Serum Interleukin-10 Levels as a Prognostic Factor in Advanced Non-small Cell Lung Cancer Patients*

Ferdinando De Vita, MD, PhD; Michele Orditura, MD; Gennaro Galizia, MD; Ciro Romano, MD, PhD; Annarita Roscigno, MD; Eva Lieto, MD; and Giuseppe Catalano, MD, PhD

Study objective: To investigate the prognostic significance of interleukin (IL)-10 serum levels in advanced non-small cell lung cancer (NSCLC) patients.

Design: IL-10 serum levels were measured before chemotherapy, on completion of therapy, and at follow-up by means of a commercially available enzyme-linked immunoassay. The results were then analyzed in comparison with other prognostic variables, and a model predicting overall survival (OS) and time to treatment failure (TTF) was finally generated.

Setting: University hospital.

Patients: Sixty consecutive patients with TNM stage III or IV NSCLC undergoing conventional platinum-based regimens.

Results: Elevated levels of serum IL-10 were found in cancer patients with respect to healthy control subjects (17.7 ± 4.4 vs 9.2 ± 1.5 pg/mL, respectively; p < 0.05), with patients with metastatic disease showing significantly higher levels than patients with undisseminated cancer (21.0 ± 4.2 vs 14.3 ± 1.2 pg/mL, respectively; p < 0.05). Following completion of treatment, patients were classified as responders if they had achieved either one of the following: complete response, partial response, or stable disease; and nonresponders, in case of progressive disease. Retrospective analysis of basal IL-10 serum levels in these two subgroups showed a significant difference between responders and nonresponders (15.2 ± 2.2 vs 21.4 ± 4.2 pg/mL, respectively; p < 0.05). Moreover, a further significant increase in IL-10 serum levels was observed in nonresponders at the end of therapy (21.4 ± 4.2 vs 26.0 ± 4.3 pg/mL, prechemotherapy and postchemotherapy, respectively; p < 0.05), whereas values in responders were found to have significantly decreased (15.2 ± 2.2 vs 14.8 ± 2.2 pg/mL, prechemotherapy and postchemotherapy, respectively; p < 0.05). Using univariate and multivariate analyses, both OS and TTF were shown to be affected by the mean pathologic levels of IL-10. Stepwise regression analysis identified IL-10 serum level and stage as the prognostic factors related to OS, and IL-10 serum level and performance status as the prognostic factors related to TTF.

Conclusions: In conclusion, this study shows that the measurement of pretreatment IL-10 serum levels is of independent prognostic utility in patients with NSCLC and may be useful for detection of disease progression.

(CHEST 2000; 117:365–373)

Key words: interleukin-10 serum levels; non-small cell lung cancer; prognosis

Abbreviations: CI = confidence interval; df = degrees of freedom; IL = interleukin; IQR = interquartile range; NSCLC = non-small cell lung cancer; OS = overall survival; PR = partial remission; PS = performance status; TTF = time to treatment failure

Animal models have demonstrated that immunization with tumor cells or purified tumor antigens can confer protective immunity against a subsequent challenge with cancer cells.1–3 However, malignant cells obviously escape immune surveillance, which suggests that they possess the ability of evading any potentially lethal effector mechanisms operated by the immune system. Potential strategies that tumors may use to avoid host immune responses include down-regulation of major histocompatibility complex molecules, expression of poorly immunogenic tumor antigens, masking or shedding of immunogenic tumor antigens, induction of tolerance via display of an incomplete antigen-presenting function, and secretion of immunosuppressive factors.4–6

From the Department of Clinical & Experimental Medicine “F. Magrassi,” Second University of Naples School of Medicine, Naples, Italy.

Manuscript received March 8, 1999; revision accepted September 14, 1999.

Correspondence to: Ferdinando De Vita, MD, PhD, Chair of Medical Oncology, Department of Clinical & Experimental Medicine “F. Magrassi,” Second University of Naples School of Medicine, Naples, Italy, c/o II Policlinico, Via S. Pansini, 5, 80131 Naples, Italy. e-mail: galizia@unina.it

CHEST / 117 / 2 / FEBRUARY, 2000 365
Recently, several studies have focused on the cytokine network involved in the tumor microenvironment. With regard to this, at least two distinct cytokine patterns are known to be generated by T lymphocytes: type 1 cytokines, which include interleukin (IL)-2 and interferon-γ and have been demonstrated to promote cell-mediated immunity, and type 2 cytokines, which include IL-4, IL-5, IL-10, and IL-13 and have been shown to suppress cellular immune responses. Among type 2 lymphokines, IL-10 has been associated with inhibition of a broad array of immune functions, such as T lymphocyte proliferation, type 1 cytokine production, antigen presentation, and lymphokine-activated killer cell cytotoxicity.

Thus, it is not surprising that a high frequency of IL-10 messenger RNA and a low frequency of IL-2 and interferon-γ messenger RNAs have been detected by means of reverse transcriptase-polymerase chain reaction analysis in various tumor samples, in addition, IL-10 has been demonstrated to be produced and secreted not only by immune cells but also by cancer cells, including lymphoma, ovarian carcinoma, melanoma, neuroblastoma, renal cell and colon carcinoma, and non-small cell lung cancer. These findings indicate that IL-10 is likely to be involved in suppressing host’s antitumor immune responses.

Recently, measurement of IL-10 serum levels in cancer patients has been suggested as a potential prognostic marker. Therefore, in this study, to evaluate the prognostic significance of IL-10 serum levels, we measured this cytokine concentrations in the sera of 60 NSCLC patients before and after chemotherapy, and at follow up; the results were analyzed in comparison with other prognostic variables, and, finally, a model predicting overall survival (OS) and time to treatment failure (TTF) was generated.

**Materials and Methods**

*Patients*

The study population consisted of 60 consecutive patients with advanced histologically proven and previously untreated NSCLC. All patients had a history of smoking, and none of them was affected by autoimmune diseases, inflammatory bowel disease, chronic liver disease, asthma, allergies, or other concomitant diseases capable of interfering with IL-10 assay. Staging was expressed according to TNM classification on evaluation of findings of physical examination, routine laboratory tests, and diagnostic imaging (chest radiograph; brain, chest, and abdomen CT scan; scintigraphic bone scan; and endoscopy). All patients were treated with cisplatin and etoposide, with courses delivered at 21-day intervals, according to hematologic tolerance. On completion of the three cycles, patients were restaged, with the continuation of chemotherapy depending on the type of response. A complete remission was defined as the disappearance of all evidence of disease that lasted at least 1 month. A partial remission (PR) was defined as a >50% decrease in the size of the longest perpendicular cross-sectional diameter of all lesion that lasted at least 1 month, without appearance of new tumor. Stable disease was considered as an objective response without satisfying the PR criteria or no change in the disease status. Finally, progressive disease was considered as a >25% increase of the measurements or the appearance of new lesions. Patients who obtained a PR or a stable disease continued the treatment until progression, while those with progressive disease were considered unresponsive. TTF was calculated from beginning of treatment until disease progression, death, or last follow-up evaluation. OS was calculated from initiation of treatment until death or the date of last evaluation.

**Serum IL-10 Determination**

Blood samples were obtained from patients before treatment, at the time of restaging, and on relapse. To exclude the possible interference of chemotherapy, blood was drawn at least 21 days apart from the last administration of cytotoxic drugs. Available to us were also serum samples from 25 age- and sex-matched healthy donors, which were used to determine mean ± SD, range, median, lower and upper 95% confidence intervals (CIs) for median, interquartile range (IQR) of IL-10 serum levels in disease-free subjects (control subjects). Serum samples were obtained by centrifugation at 3,000 revolutions/min for 10 min and stored at −80°C until use. Serum levels of IL-10 were determined using a commercially available sandwich enzyme immunoassay kit (Endogen IL-10 ELISA kit; Endogen; Cambridge, MA). Samples were prepared and tested in duplicate according to the instructions of the manufacturer. Briefly, samples or standards were added to an anti-human IL-10 monoclonal antibody-coated 96-well microplate. IL-10 present in the samples was immobilized by the primary antibody during incubation at room temperature. After thorough washings to remove unbound proteins, a horseradish peroxidase-conjugated polyclonal antibody was added to the wells. Following further washings to remove unbound conjugate, IL-10 capture was manifested by adding a chromogenic substrate that yielded a colored product in proportion to the amount of IL-10 present in the samples. Absorbance was read against a blank using a microtiter enzyme-linked immunosorbent assay reader set at 450 nm, and a standard curve was prepared from the group of serially diluted standard samples of IL-10. Unknown values were determined from the standard curve and expressed in picograms per milliliter. As reported by the manufacturer, this assay is specific for human IL-10 and does not cross-react with other known cytokines. The detection limit of the enzyme-linked immunosorbent assay reader for IL-10 is <3 pg/mL, with an intra-assay and an interassay variation <10%. Values were considered elevated when they exceeded the mean of control subjects plus two SDs.

**Statistical Analysis**

Statistical analysis was performed using the BMDP statistical package (BMDP Statistical Software; Los Angeles, CA). In all analyses, the significance level was specified as p < 0.05. Comparisons between continuous variables (eg, IL-10 serum levels in control group vs basal [that is, prechemotherapy]) IL-10 serum levels in cancer patients) were performed using the Mann-Whitney U test for unpaired data and Wilcoxon signed rank test for paired data. For all data are provided mean ± SD, range, median, lower and upper 95% CI for median, and IQR. The seven prognostic factors considered were as follows: sex,
age, stage, grade, performance status (PS) according to the Eastern Cooperative Oncology Group scale, basal IL-10 serum levels, and histology.

Univariate analysis related to OS and TTF was performed on each variable, and significance was determined by log-rank test (Cox-Mantel).

Univariate analysis for continuous variables was performed by subclassifying the patients into two subgroups according to their value of serum IL-10 relative to an arbitrary cut-off (high or low, see below).

Survival curves were plotted using the product-limit method (Kaplan-Meier) and analyzed using the Generalized Savage test or Cox-Mantel test with computer software (BMDP1 L; BMDP Statistical Software, Inc).

The independent significance of every prognostic variable related to OS and TTF was determined by multivariate analysis using Cox’s proportional hazards model. Basal IL-10 serum level was analyzed as a continuous variable. Coefficient, SE, and hazard rate were considered; level of significance was obtained by score test (BMDP2 L; BMDP Statistical Software, Inc). Finally, a stepwise multivariate analysis was performed and a predictive model of the best linear combination of variables predicting OS and TTF was developed; maximum partial likelihood ratio test was used with remove and enter limits of 0.15 and 0.10, respectively (BMDP2 L). Again, basal IL-10 serum level was analyzed as a continuous variable.

RESULTS

IL-10 Serum Levels

IL-10 serum levels were significantly higher in cancer patients prior to chemotherapy (17.7 ± 4.4 pg/mL; range, 12 to 35 pg/mL; median, 16.3 pg/mL; 95% CI, 15.0 to 19.5; IQR, 6) with respect to control subjects (9.2 ± 1.5 pg/mL; range 7.4 to 12.0; median 9.0; 95% CI, 8.4 to 10.0; IQR, 1.86; p < 0.05). In addition, IL-10 serum levels were shown to be significantly increased in stage IV patients (n = 32; 21.0 ± 4.2 pg/mL; range, 14 to 35; median, 20.0; 95% CI, 19.5 to 22.5; IQR, 4.1) as compared to stage III patients (n = 28; 14.3 ± 1.2 pg/mL; range, 12.0 to 16.5 pg/mL; median, 14.0 pg/mL; 95% CI, 13.5 to 15.0; IQR, 2; p < 0.05).

Following completion of chemotherapy, 36 patients were classified as responders, as they were shown to have achieved either one of the following: stable disease or PR (no complete remission was recorded); and 24 patients were regarded as nonresponders because of disease progression. When basal IL-10 serum levels in cancer patients were analyzed according to this subclassification, we observed a significant difference between responders (15.2 ± 2.2 pg/mL; range 12.0 to 19.8; median, 14.5; 95% CI, 14.0 to 15.5; IQR, 2.7) and nonresponders (21.4 ± 4.2 pg/mL; range, 15 to 35 pg/mL; median, 20.7 pg/mL; 95% CI, 19.9 to 22.5; IQR, 3.4; p < 0.05). Both subgroups had still higher IL-10 serum levels than the healthy subjects (p < 0.0001 for both vs control subjects).

Furthermore, IL-10 serum levels were shown to have significantly decreased in the responder group at completion of therapy (basal values, 15.2 ± 2.2 pg/mL; posttreatment values, 14.8 ± 2.2 pg/mL; range, 11.5 to 19.2; median, 14.0; 95% CI, 13.8 to 15.1; IQR, 3; p < 0.05), whereas they were found to have significantly increased in the nonresponder group (basal values, 21.4 ± 4.2 pg/mL; posttreatment values, 26.0 ± 4.3 pg/mL; range, 17.9 to 38.6; median, 25.6; 95% CI, 24.5 to 28.2; IQR, 4.45; p < 0.05; Fig 1).

Based on these results, we retrospectively decided to use 19.6 pg/mL (mean ± 2 SD of basal IL-10 serum levels in responder patients) as the cutoff value for analysis of survival rates and TTF, since values > 19.6 pg/mL appeared to identify nonresponder patients only (see below).

Analysis Related to Survival Rate

None of the patients were lost at follow-up; at the end of the study, 5 patients (8.3%) were still alive and 55 (91.7%) had died. The mean OS was 13.3 months (range, 2.57 to 43.38). Seventy-fifth quartile survival time was 7.43 months; 50% of the patients

![Figure 1. IL-10 serum levels before and after chemotherapy. Following completion of therapy, IL-10 serum levels were shown to be decreased in responders (n = 36), whereas a significant increase was observed in nonresponders (n = 24; p < 0.05; Wilcoxon signed rank test).](http://journal.publications.chestnet.org/pdffaces.asmx?url=/data/journals/chesteri/21939/)
survived 12.35 months. None of the patients died of causes other than NSCLC. Mean follow-up time was 12.8 ± 7.8 months. Survival rates were not significantly different among groups stratified for sex, age, grade, and histology. On the contrary, stage, PS, and basal IL-10 serum levels significantly affected survival rate (Figs 2–4 and Table 1).

Product limit survival analysis demonstrated a statistically significant association between IL-10 serum levels and OS: patients with IL-10 serum levels < 19.6 pg/mL (group A; n = 39; 36 responders and 3 nonresponders) had a significantly longer survival than patients with IL-10 serum levels > 19.6 pg/mL (group B; n = 21; all nonresponders); mean survival time was 16.7 and 6.8 months, respectively, in group A and group B patients. Seventy-fifth quartile, median, and 25th quartile survival times were 12.15 and 4.68 months, 14.32 and 6.58 months, and 16.93 and 7.97 months, respectively, in group A and group B patients, respectively. Group B patients had almost a fivefold increased risk of death as compared to group A patients (Fig 4).

Using multivariate analysis, IL-10 was demonstrated to be the only covariate independently associated with OS (Table 2). The log minus log plot was used to check the proportionality assumption. On
stratification of basal serum IL-10 levels (≤ 19.6 pg/mL and > 19.6 pg/mL), the plot exhibited constant differences between strata (global $\chi^2$, 13.92; degrees of freedom [df], 6; p = 0.03), thus demonstrating that the proportionality assumption held. After backward elimination, sex, age, grade, PS, and histology were removed from the model; stepwise regression selected basal serum IL-10 level (p = 0.0003) and stage (p = 0.0393) as the best combination of variables to predict survival.

**Analysis Related to TTF**

Of the five patients still living at the end of the study, one had progression of his disease; therefore, overall progression rate (living and dead patients)

![Graph showing survival rates](image)

**Figure 4. OS according to basal IL-10 serum levels.** Group A patients (n = 39; IL-10 serum level ≤ 19.6 pg/mL) had a statistically significant longer survival than group B patients (n = 21; IL-10 serum level > 19.6 pg/mL; test statistic = 49.060; p = 0.0001 [Cox-Mantel test]).

| Table 1—Individual Prognostic Significance Related to Survival (Univariate Analysis by Log-rank Test) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables       | Patients, No.   | Living, No.     | Dead, No.       | Hazard Rate (Cox-Mantel Test) | p Value        |
| Sex             | 50              | 2               | 48              | 1.09                         | 0.20           |
| Male            | 49              | 2               | 47              |                              |                |
| Female          | 11              | 3               | 8               |                              |                |
| Age, yr         | 60              | 28              | 4               | 24                           | 0.99           |
| ≤ 60            | 28              | 4               | 24              |                              |                |
| > 60            | 32              | 1               | 31              |                              |                |
| Stage           | 32              | 4               | 24              | 0.59                         | 0.0001*        |
| III             | 28              | 4               | 24              |                              |                |
| IV              | 32              | 1               | 31              |                              |                |
| Grade           | 36              | 4               | 32              | 2.12                         |                |
| 1               | 7               | 0               | 7               | 0.93                         | 0.8            |
| 2               | 44              | 4               | 40              | 1.06                         |                |
| 3               | 9               | 1               | 8               | 0.83                         |                |
| PS              | 36              | 4               | 32              | 3.00                         |                |
| 0               | 18              | 3               | 15              | 0.53                         | 0.0001*        |
| 1               | 33              | 2               | 31              | 1.30                         |                |
| 2               | 9               | 0               | 9               | 3.00                         |                |
| Pathology       | 36              | 4               | 32              | 0.88                         | 0.47           |
| Adenocarcinoma  | 22              | 4               | 18              |                              |                |
| Epidermoidal    | 38              | 1               | 37              |                              |                |
| Prechemotherapy serum IL-10 concentration† | 39              | 4               | 35              | 0.71                         | 0.0001*        |
| Group A         | 21              | 1               | 20              | 3.28                         |                |

*Significant difference.
†Group A, IL-10 ≤ 19.6 pg/mL; group B, IL-10 > 19.6 pg/mL.
was 91.6% (56 patients). Mean of TTF was 10.7 months (range, 1.31 to 30.72). Seventy-fifth quartile of TTF and median time were 4.16 and 9.89 months, respectively.

As in the previous statistic study related to survival, univariate analysis selected stage, PS, and basal IL-10 serum levels as variables with prognostic value (Figs 5–7 and Table 3). Using multivariate analysis, IL-10 serum level \( (p = 0.0020) \) was considered as the only independent covariate related to TTF (Table 4). The best model to predict likelihood of disease progression, selected by stepwise regression analysis, included IL-10 serum level \( (p = 0.0001) \) and PS \( (p = 0.0623) \).

Product limit survival analysis demonstrated a statistically significant association between IL-10 serum levels and TTF; mean TTF was 13.9 and 4.57 months in group A and group B patients, respectively. Seventy-fifth quartile, median, and 25th quartile were 9.36 and 2.56 months, 11.6 and 4.0 months, 13.57 and 5.31 months, respectively, in group A and group B patients. Group B patients had a fivefold increased risk of disease progression as compared to group A patients.

**DISCUSSION**

NSCLC is characterized by an aggressive clinical course and poor response to immunotherapy,\(^{24,25}\) probably because of the ability of NSCLC cells to produce a wide variety of immunosuppressive factors that may allow escape from immune recognition. A candidate for such a factor in humans is IL-10, a pleiotropic type 2 cytokine with suppressive activity against various aspects of the cellular immunity, namely, inhibition of proinflammatory cytokine production, reduction of antigen-specific activation of T lymphocytes by impairment of antigen-presenting cell function, down-regulation of production of tumoricidal molecules such as tumor necrosis factor-\(\alpha\), and inhibition of natural killer cell cytotoxicity.\(^{12–14,26,27}\)

![Figure 5. TTF according to stage. Time to disease progression was found to be statistically longer in stage III \((n = 28)\) than stage IV patients \((n = 32); \) test statistic = 24.771; \(p = 0.0001\) [Cox-Mantel test].](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21939/ on 06/21/2017)
Indeed, elevated levels of IL-10 have been reported in sera from patients with NSCLC as well as different histotypes of solid and hematopoietic tumors, suggesting that a predominant T-helper type 2 pattern of cytokine secretion is commonly operative at the tumor site regardless of the histology; this observation that different malignancies can adopt the same strategy in their struggle against immunorecognition suggests that this mechanism is likely to turn out very rewarding to cancer cells.

In agreement with the above-mentioned data, in this study, serum levels of IL-10 were found to be elevated in NSCLC patients when compared to healthy controls; moreover, IL-10 serum levels were demonstrated to be higher in patients with metastatic disease as opposed to the values recorded in patients with undisseminated cancer.

Interestingly, when we analyzed the patient population based on the type of response to therapy, we observed that the mean of basal values in patients for whom treatment proved to be ineffective was significantly higher than that of responder patients. With time, moreover, we recorded a further significant increase in IL-10 serum levels in nonresponders, and
a significant decrease in responders at the end of the follow-up period. Although we did not search for the origin of IL-10, we hypothesize that the main source of this cytokine in our patients was the tumor itself rather than the inflammatory infiltrates, based on the observation that tumor shrinkage was paralleled by a decrease in IL-10 levels and on recent reports documenting the ability of NSCLC cells to produce IL-10.19,20

Using univariate analysis, both OS and TTF appeared to be affected by the mean pathologic levels of IL-10, with patients with values ≤ 19.6 pg/mL living longer than patients with values > 19.6 pg/mL. These results appear to support our hypothesis of IL-10 being a potential prognostic factor. However, they might be considered misleading, since 36 out of 39 patients with IL-10 serum levels ≤ 19.6 pg/mL were responders, whereas all the 21 patients with IL-10 values > 19.6 pg/mL were nonresponders; in other words, it may appear that the only reason why group A patients had a better survival was that they were almost all responders. Yet the interest of the previous observation was reinforced by the evidence that the IL-10 serum level maintained its prognostic significance using the multivariate analysis. Finally, we performed a stepwise regression analysis with backward elimination without covariates forced into the regression model at the first step; that is, we chose not “force” the statistical program to consider IL-10 as an important variable. Nevertheless, IL-10 serum level came out again along with other prognostic factors affecting OS and TTF (namely, stage and PS, respectively), suggesting that IL-10 was not redundant with other commonly accepted prognostic parameters for NSCLC, such as those reflecting tumor mass or the patient’s condition.

Under a biological standpoint, IL-10 immunosuppressive properties may help explain, at least in part,
NSCLC progression in vivo, for its precise role among all factors involved in cancer progression is still under investigation; under a clinical standpoint, the correlation between OS/TTF and IL-10 serum levels also may appear consistent, as patients with higher serum levels of this cytokine may be regarded as bearing a more widespread disease and/or as having a more profound immunodepression resulting in a shorter life expectancy.

Clearly, the IL-10 cut-off point for discriminating subgroups of patients with different prognosis was set retrospectively, and, for this reason, needs to be validated in prospective studies.

In conclusion, this study shows that the measurement of basal levels of serum IL-10 is of independent prognostic utility in advanced NSCLC patients; its usefulness in detection of disease progression awaits further, prospective studies.

REFERENCES
14 Kaznuki T, Mostowski H, Tosato G. Human interleukin-10 can directly inhibit T cell growth. Blood 1993; 81:2964–2971