

## The rate of natural killer cells and their cytotoxic activity in patients with advanced pharyngeal and laryngeal cancer

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*The activity of natural killer (NK) cells was assessed in 46 patients with previously untreated advanced squamous cell carcinoma of the head and neck region. The mean NK cell activity of these patients was lower than that observed in a group of 32 age-matched controls (62.5 vs 90), however, the difference was not statistically significant. Patients with regional metastases that grew through the lymph node capsule and those with primary tumor directly invading the surrounding tissue had significantly lower values of NK cell activity than patients with locoregionally controlled tumors ( $p = 0.02$ ).*

*Key words:* pharyngeal neoplasms; laryngeal neoplasms; killer cells, natural

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### Introduction

The role of natural killer (NK) cells in patients with malignomas of the head and neck has not been fully explained yet. Many facts support their possible influence in the destruction of circulating tumor cells,<sup>1</sup> and some authors believe that a decreased cytotoxic activity (CA) of these cells attributes to the appearance of distant metastases.<sup>2-5</sup> The latter observation seems to be of particular importance and interest in the case of patients with head and neck tumors, as such indicators of the probability of distant metastatic spread would be very helpful for correct treatment selection.

In our prospective study we were determining the number of NK cells and their CA in the peripheral blood of patients with advanced laryngeal and pharyngeal cancer, in order to assess their possible relevance for prognosing the course of disease.

### Methods

The study was carried out in 46 patients, 40-81 years of age, with previously untreated and histologically confirmed advanced carcinoma of the pharynx and larynx. The disease was classified as stage 3 or 4 in all patients.<sup>6</sup> In 23 of them the site of origin was the oropharynx, in 15 hypopharynx and in 8 larynx. The prevailing histologic type was poorly differentiated or non-keratinizing planocellular carcinoma (37 patients), whereas well differentiated keratinizing planocellular carcinoma was established in 9

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cases. Twenty-two patients were treated by radiotherapy, 20 were operated on – of these 18 also received postoperative irradiation, and 4 patients were treated by a combined radio- and chemotherapy.

The control group consisted of 32 patients admitted to the University Department of Otorhinolaryngology and Cervicofacial Surgery because of some other, non-malignant diseases; the controls were matched to the studied cancer patients by age and sex.

CA of NK cells was determined by means of K 562 tumor cell killing test. These cells were mixed with lymphocytes isolated from the peripheral blood of patients with laryngeal and pharyngeal cancer. After 4-hour incubation, the rate of killed target cells was evaluated, and calculated in lithic units, where their higher value was associated with a higher K 562 tumor cell killing potential of NK cells.

### Results and discussion

In patients with advanced cancer NK activity is usually depressed.<sup>7-11</sup> Also in our patients, the average CA of NK cells was lower than that observed in the control group (62.5 vs 90), though the difference between both groups was not statistically significant. The average rate of NK cells in patients with laryngeal and pharyngeal cancer was 16.1%, being lower from that in the control group, though the difference between both groups was not statistically significant.

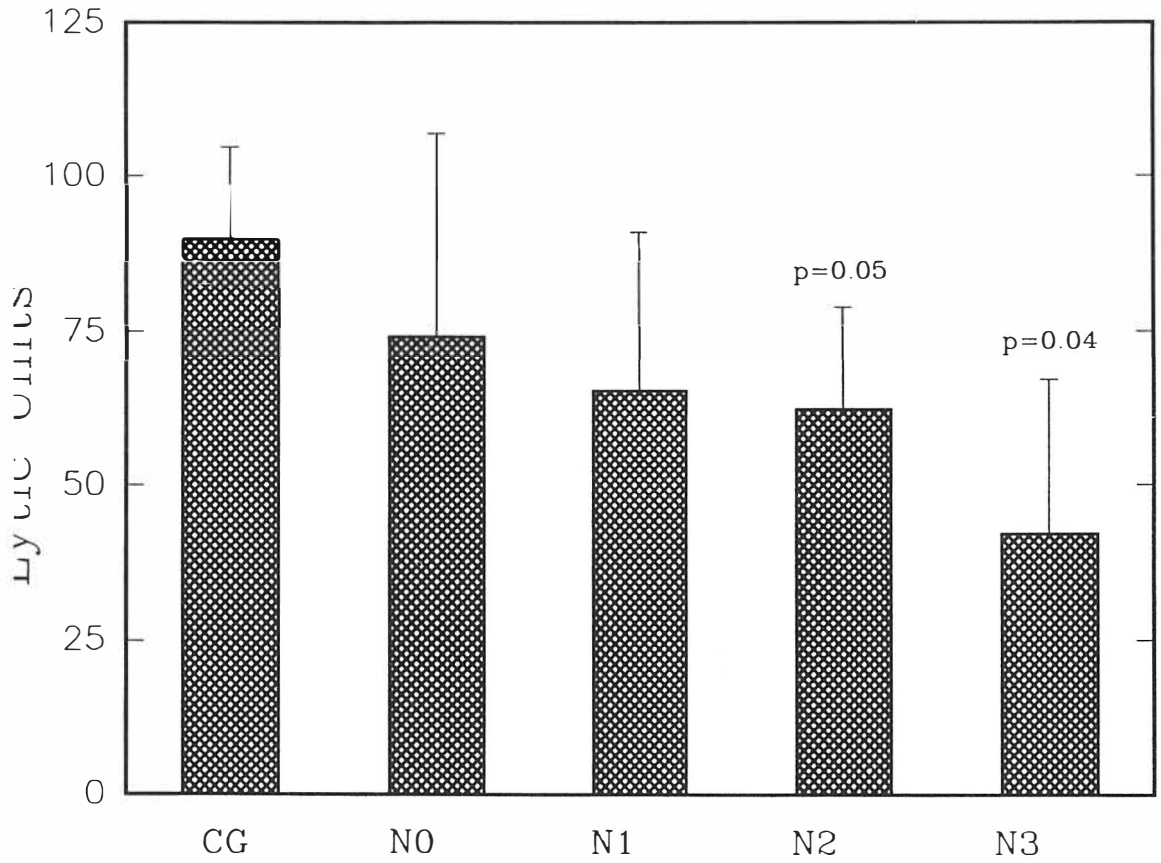
Comparison of the average rates of NK cell counts and their cytotoxic activity by tumor site and histologic type did not show statistically significant differences, and neither confirmed a correlation between the average rate of these cells and their CA. Similarly, data from single-cell cytotoxicity assays suggest that the defect rests in the activity and not in the number of NK cells.<sup>12</sup>

Since our investigation was aimed to assess a possible prognostic relevance of NK cells, and since in patients with laryngeal and pharyngeal cancer the best indicator of the probability of

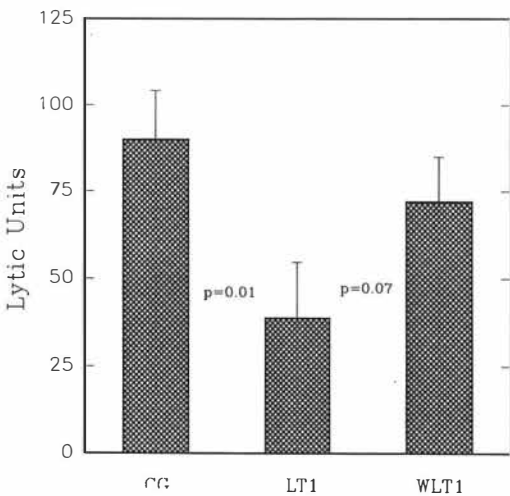
distant dissemination is known to be local and regional tumor spread,<sup>13-15</sup> our analysis of the results was centred particularly on these issues. The available literary data on the CA of NK cells in patients with head and neck carcinomas are relatively scarce. There are, however, even less reports on the role and significance of CA of these cells for the appearance of local metastases. Cortesina et al.<sup>16</sup> report a decreased CA of NK cells in advanced head and neck cancers. Pross and Baines<sup>7</sup> established a decreased CA in patients with metastases in comparison with cases without regional tumor dissemination. Schantz and coworkers,<sup>17</sup> however, found an increased CA of NK cells in patients with local or regional tumor invasion of the surrounding tissue.

In our study, the average CA of NK cells in patients with regional metastases in N3 and N2 was found to be significantly lower than that in the control group ( $p = 0.04$  and  $0.05$  respectively), whereas in patients with N0 and N1 no statistically significant difference could be established (Figure 1). The extent of local dissemination (control group – N0-N1-N2-N3) was in correlation with the values of average CA in individual groups: thus, a 5% risk was associated with negative correlation ( $KK = -0.274$ ).

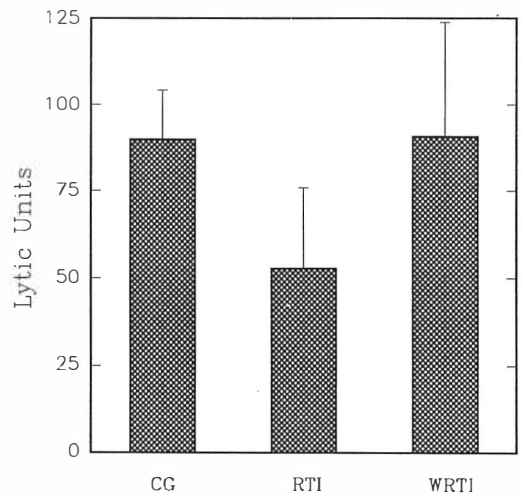
The comparison of NK cell rates and their CA in the group of patients with local or regional spread, and those without evidence of dissemination revealed interesting differences. In the cases when tumor did not show evidence of local or regional invasion into the surrounding tissues the rate of NK cells was on average lower than that in the group with local infiltration (13.5 vs 18.2), though the difference was not statistically significant ( $p = 0.08$ ). An average CA of NK cells in the group of patients with local tumor invasion (39) was significantly lower than the relevant values in the control group (90) ( $p = 0.01$ ), and borderline significantly lower than in patients without local infiltration (73) ( $p = 0.07$ ) (Figure 2). Also, an average CA in the group of patients with regional invasion (44) was significantly lower than that in the control group (90) ( $p = 0.02$ ) and borderline significantly lower than in the group



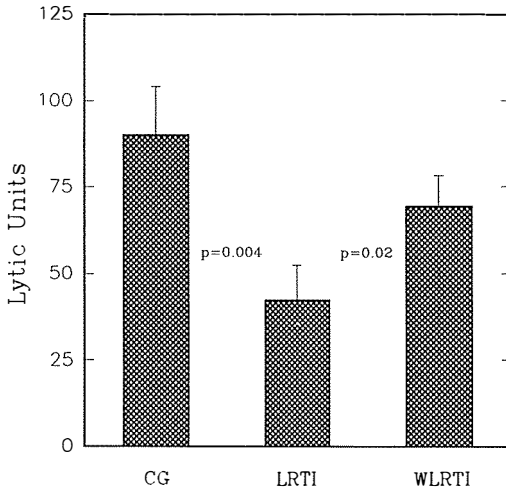
**Figure 1.** Mean CA of NK cells according to N stage (CG = control group).



**Figure 2.** Mean CA of NK cells according to local tumor infiltration into surrounding tissue (CG = control group; LTI = local tumor infiltration; WLT1 = without local tumor infiltration).



**Figure 3.** Mean CA of NK cells according to regional tumor infiltration into surrounding tissue (CG = control group; RTI = regional tumor infiltration; WRTI = without regional tumor infiltration).



**Figure 4.** Mean CA of NK cells according to local and regional tumor infiltration into surrounding tissue (CG = control group; LRTI = local and/or regional tumor infiltration into surrounding tissue; WLRTI =  $\pm$  without local and/or regional tumor infiltration into surrounding tissue).

without regional invasion (75) ( $p = 0.07$ ) (Figure 3). The difference is apparent also in the group of operated patients in whom the removed lymph nodes were histologically examined.

When the average CA in the group without locoregional invasion into the surrounding tissues was compared with that found in the group with locally and regionally infiltrating tumor the difference was statistically significant ( $p = 0.02$ ) (Figure 4). These findings can be presented from yet another aspect: in only one of 18 patients without evidence of locoregional invasion CA was lower than 30 lithic units (LU) (5.5%). On the other hand, in the group of 28 patients with tumor invasion into the surrounding tissues CA was below 30 LE in as many as 17 patients (61%). Therefore CA lower than 30 LE could be considered as unfavourable prognostic sign.

### Conclusion

The findings of our investigation indirectly support the hypothesis that in patients with laryngeal and pharyngeal cancer the course of disease is reflected in the CA level of NK cells.

Nevertheless, it should be kept in mind that our results are only preliminary, and therefore their final evaluation would require further follow up of the patients. Only in this way the hypothetical correlation between the CA of NK cells and prognosis of the disease could be confirmed.

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