

**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND
PHARMACY OF TIMIȘOARA**

MOȚ ION CRISTIAN



PhD. THESIS

**CLINICAL, HISTOPATHOLOGICAL, AND
IMMUNOHISTOCHEMICAL ASPECTS OF
SYNCHRONOUS AND METACHRONOUS
TUMORS IN OTORHINOLARYNGOLOGY**

Scientific leader

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The doctoral thesis entitled “Clinical, Histopathological, and Immunohistochemical Aspects of Synchronous and Metachronous Neoplasms in Otorhinolaryngology”, supervised by University Professor Dr. Mărioara Poenaru and developed in the Otorhinolaryngology Clinic pertaining to the University of Medicine and Pharmacology “Victor Babeș” of Timisoara, in collaboration with the Department of Pathological Anatomy of Timisoara’s Municipal Emergency and Clinical Hospital, proposes the study of new clinical and histological aspects of head-and-neck squamous cell carcinomas associated with neoplasms with other localizations (secondary primary tumors), either synchronous or metachronous.

The thesis is divided into three parts: the general part (comprising epidemiological data, etiology, prognosis, risk factors of head-and-neck squamous cell neoplasms and multiple primary tumors and their evolution, their molecular profile, relevant histological and immunohistochemical data, as well as possible treatment options), the specific part, containing information regarding the materials, methodology, results, and discussions, as well as a final chapter addressing the study’s conclusions.

Neoplasms in Otorhinolaryngology associated with other synchronous or metachronous tumors are challenging from both a surgical as well as a radiotherapy standpoint. While the development of successful cancer treatments has led to increased life expectancy, an increased incidence of both synchronous and metachronous tumors has been observed, with an exponential growth over recent periods. Thus, a detailed study of the relevant pathology may lead to an improvement of the diagnosis and the therapeutic approach.

The research protocol followed clinical and statistical, retrospective and prospective evaluations of patients admitted to the Otorhinolaryngology Clinic of Timisoara, and diagnosed with localized neoplasms that correlated with synchronous and/or metachronous tumors, both within and outside the head-and-neck regions. Furthermore, the study analyzed the clinical-

pathological, histological, and immunohistochemical particularities of these neoplasms.

The incidence of multiple primary tumors (MPT) falls between 0.7 and 11% of the total number of neoplasms, and may appear in any region of the body.

Statistically, the incidence rate of secondary primary tumors is 15% for synchronous tumors and 4% for metachronous tumors.

According to the World Health Organization, head-and-neck squamous cell carcinomas (arising in the oral cavity, the oropharynx, rhinopharynx, laryngopharynx, larynx, nasal cavity and paranasal sinuses) represent 6% of the annual incidence of tumors. Recent studies have demonstrated a world-wide increase in 2012 of head-and-neck tumors, with 686,000 new cases and 404,000 deaths in a single year.

Patient survival rates are significantly lower when diagnosed with a primary metachronous tumor, than after the discovery of a primary head-and-neck neoplasm, because the most frequent sites of secondary primary metachronous tumors, outside of the head-and-neck region, are the esophagus and the lung, both of which present a highly unfavorable survivability.

Five-year survival rates of patients with solitary head-and-neck neoplasms is roughly 50%. By comparison, the survival rate following the discovery of a secondary primary metachronous tumor is a mere 20%.

Squamous cell carcinomas represent the most frequent type of neoplasm in Otorhinolaryngology, with a prevalence of 90%. It is most frequently diagnosed in adult males presenting with chronic tobacco use and alcoholism, according to the World Health Organization. The remaining 10% of neoplasms comprise adenocarcinomas, melanomas, soft-tissue tumors, and lymphomas.

Furthermore, these carcinomas occur mainly in the sixth and seventh decades of life, and most frequently among patients living in an urban environment.

A study showed that the development of secondary primary tumors depends on a high alcohol and tobacco consumption (greater than 20 cigarettes daily).

Laryngeal and laryngopharyngeal squamous cell carcinoma incidence is increasing on a global scale, in both male and female patients, largely due to an increase in alcohol and tobacco consumption. Therefore, the most important prevention method is the reduction of such behaviors.

The study demonstrated that patients with oropharynx and laryngopharynx cancers were statistically prone to develop esophageal secondary primary neoplasms, while patients with laryngeal cancers tended to later develop pulmonary secondary primary neoplasms.

Treatment options for incipient head-and-neck squamous cell cancers are generally limited to either surgery or radiotherapy. A multimodal approach may be applied in cases of locally-advanced head-and-neck tumors, and include either surgical intervention followed by adjuvant radio- or chemotherapy (as indicated by the histopathology findings), or definitive chemoradiotherapy for irresectable tumors. Chemotherapy, with or without a biological agent, is reserved for metastatic disease or unapproachable, recurring tumors in which local or regional salvage is unattainable.

The specific portion of the doctoral thesis covers information regarding the materials used, implemented methods, results and the relevant discussion, and ultimately, the conclusions.

This prospective and retrospective study was carried out between 2008 and 2018, at the Otorhinolaryngology Clinic within the Municipal Emergency and Clinical Hospital in Timisoara, and covers patients diagnosed with and treated for head-and-neck neoplasms of various localizations, in collaboration with the Anatomical Pathology Department of the Hospital.

During the study's period, 902 patients were diagnosed with head-and-neck tumors, of which 12 cases presented metachronous neoplasms, representing 1.33% of the sampled population.

Gender distribution of those 12 patients was found to be largely skewed towards male, with 11 males and one female presenting metachronous neoplasms.

Patient enrollment criteria took into account the 1932 Warren and Gates definition of multiple cancers:

- tumors that have been histopathologically confirmed as malignant;
- patients have been excluded by histopathological findings as being metastatic tumors or a case of recidivism;
- exclusion of patients with inconclusive histopathological findings regarding the secondary tumor, which may have been a metastasis of the primary tumor;
- tumors arising in the same organ were considered metachronous neoplasms if the histopathological findings demonstrated two distinct diagnoses;
- tumors were clinically and histopathologically defined at an interval greater than 6 months from the initial diagnosis.

Both the number and localization of the studied tumors was varied. Nine patients presented two tumors, one patient presented three tumors, another patient presented four tumors, while yet another patient presented with five neoplasms.

Metachronous neoplasms varied in their distribution: tumors located in the oral cavity, pharynx, or larynx presented secondary localizations in the prostate, kidney, ureter, pulmonary tree, salivary glands, thyroid gland, meninges, skin, colon, or in the rectum.

The head and neck neoplasms studied exhibited a varied distribution: one tumor was found at the intermaxillary commissure, another at the maxillary apex, yet another within a palatine tonsil, five tumors in the pharynx, and four in the larynx.

A single patient presented with two metachronous neoplasms in the head-and-neck region: one at the right vocal cord, and a second localization in the rhinopharynx.

Another frequent localization was found to be the skin. Of the 12 patients diagnosed with secondary primary tumors, five of these presented a second malignancy on the skin, representing 41.66% of the sampled population.

In our study, secondary primary tumors appeared predominantly in males (11 patients, or 91.67%), with only a single female qualifying into the study, representing 8,33%.

From a behavioral standpoint, nine patients (75%) admitted to chronic alcohol consumption and ten patients (83.33%) acknowledged smoking more than 20 cigarettes daily. We consider that the increased incidence of metachronous head-and-neck tumors in males to be secondary to a penchant for alcohol and tobacco consumption, although female preference for this type of behavior appears to be continually amplifying.

Age distribution of patients showed that 75% of subjects were between 60 and 69 years old, two were in their seventh decade, and a single patient was younger than 60 years.

Of the patients who qualified into the study, nine arrived from rural environments, with only three subjects coming from urban settings.

With respect to tumor staging, a single case was found in stage I, three cases with stage II, six cases in stage III, and two cases in stage IV.

Histopathological biopsy results revealed all primary metachronous neoplasms in head-and-neck regions to be keratinizing squamous cell carcinomas (SCC). Of these, one case was categorized as well-differentiated G1, eight samples were moderately-differentiated G2, and three results showed high-grade dysplasia G3

Examination of the tumoral stroma revealed a diverse composition, with both regions of fibroblasts and collagenous fibers, as well as loose fibronectin and collagen. The stroma was found to be inhomogenously occupied by chronic inflammatory infiltrate, composed mainly of lymphocytes, plasmocytes, macrophages, and occasionally mastocytes. The extent of angiogenesis correlated with the density of the infiltrate.

Immunohistochemical analysis revealed relatively high Ki-67 proliferation indices, especially in poorly- and moderately-differentiated carcinomas. Furthermore, intense reactions of anti-p53 and anti-endomysial antibodies were observed.

Cytokine studies demonstrated positivity for pan-Cytokeratin AE1/AE3 in all samples; however, the reaction intensity was inversely correlated with the degree of dysplasia. By contrast, Cytokeratin-7 and CK-20 reactions were negative for each SCC.

Metachronous neoplasms localized in other organs tested histopathologically and immunohistochemically distinct from primary tumors located in the head-and-neck regions. Here, we present a single, metachronous pulmonary malignancy, which was revealed to be a poorly-differentiated G3 squamous cell carcinoma.

Primary tumor localization and TNM staging are used to determine possible therapeutic approaches, including surgery, radiotherapy, and chemotherapy. A combination of surgery and radiotherapy are well-suited to treat malignancies in the first two phases, where approximately 40% of patients are placed. Locally advanced cancers (those in stages III and IV B), plead for a chemotherapeutic approach. Metastatic disease also mandates chemotherapy for tolerant patients. Local or regional tumoral recidivism treatment depends upon the site of recurrence, the tumoral mass itself, and previously-administered treatment. This may vary from surgical rescue to radiotherapy or re-irradiation associated with chemotherapy to chemotherapy alone. Unfortunately, each of these options is associated with increased toxicity and late-phase organ dysfunction.

Multiple head-and-neck malignancies are not as rare as expected. There has been a notable increase in the incidence rates of regional synchronous and metachronous neoplasms in previous years.

Due to increased life expectancy, there is an urgent need to screen and investigate every oncological patient regarding tumor evolution; furthermore, the entirety of respiratory and digestive tracts must be monitored closely to

ensure early detection of secondary primary synchronous or metachronous neoplasms.

In Romania, there is a concerning lack of surveillance of head-and-neck synchronous or metachronous tumors. This is partly caused by the reduced number of patients and associated histories to which clinicians and surgeons have access.

Following this study, several conclusions may be reached:

- Multiple primary metachronous tumors represented only 1.33% of head-and-neck tumors admitted to the Otorhinolaryngology Clinic of the Municipal Emergency and Clinical Hospital of Timisoara, between 2008 and 2018. Of the 12 patients with metachronous tumors, nine presented two primary tumors, one patient presented three tumors, another patient showed four tumors, and yet another patient presented five malignancies.

- The second most common site of a primary tumor was found to be the skin.

- In our study, primary metachronous tumors appeared predominantly in males over 60 years of age.

- The chief risk factors in the development of metachronous neoplasms were tobacco smoking and alcohol consumption, leading to the urgency to monitor patients greater than 60 years of age who continue such behaviors.

- The majority of patients were diagnosed with TNM stage III malignancies.

- Moderately-differentiated G2 squamous cell carcinomas were most frequent.

- Histopathological examination of excised tumoral fragments revealed a desmoid-type stroma formed by both regions rich in fibroblasts and collagenous fibers as well as areas of loose fibronectin and collagen, with unevenly distributed chronic inflammatory infiltrate that showed extensive angiogenesis. The infiltrate was primarily composed of lymphocytes, plasmocytes, macrophages, and less frequently, mastocytes.

-Metachronous tumors localized in other organs were shown to be histologically and immunohistochemically distinct from the primary head-and-neck tumors.

-Patients were clinically and paraclinically monitored through panendoscopy, CT, PET-CT, and MRI investigations of the respiratory and digestive systems to screen for possible secondary primary synchronous or metachronous recidivism.

-Immunohistochemical analysis revealed patients' tumor cells to be positive for Cytokeratins AE1/AE3 and negative for CK-7 and CK-20 antibodies.

-Anti-CD-20 antibody marking showed the peritumoral stroma to be infiltrated with B-lymphocytes.

-Anti-CD-34 antibody marking revealed the presence of variable-caliber neo-vessels in the peritumoral stroma of moderately-differentiated carcinomas, as well as an abundant inflammatory infiltrate and extensive angiogenesis in poorly-differentiated neoplasms.

-Due to the reduced number of synchronous and metachronous tumors in the respiratory and digestive tracts, as well as the reduced access to patients presenting such recidivism, data on such cases cannot be statistically significant.

-Owing to the problems posed by this category of patients, we propose the creation of a national online database that would include histological and immunohistochemical aspects of synchronous and metachronous tumors within the upper respiratory and digestive systems, in order to readily establish diagnosis, treatment, and out-of-hospital monitorization.