

**UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA**

**PhD THESIS**

**THE IMPORTANCE OF MOLECULAR TUMOR MARKERS IN THE DIAGNOSIS,  
PROGNOSTIC ASSESSMENT AND TREATMENT MONITORING AND OF HEAD AND NECK  
CANCER EVOLUTION**

**-SUMMARY-**

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**CRAIOVA 2014**

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## **INTRODUCTION**

Head and neck cancers represents 10-15% of the total of neoplasias and determines 4-5% of the total cancer deaths.

Taking into account the permanent increasing incidence and mortality of malignant neoplasias in head and neck area, the necessity of knowing the causes of those parameters increase, as well as the impact of genetic and molecular changes in the prediction of therapeutic response and in patients prognostic, is imposed.

Mr.Prof.Univ.Dr.Florinel Badulescu's suggestion approached the actual interests in defining the therapeutic algorithm of head and neck cancers in locoregionally advanced stages, taking as reference the clinical and histoprognostic phenotypes with impact in the prediction of the therapeutic response and the patients prognostic.

**Key words** : cancer, head and neck area, incidence, prognostic, treatment, clinical and histoprognostical phenotypes.

**Chapter 1, „KNOWLEGDE STAGE”**, clearly and didactically expressed within 80 pages emphasizes elements regarding the epidemiology, histopathogenesis, natural history, diagnosis, staging criterias, anatomic and evolutive particularities, prognostic, as well as the therapeutic methods applicable in head and neck cancers.

At the same time, the role of complex and individualized treatment of head and neck cancers, of chemo-radiotherapy respectively, as well as the role of molecularly targeted therapy within this combination is emphasized. The radiotherapy, as main therapeutic means in head and neck cancers in locoregionally advanced stages, was debated as actual therapy, as well as from the therapeutic perspectives in the radiotherapy domain and their accesibility and proved statistical benefits respectively.

**Chapter 2, „PERSONAL CONTRIBUTIONS”**, opens with the enunciation of the study objectives:

- a) Establishing some clinical phenotypes with predictive value in head and neck cancers by reference to the treatment response rate;
- b) Establishing some clinical phenotypes with prognostic value in head and neck cancers by reference to the survival rate and the disease-free survival;
- c) Establishing connections between the clinical factors with prognostic value and the histoprognostic factors.

**Chapter 2.2, „Resources and Methods”**, describes the features of patient groups, having as reference demographic criterias (age, sex, origin, performance status) and clinical criterias (tumor location, disease stage, treatment applied) that allow the personalization of each particular case, by shaping a clear and real expression of personalized oncology within this study.

Within the immunohistochemical study, for the immunomarking achievement, LSAB-HRP technique was used by means of monoclonal antibodies: anti-EGFR, clone E30 DAKO (dilution 1 : 50, 16 minutes at 37°C), anti-p53, clone DO-7 DAKO (dilution 1 : 50, antigen reactivated through dilution in citrate solution pH 6) and anti-Ki67, clone MIB-1 DAKO (dilution 1 : 50, antigen reactivated through immersion in EDTA solution pH 9).

The immunoreaction for EGFR was membranary and quantified through Altkins method and the global score was calculated through sum score P (the proportion of immunomarked cells) and I (the intensity of the immunomarking), and the interpretation was: score 0 = absence of immunomarking in tumor cells; score 1 - 3 = slightly positive immunomarking; score  $\geq 4$  = intensely positive immunomarking.

The quantification of nuclear immunomarking for the proliferation marker Ki67 was achieved semi-quatitatively by using the following assessment scores: score 1 - index  $\leq 15\%$ ; score 2 - index 16 - 44%; score 3 - index  $\geq 45\%$ .

For the assessment of nuclear immunomarking for p53 protein, the following semi-quantitative scale was used: score 0 = negative; score 1 = immunomarking in less than or equal to 10% of tumor cells; score 2 = nuclear immunomarking in 11 - 54% of tumor cells; score 3 = immunomarking positive in at least 55% of tumor cells; scores 0 and 1 were considered to be negative for the existance of mutations at the level of gene p53, and scores 2 and 3 were considered positive for these mutations.

For the analysis of variables taken into account, known statistical methods were used, logrank test respectively, in order to compare the existing differences in survival between sublots and the test t-test for the comparative analysis as regards the disease-free survival. The statistical analysis was made computerized (Epi INFO 2000) and the statistical meaning was achieved for  $p < 0.05$ .

**Chapter 2.3, „Results”**, is focused on the results of three studies- epidemiologic, clinical and immunohistochemical.

### **The results of the epidemiologic study**

The statistical analysis of epidemiologic data was achieved for tumor locations where the group of patients was numerically significant and balanced, including oropharyngeal carcinoma (tongue base and tonsil), hypopharyngeal carcinoma, laryngeal carcinoma and rhinopharyngeal carcinoma, in any evolutive stage, except for the metastatic stage (stage IVC).

After the separate delimitation of the global incidence (3.23‰) and of the balance of each tumor location (hypopharynx: 210 patients-40.07%, larynx: 195 patients-37.21%, oropharynx: 85 patients-16.22%, rhinopharynx: 34 patients-6.48%), for a better connection with the risk factors, the distribution of cases was made according to sex, age group and origin, by shaping the descriptive epidemiologic frame specific to head and neck neoplasias, that includes men (463 men/61women, index ratio 7.59/1), old men (average age 51.7 +/-13.46 years), originary from rural environment (rural/urban ratio:1.81, 338/186 patients), peasants, alcohol cronic consumers and chronic smokers, with poor oral hygiene, which handle field works, thus being exposed to the sunlight.

### ***The results of the clinical study***

The statistical analysis of the therapeutic results was achieved on a group of 524 patients clinically and imagistically diagnosed and histopathologically confirmed with malignant neoplasias in head and neck area, patients which were randomized and treated with radiotherapy (270 patients) vs radiochemotherapy (254 patients) within The Oncology Clinic Craiova, between 2000-2008.

The majority of the patients presented the disease in evolutive locoregionally advanced stage (stages III, IVA,B: 445 patients-84.92% vs stages I, II: 79 patients -15.07%).

The multivariate statistical analysis of *the therapeutic response rate* distinguished statistically significant differences depending on the tumor location ( $p=0.053$ ), the disease stage ( $p=0.0001$ ) and the treatment applied ( $p<0.0001$ ), thus proving the predictive value of the clinical factors analyzed in terms of the therapeutic response rate.

*The disease-free survival* was taken into consideration and calculated for patients which didn't show post-therapeutic disease signs anylonger and it represented disease-free period until the emergence of disease signs, being analyzed after a 5-year monitoring period considered to be statistically significant.

Thus, of the total of 188 patients (35.87%), having post therapeutic complete remission, 95 patients (50.53%) presented therapeutic failures, after a median disease –free survival of 7 months.

The majority of the patients (89 patients-93.68%) showed either the progress of the locoregional evolution of the disease, as a result of therapeutic failures and of the surgery applied as primary therapeutic step in the locoregionally advanced stages of the disease respectively (73 patients-76.84%) or the recurrence of the locoregional evolution of the disease, in the absence of clinical and imagistic assessment of the reconversion to surgery in the locoregionally advanced stages of the disease, but with post-radio-chemotherapy complete remission (16 patients-16.84%).

The therapeutic failures recorded as recurrence of the locoregional evolution of the disease were registered for patients treated with radiotherapy (6 patients-6.32%), as a result of diagnosis errors, with the sub-degree stadialization of the disease in the absence of the imagistics needed for the assessment of the disease real extension and the non-compliance with the therapeutic protocols accordingly .

The multivariate statistical analysis of disease-free survival distinguished statistically significant differences depending on the tumor location ( $p=0.0002$ ) and the disease stage ( $p=0.0091$ ), thus proving the predictive value of the clinical factors analyzed in terms of disease-free survival.

The multivariate analysis of disease – free survival depending on the therapy applied didn't achieve the level of statistical significance ( $p = 0.3179$ ); in each therapeutic case, the failures were registered after a median disease-free survival of 7 months, but the percentage of therapeutic failures for patients treated with radiochemotherapy was reduced 3 times (35.41% vs 100%).

At the analysis of *5-year survival rate*, data reporting was achieved after a 5-year post-therapeutic monitoring time, the head and neck cancer mortality rate being 40.07% (210 deaths), with a median survival of 19 months.

The therapeutic errors practiced in the locoregionally advanced stages of head and neck carcinomae were responsible for the patients death registered at the time of data reporting, as a result of the recurrence of the disease locoregional evolution, palliative first-line post-chemotherapy, 5FU/Cisplatin regimen(16 patients) or as a result of the disease evolution at distance, palliative second-line post-chemotherapy, Paclitaxel/Carboplatin regimen (bone metastases - 44 patients and/or pulmonary-29 patients).

The diagnosis errors practiced in the localized stages of head and neck carcinomae were responsible for the patients death registered at the time of data reporting, as a result of the recurrence of the disease locoregional evolution, palliative first-line post-chemotherapy, 5FU/Cisplatin regimen (6 patients). Furthermore, 7 patients diagnosed with oropharyngeal

carcinoma (palatine tonsil), in localized stages with complete remission on primary therapy, presented the recurrence of the disease locoregional evolution after 19 months, death being registered at the time of data reporting, as a result of the recurrence of the disease locoregional evolution, palliative first-line post-chemotherapy, 5FU/Cisplatin regimen.

Deaths were also registered to patients showing aspect of stabilized disease after primary therapy (38 patients post-radiochemotherapy, 70 patients post-radiotherapy); death was registered at the time of data reporting, as a result of the recurrence of the disease locoregional evolution, palliative first-line post-chemotherapy, 5FU/Cisplatin regimen.

The multivariate statistical analysis of *the 5-year survival rate* distinguished statistically significant differences based on the neoplastic location ( $p < 0.0001$ ), the disease stage ( $p < 0.0001$ ) and the treatment applied ( $p < 0.0001$ ), thus proving the predictive value of the clinical factors analyzed in terms of 5-year survival rate.

Therefore, *the clinical factors with prognostic and predictive value* are represented by the disease stage, tumor location and treatment applied; consequently, the patients diagnosed with rhinopharyngeal and oropharyngeal carcinomae, in localized stages, treated with radiochemotherapy, proved statistically superior results in terms of therapeutic response rate ( $p = 0.053$ ) and general survival ( $p < 0.0001$ ), reducing 3 times the rate of local recurrences.

#### ***The results of the immunohistochemical study***

The selection of the patients for the immunohistochemical study had as reference the data obtained within the clinical study, where the results achieved the level of statistical significance within the group of patients treated with radiochemotherapy, allowing the distribution of the patients into prognostic groups, as follows:

- a) unfavourable prognostic (51 deceased patients at the time of data reporting, of which 35 patients having as primary therapeutic step a surgery incorrectly performed and 16 patients without clinical and imagistic assessment for the reconversion to surgery);
- b) intermediate prognostic (38 patients with aspect of disease stabilized after radiochemotherapy, for which the death was recorded at the time of data reporting );
- c) favourable prognostic (4 patients with complete remission after radiochemotherapy and with disease monitored locoregionally and at distance, at the time of data reporting).

The immunohistochemical colourings performed on fragments resulted from the biopsy of the tumoral lesion distinguished a liniary relation between the tumor staging degree and the membrenary expression for EGFR and for the nuclear immunomarking for p53 and Ki67 respectively, in the case of poorly differentiated epidermoid carcinomae.

Furthermore, the statistical analysis within the immunohistochemical study distinguished a linear relation between the intensity of the membrane expression for EGFR, the percentage value of the nuclear immunomarking for p53 and ki67 and the clinical prognostic category.

The clinical histoprognotic factors with impact on the patients prognostic (anatomic location of neoplasia, disease stage, treatment applied, histopathologic type, tumor staging degree and expression of tumor markers) create the premise of a proper therapeutic randomization based on the histopathologic and immunohistochemical medical bulletins (the intensity of EGFR colouring and the percentage value of p53 and ki67 expressions), as follows:

- a) favourable prognostic: negative immunomarking of membrane for EGFR, nuclear immunomarking for p53, ki67 absent or present within < 10% of the tumor cells;
- b) intermediate prognostic : poorly positive immunomarking of membrane for EGFR, nuclear immunomarking for p53 within < 55% of the tumor cells (10-55%) and nuclear immunomarking for ki67 within < 45% of tumor cells (10-45%);
- c) unfavourable prognostic: strongly positive immunomarking of membrane for EGFR, nuclear immunomarking for p53 positive within > 55% of the tumor cells and nuclear immunomarking for ki67 positive within > 45% of the tumor cells;

**Chapter 2.4 „ Discussions ”**, goes deeply into the significant aspects of the study that proved the impact of simultaneous radiochemotherapy in the improvement of therapeutic response and the patients prognostic and defined the clinical histoprognotic factors, by creating the premise of a proper therapeutic randomization based on the histopathologic medical bulletins (histopathologic type, tumor staging degree) and the immunohistochemical bulletins (the intensity of EGFR colouring and the percentage value of p53 and ki67 expressions).

**Chapter 2. 5, „Conclusions”**, 5 in number, clearly and suggestively stated, represent a logical and natural succession of the study results.

- 1) The functioning at optimal parameters of the Regional Cancer Registry demands a clinical and imagistic diagnosis and a histopathologic confirmation.

The clinical factors with predictive and prognostic value are represented by the disease stage, tumor location and treatment applied.

- 2) The defining elements of personalized oncology that handles the individualized approach to diagnosis and treatment for patients with head and neck cancers are clearly

defined, having direct involvements on the therapeutic response and the patients prognostic.

3) The immunohistochemical analysis distinguished a linear relation between the histopathologic type, the tumor staging degree and the expression of tumor markers, on one side, and between the clinical factors with prognostic value and the immunohistoprostic factors on the other side, thus creating the possibility of defining the clinical and immunohistoprostic phenotypes.

4) Furthermore, the prognostic role of HPV determination is mentioned, its presence being related to the therapeutic response that will represent the domain of approach for future studies .

5) The study attests the necessity of a simultaneous complex therapeutic approach for head and neck cancers that imposes as therapeutic standard.

The improvement of the locoregional monitoring of the disease remains the objective of the performant and conformational radiotherapy, with hyperfractionated regimen.

The proper therapeutic randomization is based on clinical and immunohistochemical criterias and on histopathologic and immunohistochemical medical bulletins respectively, the group with unfavourable prognostic that requires the combination radiotherapy-antiEGFR therapy as therapeutic standard, including patients diagnosed with poorly differentiated hypopharyngeal and laryngeal epidermoid carcinomae in advanced stages.