The Diagnostic and Prognostic Value of sIL-2R as an Immune Biomarker in Head and Neck Cancers

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Abstract. *Background/Aim: Head and neck cancer (HNC) patients are usually diagnosed with advanced disease and multimodality therapies are required, as well as prognostic biomarkers to predict their response and assess survival. In this study, we aimed to evaluate the ability and clinical significance of the immune biomarker sIL-2R in HNC patients, to assess therapy response and prognosis. Materials and Methods: We evaluated 328 blood samples from 145 head and neck cancer patients (HNC) from several subgroups: 84 larynx carcinomas pre- and 39 post-therapy, 46 oral cavity carcinomas pre- and 29 post-therapy, 12 nasopharynx carcinomas, 16 parotid and other salivary gland carcinoma patients. The control group included 45 healthy subjects. Serum sIL-2R levels were evaluated by ELISA assays and correlated to disease stage, lymph nodes, response to therapy, survival and cancer differentiation. Results: Significantly higher sIL-2R levels were recorded in all HNC patients, as opposed to controls, in advanced versus early-stage disease that decreased following therapy. sIL-2R distinguished best, in comparison to other tumor markers, between HNC patients and controls. Survival was strongly associated to lower sIL-2R levels in patients entering the study. Conclusion: sIL-2R is a sensitive immune marker for HNC patients. Its levels correlate to disease stage, assess response to therapy and are predictive of recurrence or better survival. We suggest, therefore, using sIL-2R as a reliable prognostic marker in HNC patients as a single marker, or in a combined panel of biomarkers.*

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Primary head and neck cancer (HNC) is estimated to exceed 500,000 cases worldwide per year. Geographic differences in HNC incidence rates persist among and within some countries (1, 2).

About 90% of the HNC cases are of the squamous cell carcinoma (SCC) type. The main etiologies of HNC are smoking and alcohol consumption. During the past decade, infection with high-risk human papilloma viruses (HPVs), in particular HPV-16 has emerged as a newly-recognized risk factor for a fraction of HNC - SCCs, specifically SCCs arising in the oropharynx. In many cases, second malignancies were described in treated HNC patients (2, 3). Prognosis of patients is estimated according to the stage of the disease, lymph nodes involvement and response to therapy. The main problem remains that approximately 60% of HNC patients present with loco-regionally advanced (stage III and IV) disease. This requires a multimodality therapy, including surgery, radiation, and/or chemotherapy. During recent years the combination of chemo-radio therapy (CRT) as part of organ preservation protocols, is the most common. However, despite recent advances in treatments for HNC, survival of patients did not improve and accounts for about 50% after 5 years (4).

Tumor markers are important tools for predicting and assessing response to therapy in cancer patients (5). Early detection of the disease and of recurrence by tumor markers, improve the clinical outcome. The most used tumor marker over the years was carcino embryonic antigen (CEA); however, additional markers were studied in various configurations over the years (5, 6).

Interleukin-2 (IL-2) is well-characterized cytokine with various immunological functions, the most important being the capacity to initiate the proliferation of activated T cells. IL-2 acts with a specific surface receptor (IL-2R), absent on resting T cells but appearing within hours of activation. Activated lymphocytes produce and release into the circulation a soluble form of the same receptor (sIL-2R) that retains the capability of binding the cytokines (7). Increased levels of sIL-2R are known to be a proxy for general immune activation. It is a nonspecific biomarker expressed by various cell populations,

including both activated Th1 and activated Th2 cells (8). Unlike the more complicated functional immune assays used to measure Th1- and Th2-specific immune responses, sIL-2R can be measured with relative ease because a portion of it is proteolytically cleaved and found in serum. Therefore, sIL–2R is an important monitoring index which can reflect the body cellular immunity function.

In diverse human malignancies, the existence of correlation between sIL-2R levels and clinical parameters, such as tumor burden and treatment response, has been reported (9, 10).

We have previously published data on the significance of sIL-2R levels in detection of Hairy Cell Leukemia (11, 12) and in staging of lymphomas (13), association with clinical status and histo-pathological grade and response to therapy was demonstrated. Other studies, most of them recruiting a low number of patients, have been searching for important prognostic Tumor Markers in HNC patients by various techniques, using cell lines and proteomic analysis (14, 15, 16, 17, and 18).

We also recently showed that CEA, Cyfra 21-1, TPS and SCC as useful and prognostic serum Tumor Markers in HNC (19). The aim of the present study was to investigate the clinical role and significance of sIL-2R as an immune biomarker in HNC patients, its correlation to disease stage, cancer differentiation, response to various treatments and survival. We also compared this immune-biomarker to the tumor markers we formerly analyzed (19), in order to suggest the best panel of combined markers, to be used for HNC patients.

Patients and Methods

Serum (received after patients' blood centrifugation) levels of sIL-2R were evaluated by a conventional ELISA assay. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-2 Rα has been pre-coated onto a microplate. Standards, samples, and conjugate are pipetted into the wells and any IL-2 Rα present is sandwiched by the immobilized antibody and the enzyme-linked polyclonal antibody specific for human IL-2 Rα. Following a wash to remove any unbound substances and/or antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-2 Rα bound. The color development is stopped and the intensity of the color is measured from Siemens Healthcare Diagnostics (Surrey, UK).

The following groups of 145 HNC patients (from whom we received 328 blood samples) were evaluated before treatment: larynx n=84, nasopharynx n=12, oral cavity n=46, parotid and other salivary gland malignancies n=16. The control group included 45 healthy subjects. Of the HNC patients, 120 were evaluated both prior to and following therapy (surgery, radiation, chemotherapy or combined modalities) and their levels were correlated to clinical status, grade, lymph nodes and response to treatment. Overall survival was estimated according to initial levels of the immune-biomarker. Sensitivity of sIL-2R compared to other tumor markers (CEA, TPS, Cyfra 21-1, SCC), was established by ROC analysis.

Table I. *sIL-2R levels in head and neck cancer patients and healthy controls.*

Head and Neck Cancer	Organ	Subjects (n)	sIL-2R (u/ml±SD)
	Laryngeal Carcinoma	145	1166±86.9
	Nasopharyngeal Carcinoma	12	838±85.7
	Oral Cavity Carcinoma	84	1219±169
	Salivary gland Malignancy	16	1306±322
	Healthy controls	45	687±94

Statistical analysis. Statistical analyses were performed using SPSS software and included non-parametric tests, as Wilcoxon matched-pairs signed rank test, Mann-Whitney test, survival analysis (Kaplan Meyer method and the log rank test) and ROC analysis. A p-value less than 0.05 were considered statistically significant.

Results

Levels of sIL-2R in the subgroups of HNC patients are presented in Table I.

Patients with HNC had significantly higher levels of sIL-2R, compared to the control group (p=0.0001), as shown in Figure 1.

sIL-2R levels were significantly higher in advanced disease (T3 and T4) compared to early disease (T1 and T2), (p=0.02), as shown in Figure 2. In addition, sIL-2R levels were significantly higher in poorly differentiated carcinoma, compared to well differentiated carcinoma (p=0.001), as depicted in Figure 3.

Comparing sIL-2R levels before and following therapy, demonstrated significant higher levels of sIL-2R in all 120 patients before therapy, opposed to decreased levels post-therapy (p=0.01), as shown in Figure 4. A significant correlation (Spearman Coefficient correlation) was demonstrated between response to therapy and sIL-2R levels that decreased accordingly, r=0.5086 (p<0.001). Figure 5 shows the kinetics of sIL-2R as an immune-biomarker, in 4 patients. While after radiotherapy the sIL-2R levels decreased dramatically, the levels of sIL-2R increased parallel to or before detection of metastasis.

sIL-2R levels did not show a significant difference between patients with lymph node involvement, compared to patients with no lymph node involvement (1013±187 *vs*. 886±73), (p=0.4), in contrast to other tumor markers (19).

Analyzing the Receiver Operating Characteristic (ROC) of sIL-2R in patients with laryngeal carcinoma, showed that sIL-2R levels distinguish by 90% between laryngeal cancer and normal controls, at a cutoff point of 533 u/ml, as compared to other Tumor Markers- SCC, TPS, Cyfra 21-1 (19), as shown in Figure 6.

Evaluating the overall survival of the HNC patients showed that lower levels of sIL-2R were correlated with a better survival rate (at a cutoff point of 533 u/ml), as shown in Figure 7.

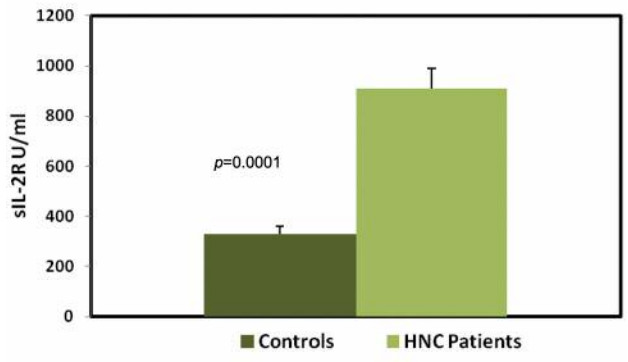

Figure 1. *sIL-2R levels in HNC patients and controls.*

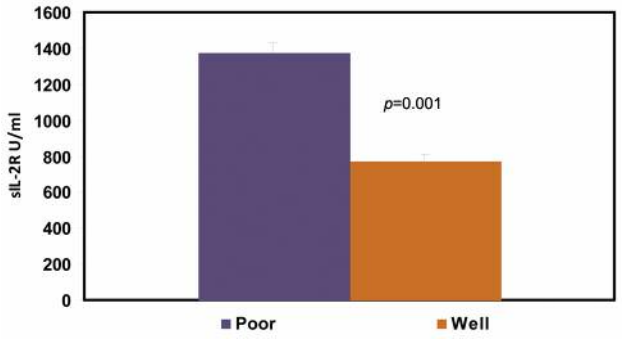

Figure 3. *sIL-2R levels in poorly and well differentiated carcinoma.*

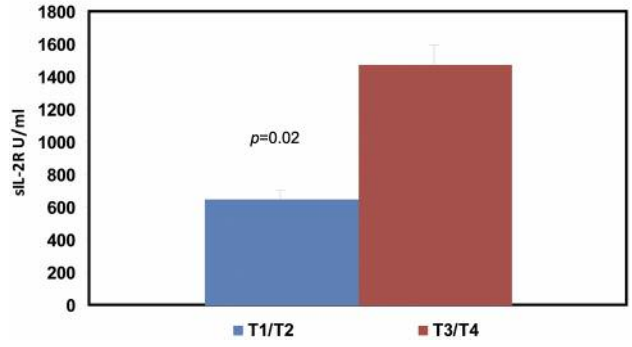

Figure 2. *sIL-2R levels in early (T1&T2) and advanced (T3&T4) HNC patients.*

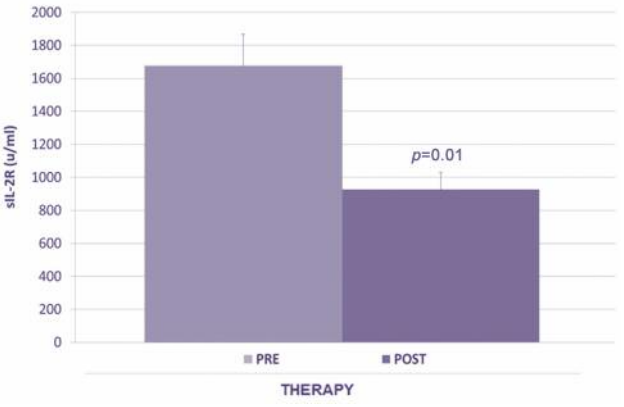

Figure 4. *Effect of therapy on sIL-2R levels of HNC patients.*

Discussion

During recent years there have been many efforts to identify new markers for HNC, using different methods, from HNC cell lines to proteomics (6, 14-18). Potential prognostic markers that can be measured in the serum are particularly interesting, as treatment monitoring and assessment to treatment response can be easily achieved.

Tumor occurrence and progression are closely associated with body cellular immunity function. sIL-2R is an important monitoring index which can reflect the body cellular immunity function (7, 8). Furthermore, sIL-2R has been reported by us and others, as an immune-biomarker in various types of cancer (9, 11, 13, 20, 21) and in various inflammatory diseases as RA, PBC, throat infections and sepsis (22-25).

In the present study, we demonstrated that serum levels of sIL-2R were significantly higher in HNC patients than in healthy controls. This increase of serum sIL-2R was significantly related to the clinical grade and the differentiation of the cancer, with higher levels of sIL-2R in advanced disease and poorly differentiated carcinoma. In addition, our results indicate that pretreatment sIL-2R level of patients with HNC was significantly higher than post-treatment levels. Those high levels or increases of sIL-2R ,were significantly related to the survival of patients - higher levels of sIL-2R were associated with negative prognostic impact and lower sIL-2R levels correlated with a better survival rate (at a cut-off point of 533 u/ml). As an immune biomarker, serum sIL-2R may detect the appearance of metastasis earlier than CTs in post-therapy patients and tumor progression, as shown in Figure 5. These findings show a significant association between sIL-2R levels and tumor burden, proliferative activity, response to therapy, and survival. Our results on a significant number of patients are in concordance with other publications showing sIL-2R as an independent prognostic marker (26, 27). However, surprisingly, another study on a large number of patients did not find sIL-2R as a prognosticator of HNC patients IL-6 was shown in their study to significantly improve outcome prediction (6).

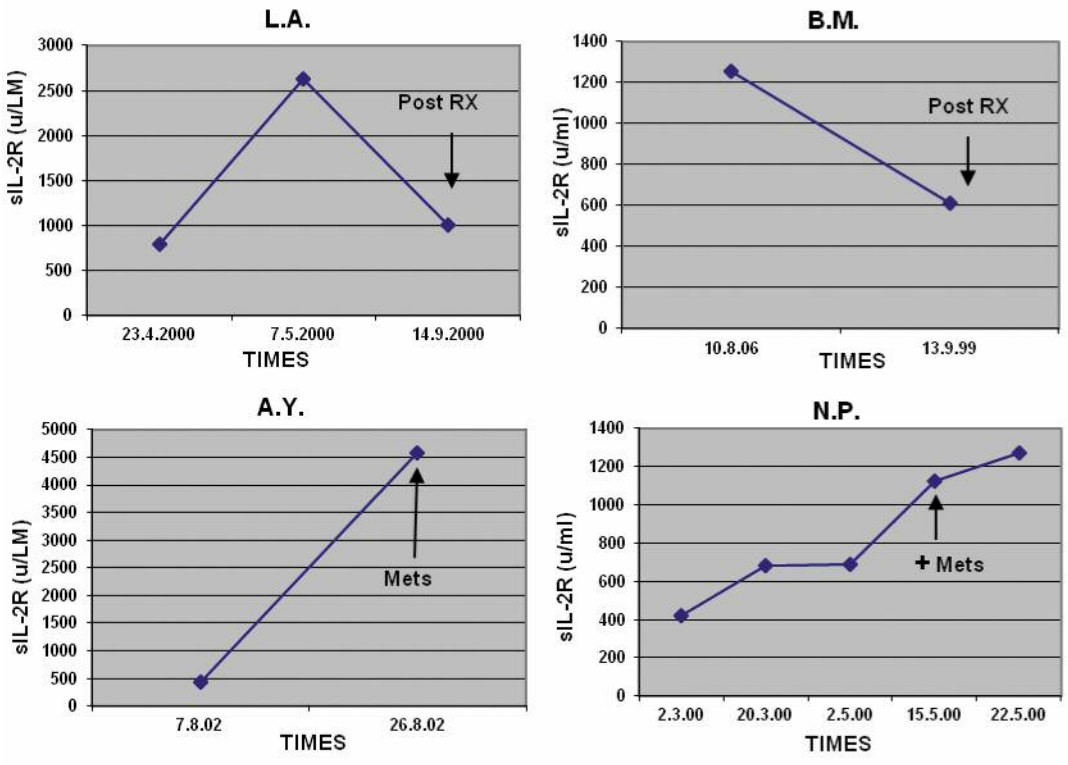

Figure 5. *sIL-2R kinetics in 4 patients following radiotherapy and metastasis detection.*

The biological mechanisms underlying the increase of sIL-2R in serum in the course of malignant processes have not been defined clearly. Cancer growth and development is associated with the stimulation of the innate immune system, including enhanced IL-2R expression on immune cells and its shedding into the circulation in a soluble form of sIL-2R. In most hematological malignancies, including different types of leukemia and lymphomas, sIL-2R has been found to be released directly from the surface of neoplastic cells, thus reflecting the tumor bulk, turnover and activity (7, 8). In these situations sIL-2R may act as a true neoplastic marker. In patients with solid tumors the biological source and role of sIL-2R is more complex. It is suggested that elevated sIL-2R levels in body fluids of patients with solid tumors reflect the augmented release of this receptor from normal lymphoid cells activated in response to tumor growth. However, some authors' postulate that increased levels of sIL-2R do not originate from activated peripheral blood mononuclear cells, but are most probably released from activated lymphoid cells infiltrating neoplastic tissues. These cells have been shown to express CD25 on their surface (28, 29). Although the precise source and biological role of sIL-2R has not been clarified definitively, pretreatment serum levels of sIL-2R have been shown to reflect the activity, advancement and biological aggressiveness of many types of cancer in adults and children, as well as to correlate with prognosis and overall survival (8, 9, 11, 26).

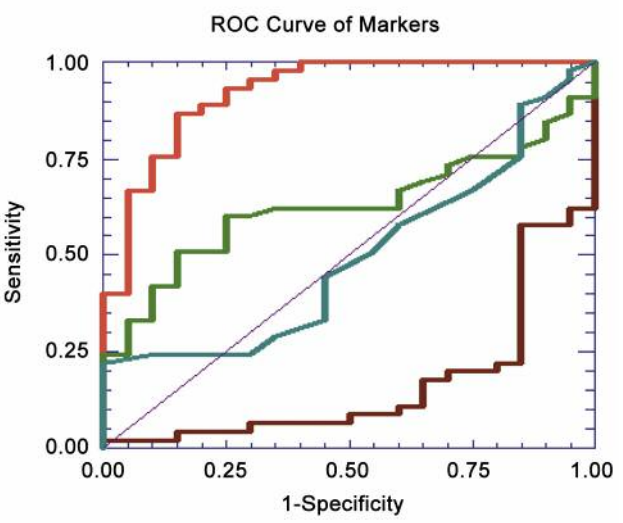

Figure 6. *ROC analysis of sIL-2R levels in laryngeal carcinoma patients, compared to other tumor markers.*

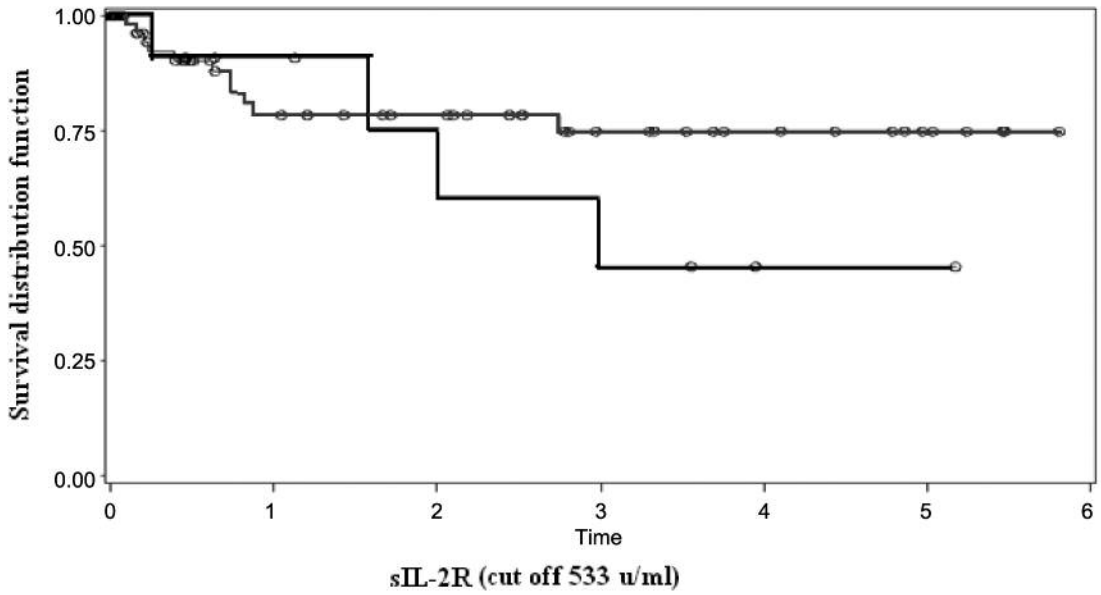

Figure 7. *Kaplan-Meyer survival analysis of HNC patients according to sIL-2R levels.*

Previous studies have shown that elevation of serum sIL-2R has a negative prognostic effect in patients with various malignant tumors, such as malignant melanoma, head and neck cancer, nasopharyngeal carcinoma, non-Hodgkin's lymphoma, non-small cell lung cancer, and clear cell renal cell carcinoma (20, 23, 29). The mechanisms responsible for the association between a high serum sIL-2R level and a worse prognosis have not been elucidated. However, it is possible that the release of this receptor might serve an important immune-regulatory role by competing for IL-2 with cell-surface receptors and thus by decreasing the local or regional immune response (30). In cancer patients and lung diseases high levels of sIL-2R are associated with signs of immune dysfunction, such as a reduced CD4/CD8 ratio and lower IL-2 serum concentration (31-34).

In conclusion, we demonstrated in the present study that sIL-2R is a sensitive and useful immune-biomarker in HNC patients. Higher levels of sIL-2R were correlated with active disease and higher stages. Significant correlations were demonstrated between sIL-2R levels, response to therapy and survival. An increase in sIL-2R levels was the most sensitive predictor of poor prognosis and best correlated to overall survival. Those results are in good concordance with our previous data on sIL-2R and its significance as an independent prognostic biomarker in other malignant diseases.

We, therefore, suggest introducing sIL-2R as a single marker into routine evaluation of HNC patients, or as part of a panel of markers, for useful follow-up, assessment of their response to therapy and early detection of recurrence, in order to improve survival. We also suggested to the EGTM society (European Group for Tumor Markers) to recommend the routine use of this immune biomarker for HNC patients in the clinical setup.

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