DOCTORAL THESIS

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL
STUDY OF GASTRIC CARCINOMAS

SUMMARY

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#### REFERENCES  

#### PERSONAL PUBLISHED PAPERS IN DOCTORAL THESIS AREA
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INTRODUCTION

Gastric cancer in most cases malignancy of epithelial origin (95%), continues to be, according to data continues to be, according to the International Agency for Research on Cancer (IARC), a major global health problem associated with high mortality and morbidity. Distinctive features of gastric cancer epidemiology, in particular regional differences and chronological changes in incidence can be partly attributed to infection with Helicobacter pylori, microbial agent included by IARC in the Class I category of carcinogens, in 1994. This study joins many other studies in the literature in an attempt to identify some specific biomarkers underlying molecular mechanisms involved in gastric carcinogenesis and at the same time, to define the behavior of biological indicators and parameters with prognostic value of these tumors.

ACTUAL STAGE OF KNOWLEDGE

Gastric carcinogenesis

Gastric cancer has a multifactorial pathogenesis that can be considered an example of the interaction of environmental factors - Helicobacter pylori, a major carcinogenic agent - and genetic factors of the host organism, the prognosis of these tumors being dictated by specific parameters appreciated with histopathological and immunohistochemical techniques.

Placing H. pylori on the onset of gastric carcinogenic sequence in the development of intestinal type carcinomas was recorded in Correa model: chronic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia, carcinoma. Contradicting the Correa model, Hattori reported the synchronous development of gastric atrophy, intestinal metaplasia, dysplasia / cancer, supporting the hypothesis of its independent development which would imply the absence of any precursor lesions.

At the molecular level, gastric carcinogenesis is a sequential process initiated by Helicobacter pylori - decisively involving bacterial virulence factors and the elements of host body's immune response, with possible involvement of other various environmental factors – and characterized by the accumulation of genetic alterations based on two distinct molecular mechanisms: epigenetic and genetic, on genes involved in growth control and cell proliferation.
THE OBJECTIVES OF THE CURRENT RESEARCH

This study has proposed a complete and thorough evaluation of the gastric carcinogenesis and natural history of these tumors, using conventional histopathological methods and modern techniques such as immunohistochemistry and morphometry. To this end we evaluated markers involved at different stages of carcinogenesis and also histopathological prognostic parameters and immunohistochemical specific markers for tumor aggressiveness.

RESEARCH, STUDIES AND PERSONAL CONTRIBUTIONS

Material and Methods

The study group included a total of 458 gastric carcinomas diagnosed between 2005-2009, in gastric biopsy fragments or resection specimens.

Biological material was investigated for histopathological diagnosis, to evaluate the prognostic value of morphological parameters and define the specific makers involved in carcinogenesis and tumor aggressiveness, for this purpose using histopathological immunohistochemical and morphometric techniques, together with the statistical analysis of resulted data.

Results

V.A. Histopathology study

The highest incidence of gastric carcinomas in this study was in the seventh decade of life (36.68%), and the most often affected were male patients (1.86:1 ratio).

Histopathological, the 458 gastric carcinomas were in most cases intestinal type tumors (80.78% vs. 19.22%), predominantly poorly-differentiated (51.35%). As a risk age groups were seventh decade (61-70ani) for intestinal-type tumors and sixth decade (51-60ani) for tumors of diffuse type.

The presence and distribution of precancerous lesions in non-tumor gastric mucosa correlated with high level of statistical significance (p <0.001), with histological type, meaning the more frequent association with phenotype tumor. Helicobacter pylori were detected against the background of chronic gastritis, in a significant number of cases, predominantly intestinal type tumors (64.08%) representing active inflammatory lesions.

Blood vessel invasion was more frequently associated with intestinal type carcinomas (21.12% vs. 17.64%), while invasion of lymphatic vessels was more frequent in diffuse type of tumors (45.58% vs. 33.45%). These prognostic parameters were correlated with the histological grade (p <0.05) being frequently associated in poorly-differentiated intestinal type carcinomas.
Residual tumor correlated with histological type (p <0.001) being more frequent in diffuse type carcinomas (25.56% vs. 13.63%).

Evaluating the tumor stage (pT) we found that gastric carcinoma stage of pT1 were represented by 4.92% of intestinal type tumors (most well-differentiated, 78.51%) and 2.94% of diffuse-type tumors, while the pT2 stage carcinomas included 27.46% of intestinal type tumors and 20.58% of diffuse-type tumors. Gastric carcinomas of pT3 stage were intestinal and diffuse type tumors in approximately equal proportion (38.02% and 36.76%); intestinal type carcinomas in this category were predominantly poorly differentiated (63.88%). Perigastric dissemination (stage PT4) was observed in 29.92% cases, frequently of diffuse type (39.70% vs. 29.92%). The tumor stage was correlated with histological grade in intestinal type carcinomas (p<0.001), advanced tumors being mostly poorly-differentiated.

Absence of lymphatic dissemination (stage pN0) was observed in intestinal and diffuse type tumors in approximately equal proportions (13.02% and 14.70%). pN1 stage gastric carcinomas included the majority of diffuse type tumors (52.94%) and the most of the intestinal tumor type (33.09%), whereas pN2 stage gastric carcinomas included predominantly intestinal type (29.92%) tumors. All pN3 stage tumors were poorly-differentiated intestinal type (2.11%). The pN stage of gastric carcinomas correlated with both histological type (p<0.05) and grade (p<0.01). pM1 category gastric carcinomas (distant metastases) included 10.56% of intestinal type (mostly poorly-differentiated) and 16.17% of diffuse type tumors.

Clinicopathological staging found the most intestinal type carcinomas as stage III tumors (A and B) (40.10%), followed as frequency by the stage IV tumors (34.37%); most of the diffuse type carcinomas were stage IV (44.44%) tumors, while stage III (A and B) included 31.11% cases.

V.B. Immunohistochemical study

Immunohistochemical study was performed for 95 cases considered representative in terms of parameters subject to assessment, using specific epithelial differentiation markers (mucins: MUC1, MUC2, MUC5AC), markers of cell proliferation (Ki-67, PCNA) and apoptosis (Bcl-2 and p53), cellular adhesion markers (E-Caderina) and tumor angiogenesis markers (VEGF, CD31).

V.B1. Markers of epithelial differentiation

► MUC1 was expressed in 82.10% cases, more frequently in intestinal type tumors (86.84% vs. 63.15%) and related with histological grade for these tumors (p<0.05). MUC1 expression was also correlated with tumor stage, noting the progressive reduction of MUC1 expression in advanced tumors (p<0.05).
► MUC2 was expressed in 85.26% of cases, in about the same proportions for intestinal and diffuse type tumors (84.21% vs. 89.47%), being also related with histological grade, observing at the same time, the over expression of this marker predominantly in extracellular and/or intracellular mucinous tumors. MUC2 expression varied statistically significant (p <0.001) with tumor stage, finding the reduced MUC2 expression with advancing tumor stage.

► MUC5AC was expressed 61.05% of cases, more frequently in intestinal type tumors (63.15% vs. 52.63%) correlated with histological grade (p <0.01) observing reduced expression of this marker in poorly-differentiated tumors. MUC5AC expression pattern varied statistically significant with tumor stage (p <0.001) with frequently reduced or absence of MUC5AC immunostaining in advanced tumors.

V.B2. Markers of cell proliferation

► Cell proliferation markers, Ki-67 and PCNA, were expressed with variable intensity in all investigated gastric carcinomas. Its over expression were found in relatively similar proportions in intestinal and diffuse type tumors (47.36% and 42.10% for Ki-67, 55.26% and 57.89% for PCNA) and correlated with histological grade (p <0.01), in that poorly differentiated tumors showing a high mitotic activity. Ki-67 and PCNA expression was also correlated statistically significant (p <0.001) with tumor stage.

Adverse prognostic parameters - blood and lymphatic vessel invasion - and lymph node and distant metastases were found predominantly in tumors Ki-67 and PCNA intense immunopozitive (p <0.05) confirming the aggressive behavior of gastric carcinomas with the increased mitotic index.

V.B3. Markers of cell apoptosis

► P53 was expressed in 64.21% of cases, much more frequently in intestinal type carcinomas compared with the diffuse type (71.05 vs. 36.84%), correlated with tumor type (p <0.01). Blood and lymphatic vessel invasion and also the distant metastases were found frequently in the p53 intense immunopozitive tumors.

► Bcl-2 was expressed in 78.94% of cases, more frequent intestinal type tumors (84.21% vs. 52.63%) statistically related with histological type (p<0.05) and grade (p<0.001), well differentiated tumors being more frequent Bcl-2 immunonegative. Among poor prognosis parameters, blood vessels invasion was more frequent in Bcl-2 intense positive gastric carcinomas (p <0.001).

V.B4. Markers of cell adhesion

► E-Cadherin was variably expressed in 68.42% of studied gastric carcinomas more frequently in intestinal type tumors (73.68% vs. 47.36%), statistically related with histological type (p<0.05) and grade (p<0.001) observing the progressive loss of E-Cadherin
immunostaining with decreasing of tumor differentiation so that 54.05% of poorly-differentiated tumors were E-Cadherin negative.

E-Cadherin expression pattern was also statistically correlated with tumor stage (p <0.001), finding frequent loss of staining in advanced tumors. Adverse prognostic parameters - blood and lymphatic vessel invasion - and lymph node and distant metastases were found predominantly in E-Cadherin weakly positive or negative tumor (p<0.01), loss of expression being the attribute of tumors with aggressive biologic behavior.

**V.B.5. Markers of tumor angiogenesis. Tumor Microvessel Density (TMD)**

► *VEGF* was expressed in 72.63% of cases, more frequently in intestinal type tumors (73.68% vs. 68.42%) related with histological grade (p<0.01), so that poorly differentiated tumors were characterized by VEGF over expression in most cases. VEGF expression pattern also correlated with tumor stage (p <0.05), advanced tumors being highly angiogenic as were tumors associated with poor prognostic parameters (blood vessels and lymphatic invasion), and lymph node and distant metastases.

► *CD31* expression for intestinal type carcinomas, showed the highest values of TMD for poorly-differentiated tumors (22.01 / hpf), observing the progressive increase of TMD values with reducing of tumor differentiation.

High TMD were also recorded for intestinal type tumors of advanced stage (21.69 /hpf in pT4 tumors) associated with poor prognostic parameters (blood vessels and lymphatic invasion), and lymph node and distant metastases (28.75 /hpf in these last cases). For diffuse-type carcinomas the TMD (with an average of 16.48 /hpf) progressively increased with tumor stage, high values being also found in tumors associated with adverse prognostic parameters.

**CONCLUSIONS**

- The highest incidence of gastric carcinomas was in the seventh decade of life (36.68%), and the most frequently affected were males (65.06%, with a ratio of 1.86:1)
- Most cases were intestinal type gastric carcinomas (80.78%), more frequently poorly-differentiated tumors (51.35%)
- The precancerous lesions of gastric mucosa were more frequently associated with intestinal phenotype carcinomas, as was active infection with *Helicobacter pylori* (64.08%)
- Vascular invasion was present more frequently in intestinal type tumors (21.12% vs. 17.64%) while lymphatic invasion prevailed among diffuse type tumors (45.58% vs. 33.45%)
- Residual tumor was found more frequently in diffuse-type carcinomas (58.82%).
- Advanced stage tumors (pT3 and pT4) predominated among both intestinal and diffuse type carcinomas accounting for 67.95% and 76.47% of cases, with a more pronounced invasiveness for diffuse type tumors.
- Compared with diffuse-type carcinomas, mostly found in pN1 category (52.94%), the intestinal type carcinomas were predominant pN2 tumors (29.92%) and were found exclusively in pN3 category (2.11%).
- pM1 category include 8.45% of intestinal type carcinomas, in most poorly differentiated tumors and 16.17% of type diffuse type carcinomas.
- Regardless of histological type, the vast majority of gastric carcinomas included in our study (74.67%), were diagnosed in advanced stages of disease (III and IV), intestinal type tumors being mostly stage III and the diffuse type, stage IV.
- Mucins MUC1 and MUC5AC expression in gastric carcinomas was found predominantly in intestinal type tumors, correlated with histological grade and tumor stage, while MUC2 was expressed more frequent in diffuse type carcinomas and associated with tumor mucinous secretion, being also correlated with histological type and grade and tumor stage.
- The highest level of Ki-67 and PCNA expression was found in advanced stage tumors, associated with adverse prognostic parameters.
- The markers of cell apoptosis in studied gastric carcinomas were expressed in relation with tumor phenotype, p53 over expression being observed in intestinal type tumors, frequent associated with blood vessel invasion and distant metastases, while Bcl-2 over expression was variable found in both intestinal and diffuse type tumors, related with tumor stage.
- E-Cadherin was more frequent expressed in intestinal type tumors, while diffuse type carcinomas were predominant E-Cadherin negative. Loss of E-Cadherin expression was the attribute of aggressive tumors, of advanced stage and associated with adverse prognostic parameters.
- VEGF was expressed more frequently in intestinal type tumors related with histological grade and tumor stage. The highly angiogenic tumors were associated with poor prognostic parameters (blood vessels and lymphatic invasion), and lymph node and distant metastases.
- Tumor microvessel density (TMD) varied according to histological type and differentiation, and also with tumor progression and dissemination.
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Published papers in doctoral thesis area

ISI Publications
2. E-Cadherin in gastric carcinomas related to histological prognostic parameters. Stănculescu D., Simionescu C., Margaritescu Cl., Stepan Al., Mitrut A, emerging article

CNCSIS Publications

Abstracts
