serial sections were obtained from each paraffin block. One section was stained with routine hematoxylin and eosin method for histopathological assessment, the other corresponding sections from each case were selected for immunohistochemistry.

Immunohistochemistry. Immunohistochemistry was performed to highlight MCs by using two types of antibodies to: MCT (monoclonal mouse anti-human, clone 10D11; Novocastra, Newcastle, UK) and CD117 (polyclonal rabbit antibody to human c-KIT; DAKO, Carpinteria, CA, USA). All immunohistochemical procedures were performed in an automatic manner by using Max Bond Autostainer (Leica Microsystems, Newcastle, UK) which worked with Bond Polymer Refine Detection system. The slides were mounted in a permanent medium and microscopically assessed.

Quantitative assessment. The cases were evaluated by three well-trained pathologists. The quantification of MCT^+ MCs and c-KIT-positive MCs ($CD117^+$ MCs) was performed inside the adjacent normal liver tissue in the peritumoral area and inside the metastatic tumor tissue. MCs were counted by choosing three areas with the highest MC density and the final value was obtained by the arithmetic mean of the values obtained in the three chosen microscopic fields (×200 magnification). The assessment of MCs was carried out using Zeiss AxioZoom ImagerA2 microscope,

Statistical analysis. MC values were correlated with the clinicopathological parameters according to TNM staging system (17) and the survival data. These procedures were carried out globally and specifically for each type of LM. Statistical analysis was performed using non-parametric test Kruskal–Wallis, and Kaplan–Meier method and log-rank test to analyze survival (SPSS software version 19, SPSS Inc., Chicago, USA). The median values of MCT^+ MCs or $CD117^+$ MCs in respective territories (intratumor and peritumor) were used as thresholds for survival analysis. Statistically significant correlation was considered for a *p*-value of less than 0.05.

Results

From a total of 71 patients enrolled in our study, 33 were females and 38 were males. All descriptive statistic data on patients are summarized in Table I.

Table I. *Clinicopathological characteristics of the patients*.

Clinicopathological characteristic	Variable description	Cases	
		Number	%
Age, years	Mean±SD	64.17±11.74	
	Median, range	65 (33-86)	
Gender			
Female	33	46.5	
Male	38	53.5	
Tumor stage			
Stage IV	TxNxM1	71	100
T			
T2		16	22.5
T3		40	56.3
T4		15	21.1
N			
N0		8	11.3
N1		34	47.9
N2		25	35.2
N3		4	5.6
Histological grade			
G1	Well-differentiated	7	9.9
G2	Moderately-differentiated	37	52.1
G3	Poorly-differentiated	27	38.0
Origin			
Colon	27	38.0	
Rectum	12	16.9	
Stomach	17	23.9	
Pancreas	15	21.1	

Overall assessment of cases. As a general picture, the MCT^+ MC population was better represented in intratumoral areas (disseminated between the tumor cells, regardless of their histoarchitectural pattern defined by the primary tumor origin and the degree of differentiation, and also within the connective tissue that formed the tumoral stroma) than in the peritumoral areas. The $CD117^+$ MC population presented a comparable distribution in both intratumoral and peritumoral territories.

For all cases which received radiotherapy/chemotherapy before surgery, we observed a high number of MCT^+ MCs, usually associated with a high inflammatory infiltrate.

Normal liver tissue adjacent to metastases contained MCs positive for both tryptase and CD117, but their distribution was different according to their phenotype. MCT^+ MCs were restricted to the connective tissue of portal spaces and at the border between healthy liver tissue and metastases, being observed in a high number in areas where the inflammatory infiltrate was present. Scattered MCT^+ MCs were distributed between hepatocyte cords in perisinusoidal area.

$CD117^+$ MCs had a different distribution compared to MCT^+ MCs in quasi-adjacent normal tissue. They were not present inside the connective tissue of normal portal spaces but they were identified in those portal spaces with a high

Table II. *Comparative differential count overview of tryptase- and CD177-positive mast cells in liver metastasis with different origin. It is noticed the highest value of mast cells density for liver metastasis derived from a primary colon cancer while the lowest was registered for liver metastasis with pancreatic origin.*

Liver metastases	MC count, mean±SD (median)			
	MCT+		CD117+	
	Intratumor	Peritumor	Intratumor	Peritumor
General assessment	13.7±17.0 (7.6)	12.1±13.2 (8.0)	11.4±15.6 (7.0)	11.0±11.4 (8.3)
Colorectal origin	16.9±18.8 (9.7)	15.2±16.3 (9.0)	13.2±18.8 (8.0)	13.7±14.1 (9.0)
Stomach origin	14.2±17.3 (8.3)	11.3±7.0 (11.3)	12.2±11.3 (7.6)	12.3±6.0 (11.6)
Pancreatic origin	4.9±6.0 (3.6)	5.3±5.4 (3.6)	6.2±8.7 (3.6)	3.8±3.1 (4.6)

inflammatory infiltrate. The number of CD117+ MCs increased with the amount of inflammatory infiltrate inside portal spaces from adjacent liver tissue. The MCT+ MC number was higher than that of CD117+ MCs from the same area (Table II).

For the whole group, the statistical analysis of the potential of MCT+ and CD117+ MCs to influence prognosis and survival revealed significant correlations for both MC types. Peritumoral MCT+ MCs and CD117+ MCs were correlated with overall survival (p=0.025 and p=0.042, respectively) (Figures 1 and 2), while intra-tumoral MCT+ and CD117+ MCs were significantly correlated with lymph node metastases (p=0.042 and p=0.043, respectively).

We also noted for intra-tumoral MCT+ and peritumoral CD117+ MCs a significant correlation with origin of metastases (p=0.001 and p=0.0002, respectively), and thus we continued with evaluation of MCT+ and CD117+ MCs separately for each type of LM.

MC characteristics in LM from colon and rectal cancer. The qualitative assessment of MCs in metastases from colon and rectal cancer revealed some particularities. Within the intratumoral areas, MCT+ MCs were distributed between tumoral cells and had a tendency to be grouped close to intra-tumoral blood vessels.

In cases with metastases from colon cancer, we noted a markedly increased number of MCs between the hepatocyte cords adjacent to the tumor nodules (areas) than in more distant areas. At the periphery of the metastatic nodules, MCT+ MCs tended to be de-granulated compared to those from the inner part of the tumor, which inhibited a non-de-granulated state. Moreover, in the peritumoral area, the accumulation of MCT+ MCs was observed in association with inflammatory infiltrate and also around large, congestive vascular spaces.

For the metastases from rectal cancer, the distribution of MCs in the peritumoral area had a special pattern, not only around the blood vessels but also penetrating inside their wall. In quasi-normal hepatic tissue, the MCT+ MCs were present inside the portal spaces mainly, without any preference for grouping around vascular or ductal components. Scattered isolated MCs were also observed outside the portal spaces between hepatocytes. Most MCT+ MCs were not degranulated – an aspect common to both intratumoral and peritumoral areas.

It is worth mentioning that for all metastases with colorectal origin, the number of intra-tumoral MCT+ MCs was higher in the cases corresponding to poorly-differentiated primary tumors than in those with moderate and well-differentiated histological grade. CD117+ MCs had a similar qualitative distribution to MCT+ MCs.

Although MCT+ and CD117+ MCs presented a high numerical variability between the 39 cases, the mean values revealed a quite similar density in both peritumoral and intratumoral areas for each MC type, but there were fewer CD117+ MCs as compared to MCT+ MCs (Table II).

The statistical analysis pointed out several correlations between MCT+ and CD117+ MCs and clinicopathological parameters and survival. Intratumor MCT+ MCs were significantly correlated with tumor grade (p=0.025) and nodal dissemination (p=0.02), while peritumoral MCT+ MCs and peritumoral CD117+ MCs were significantly correlated with overall survival (p=0.001 and p=0.035, respectively) (Figures 3-5).

MC characteristics in LM from gastric cancer. From the qualitative point of view, the distribution of both types of MC generally respected the already described pattern. Nevertheless, an unexpected aspect was observed in six cases – namely a peculiar positivity (membraneous and cytoplasmic) of the tumor cells for CD117, with CD117+ MCs present in a very small number and only in the stromal component of tumor nodules, or completely absent.

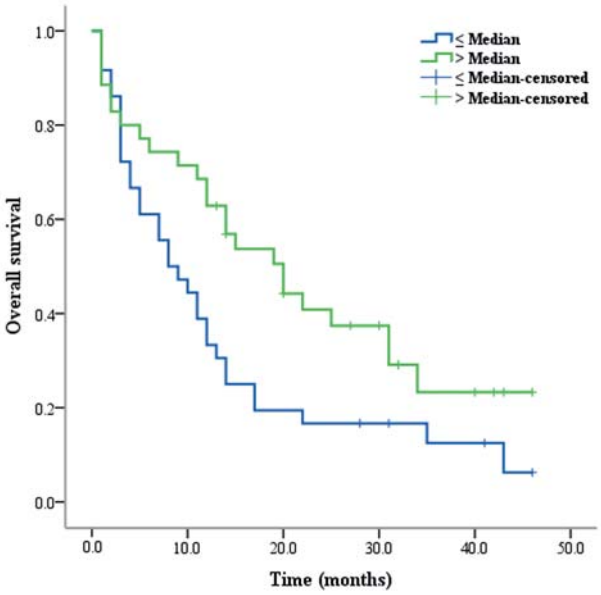

Figure 1. *Kaplan–Meier curves showing the prognostic value of peritumoral MCT+ MCs in LM (p=0.025). Cut-off at a median MC count of 8.*

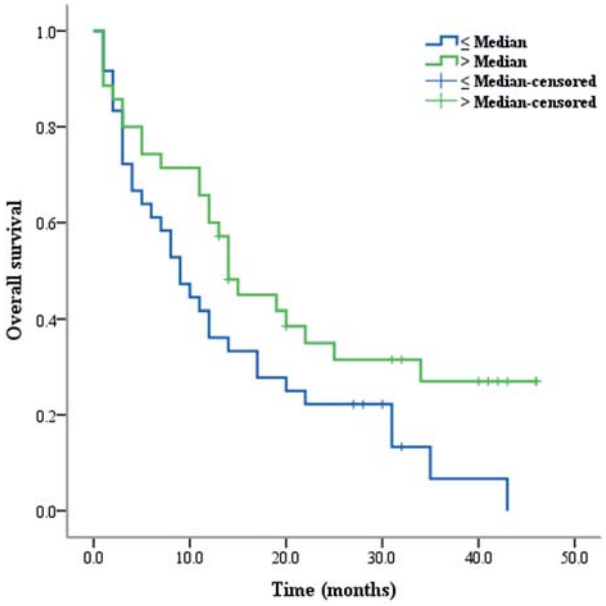

Figure 2. *Kaplan–Meier curves showing the prognostic value of peritumoral CD117+ MCs in LM (p=0.042). Cut-off at a median MC count of 8.3.*

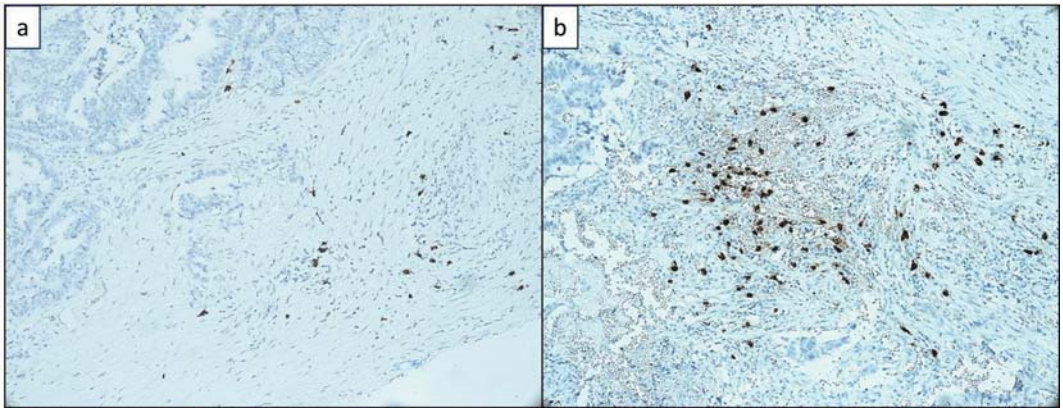

Figure 3. *Comparative assessment of CD117+ (a) and MCT+ mast cells (b) in the peritumoral area from LM of colorectal origin.*

For the 17 cases of LM from gastric cancer, the mean number of intra-tumoral MCT+ MCs was higher than the peritumoral ones, whereas those of intra-tumoral and peritumoral CD117+ MCs were almost equal (Table II).

The statistical analysis showed no significant correlations between MCT+ and CD117+ MC number and clinicopathological parameters or survival.

We also analyzed the association between CD117 expression in tumor cells (considered a possible influence of c-KIT expression) with the above mentioned parameters but obtained no statistical significant correlations.

MC characteristics in LM from pancreatic cancer. From the qualitative point of view, the distribution of both types of MC generally respected the already described pattern.

The quantitative assessment of the 15 cases of LM from pancreatic cancer revealed, for both MCT+ and CD117+ MCs, mean numbers lower than in LM of colon, rectal and

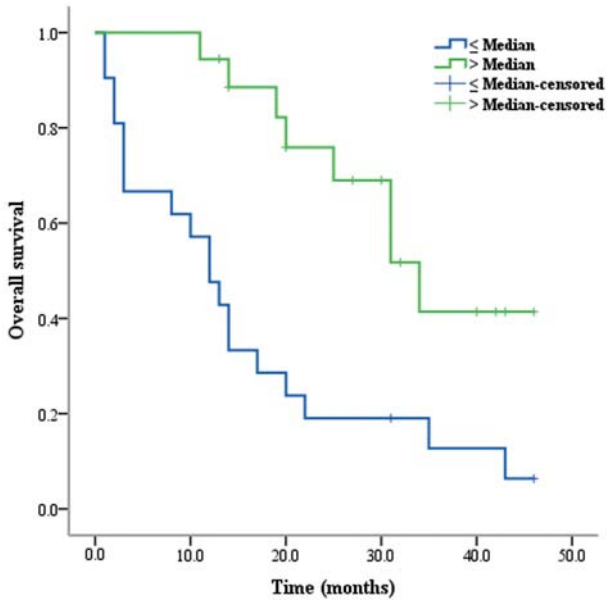

Figure 4. *Kaplan–Meier curves showing the prognostic value of peritumoral MCT+ MCs in LM of colorectal origin (p=0.001). Cut-off at a median MC count of 9.*

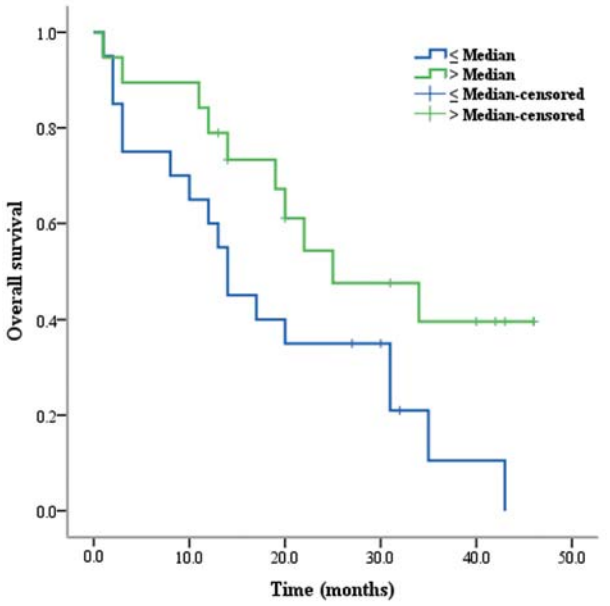

Figure 5. *Kaplan–Meier curves showing the prognostic value of peritumoral CD117+ MCs in LM of colorectal origin (p=0.035). Cut-off at a median MC count of 9.*

gastric origin. For MCT+ MCs, the number was almost equal in intratumoral and peritumoral areas, while for CD117+ MCs, the intratumoral number was approximately twice the peritumoral (Table II).

Statistical analysis showed significant differences only for peritumoral MCT+ MCs and overall survival (p=0.018) (Figure 6).

Discussion

LM represent a challenge for cancer therapy of various malignant tumors. It has been shown that for some malignancies, metastatic tumor cells change their molecular profile and become resistant to conventional chemotherapy, radiotherapy or adjuvant targeted therapies (18, 19). The seed and soil hypothesis of cancer metastasis characterizes not only the ability of tumor cells to proliferate and form tumor masses in sites other than those of their origin but also the acquisition of a proper microenvironment for this process. Usually, the connective tissue that represents the metastatic stroma looks microscopically similar to that from the site of origin of the primary tumor and contains similar cellular components.

One of the controversial cells of tumor stroma of primary and metastatic sites is the MC. Known as cells with a heterogeneous content of bioactive compounds, MCs are intensely studied as targets for therapy of allergic diseases and other inflammatory conditions (20). MCT and CD117 are the most frequently used markers to identify MCs in normal and

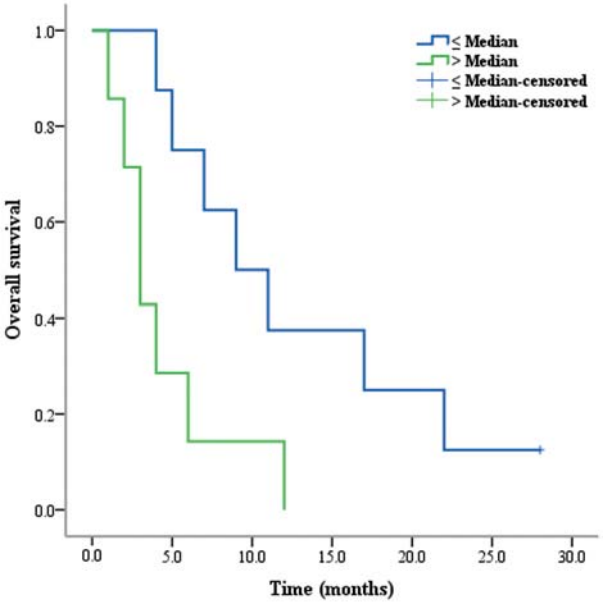

Figure 6. *Kaplan–Meier curves showing the prognostic value of peritumoral MCT+ MCs in LM of pancreatic origin (p=0.018). Cut-off at a median MC count of 3.6.*

pathological tissues. They are strongly inter-connected because tryptase release is conditioned by the activation of CD117 (21). The MC profile in the normal liver tissue microenvironment was studied in relation to the aging process

(22) and liver innervation (23), the results showing the relationship between the released mediators and the immunosenescence process and nerve fibers, respectively.

If for the primary tumors MCs are extensively used as parameters to assess tumor angiogenesis, lymphangiogenesis, prognostic or therapeutic impact (24), their role in tumor metastases with different origin is less known.

Few data are available about MC involvement in lymph nodes (21), bone (1) and LM (15, 16), and their impact on prognosis and survival is not yet stated. Supplementary indirect evidence support the role of the MCs located in the normal mucosa adjacent to colon cancer areas in carcinogenesis and development of LM (25).

No comparative assessment of MCs in different LM was recently published in the literature, moreover, no data about influence of MCs in LM on survival and prognosis were reported.

All the above mentioned data strongly motivated the comparative study of MCs in LM originating from different primary malignant tumors. Our results support a complex role for MCs in tumor cell metastasis, probably though the local immunological response of the hepatic microenvironment to the metastatic invasion process.

Our data point-out the differences in MCT and CD117 MC distribution in LM that seems to be related to the primary site of origin. We underline the dissimilarities between the MC frequency in colorectal and gastric LM (high number, quite equal) and pancreatic LM (low number). Moreover, the results obtained in LM of pancreatic origin opens interesting perspectives in the study of corresponding primary tumors, because the number of MCs counted in these cases was very low as compared to the number reported (relatively recently) in pancreatic cancer (26).

The potential prognostic value of MCs was demonstrated by the significant correlation between intratumoral MCT^+ MC and $CD117^+$ MC counts and the lymph nodes involvement (N parameter from TMN staging of the primary tumors) in the whole group and also for colorectal LM. These results can be interpreted as a strengthening of the data reported by Xia and coworkers (25) showing the relationship between MCs in the adjacent normal tissue of primary colon cancer and the metastatic process.

Furthermore, our data support the fact that LM are strongly correlated with lymph node metastases at the time of diagnosis of primary tumor and that MCs could be the promoters of local and distant metastases.

Nevertheless, we consider the most valuable results to be the significant correlations registered between the peritumoral MC count and survival in patients with LM from colorectal and pancreatic cancer. Consequently, our comparative assessment of MCT^+ and $CD117^+$ MCs in LM, correlated with their known functional interrelation and integrated with the clinicopathological parameters and survival data may help appreciate the active state of metastatic development. Our observation of CD117 positivity not only in MCs but also in tumor cells could suggest the possibility of a novel therapeutic approach with dual targets: metastatic tumor cells and MCs.

The significant correlations between both MCT^+ and $CD117^+$ MC counts from peritumoral areas with overall survival on the one hand, and the association of a high number of MCs with a rich inflammatory infiltrate around metastases on the other hand can be interpreted as solid arguments for the activation of a local immunological defense, strongly involved in tumor behavior and subsequently in the patient's survival.

Within the framework of our discussion on the MC profile in LM, a final remark on a controversial issue, namely their origin, is compulsory. Our data support there being a dual origin. Firstly, the significant correlation between the intratumoral MCT^+ MC count and the different origins of LM analyzed in the present study give rise to the possibility that these MCs come from the primary tumor tissue itself and, probably, travel to the metastatic sites by a mechanism not yet well-known. Secondly, the peritumoral aggregation of MCs parallel to their presence in the portal spaces in adjacent normal liver tissue, and their presence within the inflammatory infiltrate suggest the concomitant existence of resident MCs, or MCs derived from bone marrow

To the best of our knowledge, this is the first report regarding MC involvement in LM from different malignant tumors with accent on their correlation with clinicopathological parameters and on the involvement in overall survival.

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