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# Soluble Interleukin-2 Receptor as a Clinical Parameter for Nasopharyngeal Carcinoma

**Mow-Ming Hsu, Yeh-Joe Chen, Yen-Liang Chang, Jenq-Yuh Ko, and Tzung-Shiahn Sheen**

**SUMMARY** **We evaluated whether the serum soluble interleukin-2 receptor (sIL-2R) may be a parameter to monitor the efficacy of treatment for nasopharyngeal carcinoma (NPC). There were 177 NPC patients and 24 healthy controls. The level of sIL-2R was measured with a sandwich ELISA kit. Higher levels of sIL-2R than for controls were found in NPC patients before treatment and in patients with distant metastasis ($p<0.001$). There was, however, no difference in sIL-2R levels between controls and NPC patients after radiotherapy in relapse-free or in primary relapse. The sIL-2R levels in sequential testing revealed good correlation with clinical response. The sIL-2R levels were found to be elevated when distant metastasis was detected. Two patients had elevated sIL-2R level up to 5 months before clinical detection of metastasis. These results indicate that serial measurements of sIL-2R levels are worthwhile for NPC patients in their clinical course. The sIL-2R level proved to be an adjunct clinical parameter to monitor the efficacy of treatment of NPC.**

Nasopharyngeal carcinoma (NPC) is the most common cancer of the head and neck in Taiwan, but it is rare in western countries. The treatment of choice for NPC is a high dosage of irradiation. The results of early stages of NPC with irradiation alone were satisfactory. However, the results of advanced stages of NPC even with high dosage of irradiation plus chemotherapy were variable.[1] More than 30% of treatment failure was due to subsequent distant metastases such as to bone, lung, or liver.[2] To these NPC patients, systemic chemotherapy in various protocols is given as our present practice. Antibodies against antigens related to Epstein-Barr virus (EBV) were proved useful as the predictor for subsequent primary recurrence and distant metastasis in NPC after irradiation.[3] However, the sensitivity was small. There is no sensitive clinical parameter to monitor the efficacy of treatment for NPC patients, especially those with bone metastasis.

Histologically, NPC is commonly associated with T-lymphocyte infiltration.[4,5] Interleukin-2 (IL-2) is produced by mature T-lymphocytes in response to lectin or antigen activation, and promotes the growth of T-lymphocytes *in vitro* by interaction with its specific cell surface receptor (IL-2R).[6] Soluble IL-2R (sIL-2R) is released from activated human lymphoid cells *in vitro*[7] and is markedly elevated in animals bearing the aggressive tumor.[8] The levels of sIL-2R have proved to be a reliable marker of neoplastic bulk in hairy cell leukemia.[9] Metastatic cancer patients show significantly larger values of sIL-2R than non-metastatic ones.[10] In our previous tests, we observed that elevated levels of sIL-2R were found in NPC and the amounts of sIL-2R were corrrelated with clinical staging,[11] and hence with the tumor burden of NPC. SIL-2R was also reported to be a reliable predictive index in NPC patients who subsequently developed distant metastasis.[12] The purpose of this work was to clarify whether sIL-2R is a clinical parameter to monitor the efficacy of treatment of NPC.

## MATERIALS AND METHODS

### Subjects and serum collection

In total 177 patients with

From the Department of Otolaryngology, College of Medicine, National Taiwan University.

Correspondence: Mow-Ming Hsu, Department of Otolaryngology, National Taiwan University Hospital, 7 Chung-Shan S. Road, Taipei, Taiwan.

histologically proven NPC were collected retrospectively. There were 127 men and 50 women. The mean age was 48 years, with range 23-68 years. The patients attended the clinic regularly after treatment. Blood samples were obtained before treatment and at regular intervals thereafter for analysis of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), IgG and IgA class antibodies against EBV capsid antigen (VCA), early antigen (EA)[13] and sIL-2R. The NPC patients comprised 84 patients before radiotherapy (pre-RT), 38 patients after radiotherapy in relapse-free state at least six months (post-RT, relapse-free), 19 patients with persistent or recurrent primary disease after rediotherapy (post-RT, primary relapse), and 36 patients with distant metastases. Serum samples from 24 healthy subjects of medical personnel were used as controls. Serum was separated from clotted blood after centrifugation at 500 x g for 10 minutes, and stored at-20°C until sIL-2R analysis.

### Soluble IL-2R immunoassay

Serum samples were thawed and a commercial enzyme-linked immunosorbent assay (ELISA) test kit (T Cell Sciences, Cambridge, Mass, Cellfree Interleukin-2 Receptor Test Kit) was used to determine concentrations of sIL-2R with two monoclonal antibodies that recognize distinct epitopes of sIL-2R molecules. Testing was perpormed according to instructions of the manufacturer. First, both standards and samples, (50 $\mu$l each and in duplicate) were placed in a microtiter plate previously coated with murine monoclonal antibody to human IL-2R. Then, to each well was added horseradish peroxidase-conjugated anti-sIL-2R antibody (100 $\mu$l) directed against a second sIL-2R epitope so as to bind the sIL-2R captured by the first antibody. After rinsing with buffer to remove the unbound components, chromogen substrate solution (100 $\mu$l) was added to the wells. The reaction was then stopped and absorbance was determined at 490 nm. A standard curve was prepared with reference preparations. all tested sIL-2R values were extrapolated from the standard curve and are expressed in units per milliliter (U/ml).

### Statistical analysis

The data were analyzed with rank-sum test, Wilcoxon sign-rank test and chi-square test when appropriate.

## RESULTS

### Soluble interleukin-2 receptor levels and disease status

Fig. 1 demonstrates the serum levels of sIL-2R in NPC patients in varied clinical status and healthy controls. The mean serum sIL-2R for normal controls was 356 ± 137 U/ml, significantly smaller than that of the NPC patients in pre-radiotherapy and those with distant metastasis ($p<0.001$). Significant elevation of the serum sIL-2R, defined as a value greater than mean + two standard deviations of the serum sIL-2R (ie 630 U/ml), was detected in 17% of the controls, whereas elevated serum sIL-2R was found in 42% of the patients in pre-radiotherapy, 18% of those in relapse-free state, 21% of those in primary relapse and 83% of those with distant metastasis respectivly. Patients in pre-radiotherapy as a whole and those with distant metastasis had a significantly greater fraction of elevated sIL-2R than controls. However, the differences between the patients of post-radiotherapy relapse-free or in primary relapse and the controls failed to reach statisical significance.

### Concentrations of serum soluble interleukin-2 receptor and EBV serology in response to treatment

The serum samples from ten patients who developed clinical remission at least six months after treatment were analyzed retrospectively to determine the variation of serum sIL-2R in response to treatment (Fig.2). The concentrations of serum sIL-2R decreased significantly with clinical remission ($p<0.05$). Eight of ten patients had elevated serum sIL-2R in pre-treatment. Six of them (75%) declined to within normal limits, and two decreased significantly although still at the category of elevated level of serum sIL-2R. The remaining two patients had normal concentrations of serum sIL-2R throughout the course of treatment.

The EBV antibody titers were determined in these serum samples.

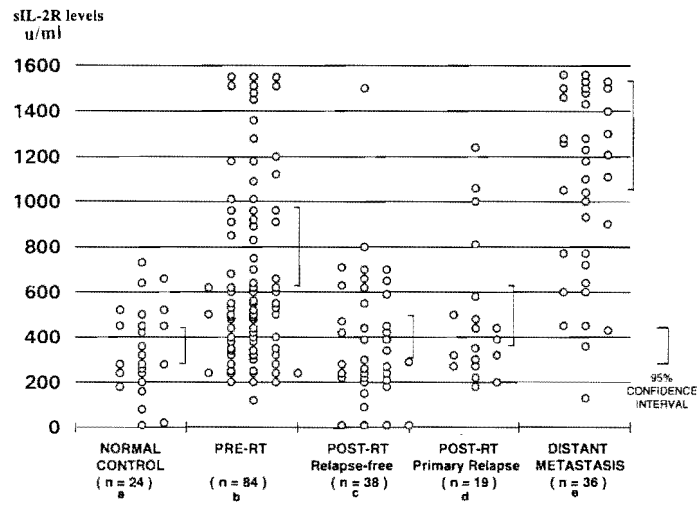


**Fig. 1.** Serum levels of sIL-2R in healthy controls and in patients with varied status of nasopharyngeal carcinoma. RT-radiotherapy.
a vs b or e $P<0.001$, a vs c or d $P>0.05$, b vs e $P<0.001$.

During clinical remission at post-treatment, negative seroconversion of VCA-IgG titre to < 1:320 was observed in only one of ten patient (10%), whereas such seroconversion for VCA-Ig (< 1:10) occurred in two of nine patients (22%). As regards EA-IgG, and EA-IgA, two of eight (25%) and none of eight (0%) showed negative seroconversion with titres of < 1:40 and < 1:10, respectively. Apparently, the sensitivity of EBV antibody titers as an indicator of tumor load is small.

**Concentrations of serum soluble interleukin-2 receptor levels in patients with metastasis**

The serum samples from 11 patients who developed distant metastasis were analyzed retrospectively to determine the prognostic value of sIL-2R in diagnosing metastasis (Fig.3). Two patients (patients 5 and 6) showed increasing serum sIL-2R up to five months before the clinical detection of metastasis. Patient 5, who had liver metastasis, showed decreased concentration of sIL-2R level to normal after chemotherapy, accompanying clinically symptomatic improvement. Patient 6 had bone and liver metastases and refused further treatment. However, four patients (patients 1-4) showed normal levels of serum sIL-2R before clinical detection of metastasis; two (patients 1 and 3) showed increasing concentrations of serum sIL-2R at the time of clinical evidence of distant metastasis. Patient 7, presenting extensive bilateral neck adenopathies, had normal level of serum sIL-2R initially; his sIL-2R level became extremely large to 2,200 U/ml soon after detection of liver and lung metastases and decrased to 860 U/ml when the clinical records showed improvement after chemotherapy. The concentration of serum sIL-2R reached 3,140 U/ml when the symptoms became aggravated before his death. The other patients (patients 8-11) revealed high concentration of serum sIL-2 throughout the clinical course with bone metastasis.

There was no significant correlation between sIL-2R and the antibody titers against EBV antigens or blood chemistry (data not shown).

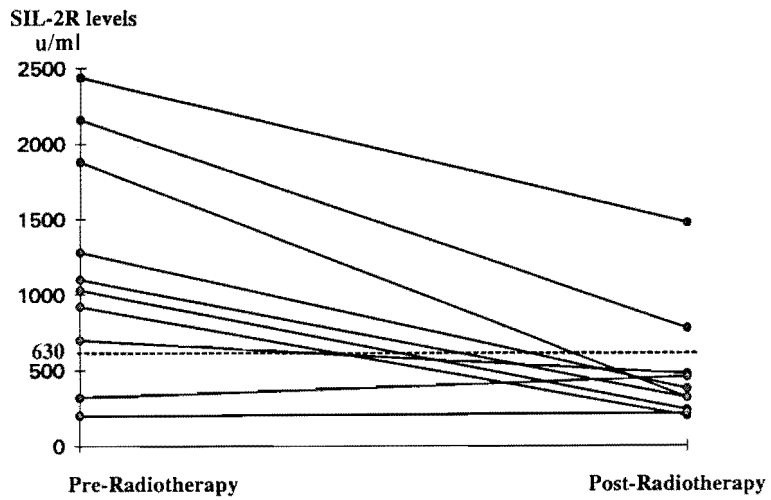


**Fig. 2** sIL-2R in ten patients who responded to treatment at pre-and post-radiotherapy. The dashed line indicates the upper limit of the normal value.

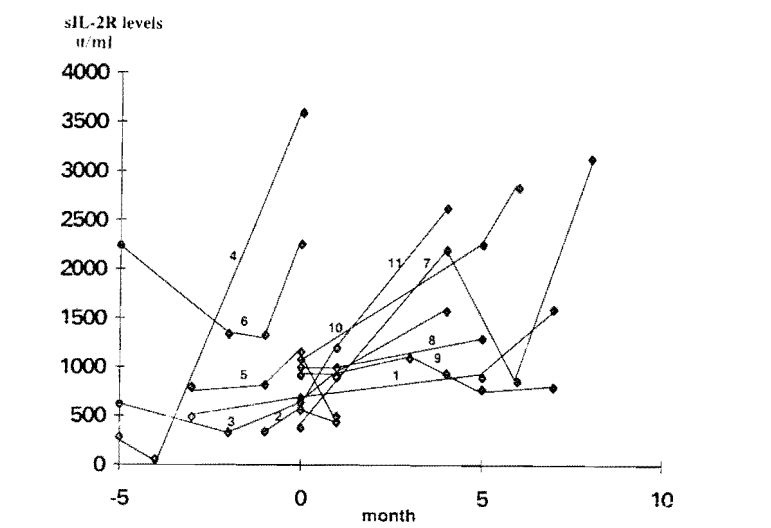


**Fig. 3.** sIL-2R in 11 patients who had metastasis. O indicates the time of clinical detection of metastasis.

## DISCUSSION

In agreement with previous reports,[11,12] in this work we found that abnormally large concentration of sIL-2R in serum commonly occur in NPC patients (Fig.1). The longitudinal tests showed that sIL-2R levels in the blood of ten NPC patients declined with statistical significance ($p<0.05$) after treatment in remission state corresponding to the clinical course. These results indicate that sIL-2R can be used as a clinical parameter to monitor the efficacy of treatment.

Eleven patients were examined for serum sIL-2R repeatedly in the post-treatment course. Two patients had persistently large concentrations of sIL-2R in serum up to 5 months before clinical detection of distant metastasis. These findings agree with those in a previous report.[12] Howere, we are reluctant to state that increased serum sIL-2R almost invariably precedes clinical detection of metastasis in NPC patients, because we found only 83% patients with distant metastasis (Fig.1) and 73% patients at the time of clinical detection of metastatic disease with increasing concentrations of sIL-2R in serum. The predictive value of sIL-2R in the development of metastasis in NPC patients should be tested further.

The mechanism responsible for increased concentrations of sIL-2R in NPC remains unclear. Histologically, primary NPC is generally infiltrated by T-lymphocytes[4,5] and these T-lymphocytes may be immunologi-cally altered due to surrounding tumor cells. This possibility is supported by its elevation before treatment, and its decrease after treatment when the tumor bulk is diminished. The question arises why primary relapse patients fail to show elevated sIL-2R level whereas patients with distant metatasis do. The reason may relate to the size of the tumor load. In the case of primary relapse, the tumor load is typically smaller than those with distant metastasis (ie bone, liver, lung). Hence, infiltrated T-lymphocytes are fewer in primary relapse than in distant metastasis. Therefore concentrations of sIL-2R levels are not elevated when the patients develop primary relapse. Although immunobiological significance is still unknown, sIL-2R could induce decreased IL-2 availability by binding it and competing for IL-2 with the IL-2 cell surface receptor.[14] We might expect an immunosuppressive effect when the elevation of sIL-2R level in NPC patients results in less potent immunosurveillance for tumor, and also an increased risk of relapse as in non-small cell lung carcinoma.[15]

In conclusion, this work shows that serial measurements of concentrations of sIL-2R in serum of NPC patients are worthwhile. The level of sIL-2R is an adjunct clinical parameter to monitor the efficacy of treatment. However, the usefulness of sIL-2R as a parameter to predict subsequent distant metastasis in NPC should be examined further.

## ACKNOWLEDGEMENTS

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