Correlative study of classical and modern prognostic factors in gastric cancer

Cancer is one of the world's leading causes of death. Although higher incidence and mortality rates were reported in developing countries, it is also the second leading cause of death in economically developed ones. By the year 2030 there are estimates of approximately 26 million new cases with 17 million cancer related deaths per year. For 2020 GLOBOCAN estimates 1,174,810 new gastric cancer (GC) cases of which 785,390 cases in men and 389,420 in women. The total number of deaths estimated is 892,622 for both sexes.

The etiology of GC is multi-factorial. It is still debatable, how much the histology (WHO histologic features) influences the patients' prognosis. In advanced stages, not only histologic type represents an independent prognostic factor but also age, lymph node involvement (pN), tumor size, localization, depth of invasion (pT stage), and grade of differentiation as well.

The personal part of this PhD thesis consisted of three main chapters that are presented below.

1. Prognostic value of classic clinicopathological factors in Romanian versus Polish patients with GC

The first aim of this chapter was to analyze the classic clinicopathological factors and their prognostic value in patients diagnosed with GC in two clinics from Romania and Poland. The second aim was to report the prognostic value of classic clinicopathological parameters in these patients.

Statistical assessment showed that there were no differences among the Romanian and Polish patients regarding the gender or histological type of the tumors. In both groups males were more frequently affected by GC and intestinal-type tumors were predominant. The mean age of the Romanian patients was 62.02±15.21 years versus 59.02±11.21 years in Polish patients ($p=0.03$). The mean age of the patients presenting tumors in the distal third of the stomach was 64.07±12.61 years in Romanian patients versus 61.95±14.24 years in Polish patients ($p<.0001$). This significantly younger age of Polish patients was also related to the proximal location of the tumors ($p<.0001$). No differences were noted regarding the age of patients with tumors of the middle third of the stomach ($p=0.21$). The Bormann types I+II was more frequent ($p<0.0001$) in Polish cases than in Romanian patients (54.80% versus 10.61%), whereas types III+IV predominated in the Romanian cases (89.39% versus 45.20%). The age of the patients (below and above 50 years) proved to be an independent prognostic factor in Polish patients only. Gender was not proved to have an independent prognostic value. In the Romanian group, patients with tumors located in the distal part of the stomach showed a better survival, compared with tumors located in the proximal part, but the small number of cases did not show a strong statistical difference. The ulcerated-type tumors showed a better OS in both groups. A reclassification of the cases showed that type I+II Bormann's types associated a better outcome than types III+IV ($p=0.0002$). Better OS was noted for pT1N0-3/pT2N0 cases, followed by pT3N0. Significant shorter OS was observed in the groups pT3N1-3, pT2N1-3, and pT4N0-3.

The conclusions are that less than one quarter of gastric cancers are diagnosed in patients below 50 years old, with a male female ratio of 2:1. Half of the patients died in first year after surgery and the 5 year survival rate is below 21% in both countries. The Polish patients are diagnosed at younger ages and in earlier stages, compared with Romanian patients. The pTN staging system is a stronger indicator of overall survival than pT or pN alone and might be used in daily practice with predictive value. Regarding the microscopic aspects, the microglandular carcinomas with diffuse growth type should be diagnosed as poorly-cohesive carcinomas.

2. Immunoprofile of gastric cancer in young versus old patients

The aim of this chapter was to examine the possible age-related particularities of clinicopathological factors and immunoprofile of GC. The reasons why we have focused the research on examining the age-related particularities is the trend of increasing number of GCs in young patients and the particular features of early-onset carcinomas.

In this chapter, the patients were grouped in two categories: group 1 (patients aged 45 and younger) and group 2 (patients older than 45 years old). In the majority of the cases from group 1 patients the tumor was predominantly located in the proximal area, whereas the distal stomach was mostly involved in cases diagnosed from group 2 of patients. The diffuse type GC was predominantly seen in group 1 patients, without associated metaplasia in majority of cases. In contrast, patients from group 2 mainly presented associated intestinal metaplasia as well as predominance of intestinal-type GC. Regarding the tumor stage, the clusters of tumor cells crossed the muscularis propria in both groups and the lymph node metastases were significantly more frequent in group 1 compared with group 2 of patients (97.3% vs. 78.6%). Total loss of E-cadherin did not prove to have an independent prognostic value. The p53 negativity was a significant independent predictor of a better survival, whereas Ki67 expression did not prove to have independent prognostic value. The positivity of HER2 did not prove to be an

independent prognostic factor. The OS was not influenced by VEGF or COX-2 expression but a slightly better OS was seen for patients with low micro-vessel density.

The conclusions are that the aggressiveness of early-onset GC, compared with cases diagnosed in older patients, is based on the predominance of proximally located diffuse-type node positive cancers in young patients. Total loss of E-cadherin is involved in histogenesis of one quarter of early-onset GCs as an indicator of epithelial-mesenchymal transition. The p53 positivity and high microvessel density are indicators of poor survival. The intestinal-type GCs seem to have a higher angiogenic activity than the diffuse type GC.

3. Maspin-related particularities in gastric cancer

One of the objectives of the newest classification systems is to understand the histogenesis of GC, as a base for individualized therapy. One of the markers used by our team to study the GC histogenesis was Maspin (mammary serine protease inhibitor). It is believed to be a tumor suppressor with antiproliferative, pro-apoptotic and antiangiogenic properties that can also induce apoptosis in endothelial cells. In this chapter we present the results related on the immunoreactivity of maspin in GC cells, in correlation with the classic clinicopathological factors.

Our results showed that the normal gastric mucosa displayed either negativity or maspin cytoplasmic positivity. Maspin cytoplasmic pattern was also preserved in glands with intestinal metaplasia. Association of high-grade dysplasia induced a combined cytoplasmic and nuclear maspin expression. From the 154 cases that expressed maspin, 76 showed only cytoplasmic expression, 61 presented mixed positivity (cytoplasm+nuclei), and 17 cases showed only nuclear maspin expression. In diffuse-type GCs, especially those that did not develop on background of intestinal metaplasia, maspin expression was predominantly nuclear with or without the associated cytoplasmic expression. In intestinal-type GCs, cytoplasmic maspin expression was more characteristic in well-differentiated to moderately differentiated adenocarcinomas which displayed a nodular growth also preserved in the deep layers. Majority of the HER2 +++ cases (48/51 cases) had displayed positivity for maspin. The predominant cytoplasmic expression of maspin was more frequent in G1/G2 adenocarcinomas that developed on background of intestinal metaplasia and displayed E-cadherin positivity. The double Maspin/E-cadherin negativity was more characteristic for diffuse-type GCs without associated intestinal metaplasia, which were proximally located. The lowest Ki67 index was displayed by the poorly-cohesive GCs that have not developed on the background of intestinal metaplasia and expressed nuclear maspin. Those cases that did not display nuclear positivity of maspin (negative or cytoplasm only expression) were mostly p53 positive. In the majority of VEGF-positive cases we observed nuclear only maspin positivity. The COX2 positivity was more frequently displayed by the cases with cytoplasmic maspin expression, especially in G1/G2 adenocarcinomas with nodular growth that also displayed bax/Ki67/p53/E-cadherin positivity.

The conclusions are that in patients with GC, loss of maspin and E-cadherin is an indicator of high risk for distant metastases and is more frequently observed in proximally located poorly-cohesive carcinomas. Patients with GCs that display cytoplasmic maspin are usually bax/Ki67/p53/E-cadherin/COX-2 positive and might respond to anti-COX-2 therapy whereas cases with nuclear maspin might be treated with conventional chemotherapeutic agents.

Finally in patients with GC, the independent prognostic factors are the macroscopic Bormann's type of the tumor, pTN stage, p53 immunoexpression, Maspin subcellular localization and microvessels density.