EVALUATION OF THE ROLE
OF THE EPITHELIAL AND
STROMAL COMPONENTS ON
THE BEHAVIOR OF
GASTRIC CARCINOMA

PhD THESIS
- abstract -
INTRODUCTION

Gastric cancer is one of the most powerful cancers worldwide with a multifactorial determinism, with a development and distribution which is also influenced by a wide spectrum of influencing factors, and a polymorphic clinicomorphological profile, all ultimately leading, to a very low life expectancy from detection. One reason for this is the very reserved prognosis of advanced forms, with survival rates below 23% and rarely above 15% [1, 2]. Results of the treatments applied are still unsatisfactory. One of the major difficulties in diagnosing and establishing effective therapeutic strategies is the lack of a consistent number of indicators for the clinical behavior [3].

Tumor stroma is not only a passive spectator but rather an active participant in the processes of cancer initiation, progression and metastasis, being able, as suggested by some authors, to be a promising therapeutic target. Although in the international scientific world many research groups have focused on tumor stroma, there are still few studies on gastric carcinoma stroma, lacking almost completely in our country.

BACKGROUND

Gastric Carcinoma. Gastric cancer is one of the most common and lethal malignancies worldwide, especially in East Asia including China, the 5 year survival rate being only 20% [4]. It is a multifactorial disease. The marked geographical variability, time course and effect of migration on gastric cancer incidence suggest that environmental factors and those related to lifestyle play an important role in the etiology of the disease. There are also differences regarding genetic susceptibility, pathologic profile, clinical manifestations and prognosis. The differences observed between the different locations of gastric cancer even suggest that they are distinct disorders with different etiologies [5].

Gastric carcinoma shows considerable variation in the histological pattern and degree of differentiation. Morphological heterogeneity of gastric carcinomas both between different tumors but even within the same tumor led to a large number of attempts to set up a classification system to include those aspects of tumor morphology that have prognostic significance. But the fact that there are numerous histological classifications of gastric adenocarcinoma suggests that none of them is entirely satisfactory [6]. The most appropriate predictor for the prognosis of gastric carcinoma remains the TNM system [7, 8].

The changing incidence with the transfer from distal forms to proximal ones, with a more aggressive phenotype, and the inability to detect early forms, limited in the past 20 years the opportunities for significant improvement in survival [9]. Individual chances of a patient surviving 5 years after diagnosis are very small [10, 11, 12].

Tumor stroma. The tumor is more than some groups solitary transformed cells. Epithelial tumor cells can grow only in a deviated microenvironment, consisting of altered extracellular matrix and many unprocessed cells that play a role in the initiation and progression of neoplasms [13, 14, 15, 16]. Tumor stroma is defined as all modified mesenchymal structures supporting cancerous tissue. As a support structure and nourishing system, the stroma accompanies any neoplastic proliferation, although it is much better distributed in carcinomas than sarcomas where it is identified with weight. It represents the reaction of the connective tissue suffering various changes when in contact with tumor parenchyma and therefore includes newly formed but nontumoral elements [17, 18].
Depending on the expression of CD34, CD31, α smooth muscle actin and high molecular weight caldesmon, in the gastrointestinal tract, in normal and pathological conditions, 5 different immunophenotypes of stromal cells can be identified, other than inflammatory cells and interstitial cells of Cajal. Angiogenesis expressed as mean vascular density is closely correlated with metastasis and a poor prognosis of gastric cancer so that DVM and lymph nodemetastasis are independent prognostic factors for gastric cancer patients [3].

PERSONAL CONTRIBUTION

MATERIAL AND METHODS

The basis for this study was originally composed of a group of 59 patients admitted and operated in the Surgical Clinics of the Emergency County Hospital and CFR University Hospital of Craiova between 2003 and 2008 in which the postoperative histopathological examination established the diagnosis of gastric carcinoma. The inclusion criteria for patients in groups and subgroups were: existence of surgical intervention, histopathologic diagnosis of gastric carcinoma. From the initial batch three study groups were individualized: Group 1a, consisting of 43 patients in whom histopathological examination revealed within the tumor mass the existence of a single morphological aspect accordin to the WHO classification [19]. Group 1b, consisting of 16 patients in whom histopathology revealed, within the tumor mass, two areas with different morphological classification according to the WHO. Group 2, consisting of 75 tumor areas. The study material was represented by two types of data sources. The first category were the medical records of patients in the study namely: clinical observation sheets, surgery protocols, histopathologic diagnosis registers. The second category included: surgical excision samples from cases operated during the study, paraffin blocks from cases operated before the begining of the study, and histological preparations obtained from all cases included in study.

The study was both retrospective and prospective, and was divided into two chapters: the study of the clinico-morphological profile, and the study of correlations between clinical and morphological parameters. Assessed parameters. "Database"-type files were created in the computer in which all parameters considered were placed. They were divided into: Clinical parameters: Gender, Age; Morphological parameters: lesion location on the surgical excision sample, macroscopic appearance of the lesion on the surgical excision sample, microscopic appearance of the surgical excision sample (WHO classification, Lauren classification, Goseki classification, Carneiro classification), degree of tumor extension (invasion of gastric wall assessed on the surgical excision sample, lymph node invasion, distant dissemination, TNM stage), degree of aggressiveness of the tumor (determination of the proliferation marker Ki67, and p53); Evaluation of the tumor stromal component, fibrillary component; Parameters for assessing the specific cell component, intratumoral vascular density assessment. Preliminary data on clinical parameters and the morphological measures were gathered in database tables using the Microsoft Excel module of the Microsoft Office XP professional software package.

Histopathological examination. Tumor tissue fragments were subjected to conventional histological processing techniques (fixation and paraffin embedding). Serial sections were cut from each block. The first five sections were stained using classical staining methods (hematoxylin and eosin, Masson’s trichrome, Gömöri, Mucicarmin, Alcian Blue). The following six sections were used for IHC labeling with the following antibodies: CD34, SMA, Ki67, p53, MUC1 and MUC2.

Acquisition of microscopic images. Histopathological aspects were selected using the X4 eyepiece. For image acquisition we used optical planapo corrected...
objectives with magnification of X4, X10, X20 and X40. The most significant images were obtained with a digital video camera, and transferred directly into the computer, and processed using a specialized image analysis software.

**Quantitative morphological measurements.** Quantitative morphometric measurements of various components of intratumoral stroma were performed with the "Measurements" module of the image analysis software. In cases with two major tumor areas, separate determinations for each of the stromal parameters were performed for each of the two tumor areas and entered separately, only for Group 2. For assessing the fibrillar component of intratumoral stroma two sets of measurements were performed on serial sections stained using two different techniques for the identification of collagen fibers: Masson’s trichrome and Gömöri. For each field and then for each case (major tumor area) we considered that the value of PCFS-M is the highest measured value on the two stains. In assessing the activity of the stromal cell component producing collagen fibers we calculated the Fibrillogenesis Index – FI. For calculating the weight for each of the types of collagen fibers we used a special algorithm. For the evaluation of the stromal cell component we performed two sets of measurements on serial sections immunohistochemically labeled with anti-SMA antibodies in order to identify SMA+ stromal cells. We used anti-CD34 antibodies to highlight vascular endothelial cells and CD34+ stromal cells. The value was the sum of two determinations. For evaluation of CD34+ stromal cell density, on the 5 fields previously labeled using the anti-CD34 antibody, labeled cells were counted for each field and the cell density/mm² was then calculated. For evaluation of vascular density, vascular structures were counted for each field on the 5 fields previously labeled using anti-CD34 antibodies, and vascular density/mm² was then calculated. The aggressiveness was assessed by calculating the Ki67 proliferation index and the p53 index. To obtain these indices at least 1,000 nuclei of malignant cells in several microscopic fields photographed with 40X objective were counted.

**Processing and interpretation of results.** In order to analyse the correlations between parameters, the filtering of the primary data with their division into groups was required. For numeric parameters the following statistical indicators were calculated: VMIN, VMAX, VMEAN, STDEV, CI - 95%. For numerical parameters we used the Pearson correlation test. For parameters divided into classes using stratification scales the "χ²" correlation test was used. The graphic expression of the results and their interpretation was carried out using specialized statistical software.

**STUDY OF THE CLINICO-MORPHOLOGICAL PROFILE**

**Clinico-morphological profile.** Even though there are independent studies that have shown that the Mixed type is a reality and not a rare one, and moreover, that it has a more severe prognosis than pure types [6, 20, 21], taking into account in routine practice classifications that include this entity is still far from becoming a common practice.

Thus tumors showing only one histological pattern within the tumor mass were usually found in older men, showed no net predilection for any of the segments of the stomach even though the antro-pyloric region was most commonly affected, expressing both as wall infiltration and protrusion in the lumen, in both cases with associated tumor surface ulceration. From the histological point of view, they usually showed an undifferentiated pattern (Diffuse according to the Lauren classification - 1965, with isolated cells or solid according to the Carneiro classification - 1995), and a tubular/glandular pattern, usually moderately differentiated and secretory. The biologic behavior was an aggressive one, with complete gastric wall invasion, frequent tumor emboli in intraparietal vascular structures, invasion into regional
lymph nodes representing almost a rule, behavior reflected in the large proportion of stages III and IV as determined by the TNM system. The necrotic phenomenon, whether macroscopically visible on the tumor surface or microscopically identifiable within the tumor mass, was also a common observation.

On the other hand, tumors with two dominant histological aspects within the tumor mass, usually revealed a younger patient, not infrequently a woman who had a tumor located almost exclusively in the antro-pyloric region, with predominantly infiltrative pattern and ulcerations on the flat surface of the tumor.

The dominant histological pattern was the tubular/glandular one, but most often poorly differentiated, often accompanied by mucinous areas, with secretory phenotype.

The biologic behavior was somewhat less aggressive, completely invading the wall but not exceeding the serous layer, with rare invasion of particular parietal structures, usually vascular and perineural, reduced lymph node invasion but with distant metastases twice as common as in other type. Overall, TNM staging was more "gentle" than in the other group. Necrotic phenomena were present, as already mentioned, but more rarely than in the group with monomorphic tumors.

Tumor markers showed comparable values, with an elevated p53 expression in tumors showing two morphological patterns within the tumor.

The difficulty, not to say the impossibility, to obtain data on development following hospital admission put us in the position of not being able to comparatively assess the outcome in the two groups of tumors and to compare our results with the literature.

**Tumor stroma profile.** Overall, the percentage of tumor stroma generally varied between 10% and 40% of the tumor area, variation explained by the great number of tumors invading the gastric wall beyond the submucosal layer. Fibrillary structures generally dominated the stromal architecture with an average ratio of the two components of "2". Thus, in almost 40% of the cases, measurements were grouped in a "2" score class, signifying a somewhat balanced distribution of the two components, from the slight prevalence of the fibrillary component to a share double that of the cellular component. It should be noted that in only 20% of determinations, the specific cellular component outweighed the fibrillar component. Although individually, each of the two types of fibers showed a share of up to 10%, overall, the fibrillar component showed a range most often between 10% and 30%, with only four cases with a very rich fibril component (over 30% of the tumor area). Reticular fibers usually dominated the fibrillary compartment, revealing a significant secretory activity of specific stromal cells.

The cellular compartment was composed mostly of specific cells that produced the MEC fibrillary material, vascular cells (endothelial, adventitial) representing around the 10\textsuperscript{th}-13\textsuperscript{th} part of the cellular compartment. The vascular compartment showed great variability in the density of intratumoral network, with an average of little less than 200 vessels/mm\textsuperscript{2}, with more than half of the values for the vascular density being below average.

**Study of Correlations between the Clinico-Morphological Parameters**

Morphological evaluation systems were correlated with each other, which is not likely to surprise us as long as the paradigm of tumor assessment still retains as the center piece the result of direct observation, that is cell morphology in step one and tumor architecture in step two, in order to finally reach the same goal that is securely anchored in the diagnostic algorithm of the pathology practitioner, namely to establish the degree of differentiation (“Does the tumor produce tubes/glands?”,
“Does it secrete or not?”), regardless of the approach of any of the classifications. We persist in placing in the center of our evaluation system(s) the degree of differentiation although none of the systems created correlated with any indicator of macroscopic and microscopic morphological and biological behavior of tumors studied, which is shown in numerous studies in literature strongly supporting the lack of predictive value of most classification systems. There is a notable exception, namely the direct and in a certain way logical correlation between the presence of tumor emboli and especially in vascular structures and the likelihood of gastric wall invasion to a lesser extent and lymph node invasion to a greater extent.

The assessment of the correlations between different components of tumor stroma bring out very strongly the active and dynamic behavior of intratumoral stroma, which tends to maintain the equilibrium between the different morphological components, probably as a result of the cell population necessities. One exception here, namely a mismatch, is the ratio of the cell population producing fibrillar structures and the percentage of fascicular collagen fibers. This type of fiber is probably the basic skeleton even in an architectural design with a high degree of disorder as the tumor, skeleton which scarcely varies.

The adaptation of the supporting fibrillary structure to dynamics of the tumor cell population is achieved by varying the number of cells producing the necessary fibrillary material dominated by reticular type fibers, confirmed by the close correlation between cell number and amount of fibers of that type that respond much better to the disordered "construction" of tumor parenchyma.

The analysis of correlations between morphological indicators and stromal components reveals that tumors with glandular differentiation and no secretory properties tend to have a more prominent stromal support, while solid and secretory forms tend to have a lower stromal support. Also, the vascular support somewhat correlated with tumor architecture, being less rich in differentiated cell types (papillary forms) or mucus producing, but well represented in forms with poor differentiation or undifferentiated. One should not overlook another obvious correlation involving again vascular embolism that is, somewhat logically, the more pregnant as the intratumoral vascular network is more developed.

**CONCLUSIONS**

Our study lead to the following conclusions:

1. The type of tumors with two dominant histological aspects present simultaneously is a reality that can not be the argued, which the authors of classification attempting its identification within the evaluation systems of malignant gastric epithelial neoplasia also have seen. Our results outlined a draft of the morphological and biological profile which proves to be different from that of monomorphic tumors, profile which needs to be completed and validated.

2. Tumor stroma component analysis revealed that the stromal microenvironment is an active participant in the neoplastic phenomenon, with the cellular compartment which remodels and continuously adapts to the needs of the supporting structure of neoplastic epithelial cells playing a central role, but only in close relation to the vascular compartment, an interrelation that is independently evolving from the morphological profile of the population of malignant cells.

3. The tumor profile should be redefined by identifying some descriptive parameters which mainly focus on the predictability of the malignant neoplastic process evolution because, as our analysis also shows, the trend to report only to morphological descriptive terms does not provide a solid support for building a therapeutic and preventive strategy.
Highlighting the phenomenon of tumor embolism as independent prognostic factor points to potential areas of exploration to identify the predictive value of descriptive parameters, namely the biological profile of malignant cells. The shift of the focus from exhaustive morphological description and the identification at all costs of an individual gene pattern, towards the identification and accurate description of their defining features of biological behavior may lead to finding those long-awaited assessment indicators for prognosis.

**SELECTIVE REFERENCES**

20. Carneiro F. **Classification of gastric carcinomas.** *Current Diagnostic Pathology.* 1997; 4:51-59

