

# Tumour necrosis factor- $\alpha$ (TNF- $\alpha$ ) in patients who have asbestosis and develop cancer

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## Abstract

**Objectives**—Concentrations of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) were assayed by radioimmunoassay in serum samples collected between 1981 and 1987 from 111 patients with asbestosis who were at a high risk of cancer. Follow up of these patients until 1993 showed that 38 had developed cancer (27 lung, three mesotheliomas, and eight diverse malignancies).

**Results**—The mean serum concentrations of TNF- $\alpha$  given in fmol/100  $\mu$ l serum in all the cases with cancer (14.1) and the cases with lung cancer (13.6) were significantly higher ( $P < 0.05$ ) than the mean concentrations in the exposed controls (10.5). A positive increase was considered to be any value that was  $> 2$  SDs above the mean of the exposed controls. 22% (six of 27) of the cases with lung cancer were positive compared with 4% (three of 73) of the exposed controls, a significant difference ( $P < 0.001$ ). The serum concentrations of TNF- $\alpha$  correlated moderately with cancer ( $r = 0.3$ ), lung cancer ( $r = 0.3$ ), and Neu oncoproteins and epidermal growth factor receptor (EGFR) ( $r = 0.3, 0.5$  respectively). Also, there was a significant correlation between development of cancer and severity or progression of asbestosis. There was no correlation between the concentrations of TNF- $\alpha$  and severity or progression of asbestosis.

**Conclusions**—These results showed high concentrations of TNF- $\alpha$  in the patients who had cancer. TNF- $\alpha$  may offer an auxiliary method in early diagnosis of cancers related to asbestosis.

(*Occup Environ Med* 1995;52:316-319)

Keywords: tumour necrosis factor- $\alpha$ ; asbestosis

Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a cytokine produced mainly by activated macrophages.<sup>1-3</sup> The human TNF- $\alpha$  is a 17 kD polypeptide, with 157 amino acids, which forms oligomeres. It contains one internal disulphide bridge and no glycosylation sites.<sup>4-7</sup> Receptors for TNF- $\alpha$  are found in macrophages and various organs but as far as is known TNF- $\alpha$  is not produced by macrophages under normal conditions.<sup>6</sup>

High concentrations of TNF- $\alpha$  have been found in ovarian cell lines,<sup>8</sup> in monocytes

from patients with cancer,<sup>9</sup> and in serum from patients who had different types of cancers, such as oat-cell carcinoma<sup>10</sup> and non-small cell lung cancer.<sup>11</sup> The biological effects of TNF- $\alpha$  include inhibition of growth of the cancer cells,<sup>8,12</sup> induction of interferons,<sup>6,12</sup> and stimulation of synthesis of other cytokines in monocytes.<sup>11,12</sup> An increase in the production of TNF- $\alpha$  has been shown in pulmonary fibrosis induced by silica in mice.<sup>13</sup>

In our study we examined the concentrations of TNF- $\alpha$  by radioimmunoassay in serum samples obtained from a group of people affected by a severe fibrotic disease caused by asbestos. The patients with asbestosis are at an unusually high risk of lung cancer and mesothelioma. Annually from 1980 to 1988 the series of patients was examined for pneumoconiosis, and serum samples were collected. The cohort was followed up for cancer until 1993 by which time several members had died. This cohort with serial serum samples offered a unique opportunity to find out whether the variation in serum concentrations of TNF- $\alpha$  depended on advancement of pneumoconiosis or development of cancer.

## Materials and methods

A cohort of 111 patients with asbestosis has been followed up with clinical and radiographical evaluations at the Institute of Occupational Health in Finland since the late 1970s. These patients received periodic follow up examinations between 1981 and 1987. Blood samples were collected and serum was separated by centrifugation and stored frozen until the time of analysis. Most of the patients ( $n = 82$ ) had multiple serum samples (two to seven) available for the analysis from this time. For various reasons some patients ( $n = 29$ ) only attended one follow up session and contributed a single sample. In the years 1978-9, 1983-4, and 1986-7 chest radiographs were evaluated according to the International Labour Organisation (ILO) classification to determine the severity of asbestosis during this time. At the ILO classification the films were analysed without comparison to other films from the same person. At a second estimation the assessment of progression was accomplished by comparing the radiographs from the different examination periods side by side. Pneumoconiosis was defined as unstable or progressive if there were signs of aggravation of the disease during the follow up period.

Incidence of cancer within the cohort was

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Accepted 19 January 1995

followed up from 1981 until the spring of 1993 through the Finnish Cancer Registry (a national registry with a complete coverage of diagnosed cancers in the country). Thirty eight patients with asbestosis developed cancer during this follow up period, including 27 lung tumours, three mesotheliomas, and eight diverse malignancies. Two cases of tumorous diseases (meningioma and skin tumour) were classified as benign and were included in the control group. All patients were white, most of them were men (95%), and the average age of the cases with cancer and the controls in 1993 was 69 years. Also, plasma samples from a group of eight non-exposed healthy controls were analysed for TNF- $\alpha$ . The average age of these healthy controls was 53 years and they were all men.

The TNF- $\alpha$  was analysed in serum samples with a radioimmunoassay kit from Amersham (code RPA532). This competitive assay was carried out according to the manufacturer's instructions. Briefly, 100  $\mu$ l of 1:2 diluted serum was incubated overnight with an equal volume of sheep anti-TNF- $\alpha$  serum at 37°C. Then 100  $\mu$ l of (<sup>125</sup>I) labelled human, recombinant TNF- $\alpha$  was added and the incubation was continued overnight at 4°C. After addition of 250  $\mu$ l of the donkey anti-sheep second antibody and a 10 minute incubation at room temperature the tubes were centrifuged at 4°C for 10 minutes at 1500 g. Serum concentrations of TNF- $\alpha$  were interpolated from a standard curve.

Analyses of epidermal growth factor receptor (EGFR) and Neu oncoproteins were performed by an enzyme linked immunosorbent assay (ELISA) as previously reported.<sup>14</sup> Differences between the mean concentrations of oncoproteins in various groups of patients were analysed by the *t* test to consider whether the samples were normally distributed. Pearson's correlations were analysed between the variables. Proportions of positive cases were compared by the  $\chi^2$  analysis.

**Results**

Table 1 shows the concentrations of TNF- $\alpha$ . This comparison was based on each person's mean protein concentrations analysed annually during the study period. Of the 111 patients with asbestosis 38 developed cancer during the study period (27 lung tumours of various subtypes, three mesotheliomas, and

Table 1 Mean concentrations of TNF- $\alpha$  in patients with cancer and controls

Cancer	n	TNF- $\alpha$ Mean (SD) (fmol/100 $\mu$ l serum)
Cancer, total	38	14.1 (9.9)*
Lung cancer, total	27	13.6 (6.5)*
Lung, non-defined	12	12.6 (5.6)
Lung, small cell	4	13.8 (6.4)
Lung, epidermal	5	16.3 (6.7)
Lung, adenocarcinoma	6	13.4 (8.9)
Mesothelioma	3	12.8 (7.6)
Other cancers	8	16.2 (18.3)
Exposed controls	73	10.5 (3.7)
Grand total	111	11.7 (6.7)

\*P < 0.05 two tailed *t* test.

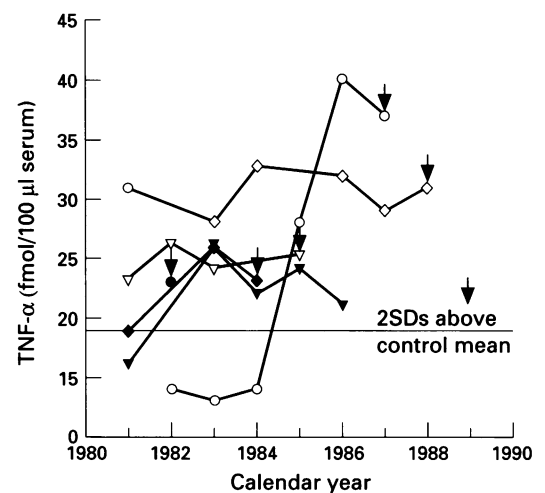


Figure 1 Increased concentrations (mean + 2SDs) of TNF- $\alpha$  in six patients with lung cancer. Arrows show the year of diagnosis of the cancer for each patient.

eight diverse malignancies). The remaining 73 patients had asbestosis but were clinically free from cancer. The mean (SD) serum concentrations of TNF- $\alpha$  in all the cases of cancer (14.1 (9.9) fmol/100  $\mu$ l serum) and cases of lung cancer (13.6 (6.5) fmol/100  $\mu$ l serum) were significantly higher (*P* < 0.05) than the mean concentrations in the exposed controls (10.5 (3.7) fmol/100  $\mu$ l serum). The mean plasma concentrations of TNF- $\alpha$  in the non-exposed control group was 13.0 (3.8) fmol/100  $\mu$ l plasma. This group is not comparable with the asbestosis cohort, because only plasma samples were available and the mean age was 16 years younger than that of the asbestosis cohort. An increase in the serum TNF- $\alpha$  was defined as any value > 2 SDs above the mean of the exposed controls. This showed that the number of cases of lung cancer in this group (six of 27, or 22%) significantly exceeded the number of controls (three of 73, or 4%, *P* < 0.001). One of the three patients with mesothelioma had a slightly increased serum concentration in the TNF- $\alpha$ . The patients who had diverse types of malignancies (*n* = 8) had no increase in serum concentrations of TNF- $\alpha$ , except for a patient with non-Hodgkin's lymphoma who had the highest concentration of TNF- $\alpha$  (60.6 (16.4) *v* 10.5 in the controls).

Figure 1 shows the annual serum concentrations of TNF- $\alpha$  of the six patients with lung cancer who had significantly high mean concentrations. For five of them there are serial samples, most of which show a slight increase in TNF- $\alpha$  before the time of diagnosis. Distribution of the mean serum concentrations of TNF- $\alpha$  seemed to be normal in the entire cohort and both groups (with cancer and exposed controls, fig 2). For each group some outliers were detected.

Correlations between each person's mean concentration of TNF- $\alpha$ , cancer, severity of asbestosis, progression of asbestosis, and lung cancer were analysed (table 2). As expected from the above results, TNF- $\alpha$  correlated moderately with both cancer and lung cancer (*r* = 0.3, *P* < 0.01). Interestingly, significant

Figure 2 Distribution of the mean serum concentrations of TNF- $\alpha$  among: (A) cancer group (B) control group.

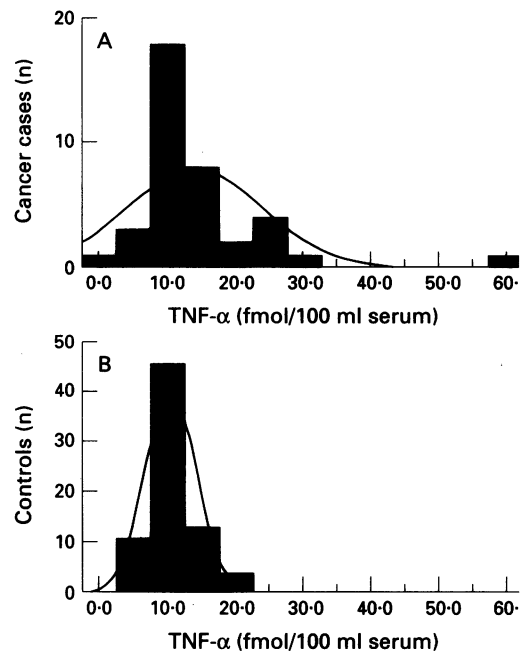


Table 2 Correlation between concentrations of TNF- $\alpha$ , cancer, and asbestosis

	TNF- $\alpha$	Severity	Progression	Lung cancer
Cancer	0.258	0.312	0.415	1.00
(n)	(111)	(67)	(79)	(98)
P value	0.006	0.010	0.000	0.000
TNF- $\alpha$		0.148	0.123	0.304
(n)		(67)	(79)	(98)
P value		0.233	0.281	0.002
Severity			0.629	0.146
(n)			(67)	(59)
P value			0.000	0.001
Progression				0.466
(n)				(71)
P value				0.000

Table 3 Correlation between concentrations of oncoprotein and TNF- $\alpha$

	Neu	NNeu	TNF- $\alpha$
EGFR	0.249	0.369	0.498
(n)	(109)	(109)	(109)
P value	P = 0.009	P = 0.000	P = 0.000
Neu		0.710	0.162
(n)		(109)	(109)
P value		P = 0.000	P = 0.093
NNeu			0.283
(n)			(109)
P value			P = 0.003

Table 4 Concentrations of TNF- $\alpha$  according to the state of asbestosis

	TNF- $\alpha$ (fmol/100 $\mu$ l serum)		
	total	Cancer	Control
<i>ILO classification</i>			
Stable:			
Mean (SD)	11.7 (5.0)	18.8 (7.3)	10.7 (3.8)
n	49	6	43
Unstable:			
Mean (SD)	13.5 (10.0)	16.9 (13.1)	10.1 (2.8)
n	30	15	15
<i>Assessment of progression</i>			
No progression:			
Mean (SD)	11.5 (5.1)	16.2 (8.2)	10.6 (3.8)
n	42	7	35
Progression:			
Mean (SD)	15.7 (14.4)	25.7 (20.3)	9.5 (2.1)
n	13	5	8

relations were noted between cancer, lung cancer, and severity or progression of asbestosis. Severity and progression had a correlation coefficient of 0.63 ( $P < 0.001$ ). Cancer and lung cancer correlated more with progression than with severity; in all the comparisons the correlation coefficients were highly significant. The TNF- $\alpha$  did not correlate with severity or progression.

Correlations between EGFR, Neu (two different assays, Neu and NNeu), and TNF- $\alpha$  were analysed based on the results from a previously reported study on EGFR and Neu.<sup>14</sup> A correlation was found between TNF- $\alpha$  and EGFR and NNeu ( $r = 0.5$  and  $0.3$  respectively, table 3).

Also, the serum concentrations of TNF- $\alpha$  were examined in relation to the severity or progression of asbestosis (table 4). There was no significant relation between the presence of advanced pneumoconiosis and TNF- $\alpha$ . The presence or absence of progression of asbestosis did not change the serum concentrations of TNF- $\alpha$ .

## Discussion

To our knowledge, high concentrations of TNF- $\alpha$  in serum samples have been found in a few previous studies. In one study, seven different cancer types were investigated with an ELISA assay for TNF- $\alpha$ . Many oat-cell carcinomas (69%) showed high concentrations of TNF- $\alpha$  in serum.<sup>10</sup> In another study two out of eight patients with non-small cell lung cancer had high concentrations of TNF- $\alpha$  in serum samples as determined by an ELISA assay.<sup>11</sup> In a third study, high serum concentrations of TNF- $\alpha$  measured by a radioimmunoassay were found in paediatric malignancies.<sup>15</sup> The increases reported coincided with active disease whereas patients with cured or stable disease had close to normal concentrations of TNF- $\alpha$ .

Our results show a significant difference between concentrations of TNF- $\alpha$  in serum from patients with cancer, particularly lung cancer, compared with controls. There was a significant difference in the proportion of the positive cases between patients with cancers and controls when an increase of serum TNF- $\alpha$  was defined as any value  $>2$  SDs above the mean value of the controls. An interesting finding in our study was that the patients who had high mean concentrations of TNF- $\alpha$  tended to have the increased concentrations throughout the entire study period—that is, several years before the diagnosis of cancer. There was some increase towards the date of diagnosis.

Due to the small numbers of diverse malignancies, including the mesotheliomas, in this study no conclusions can be drawn about the role of TNF- $\alpha$ . One person who had non-Hodgkin's lymphoma had the highest values of TNF- $\alpha$  found in this study. The same person had high EGFR and Neu concentrations in a previously reported study.<sup>14</sup>

A correlation was found between TNF- $\alpha$  and EGFR and Neu. The correlation was

strongest with EGFR. In patients with cancer EGFR was higher than in the controls, which may partially explain the correlation.<sup>14</sup> In patients with cancer Neu was not significantly increased but it correlated with EGFR, which may also be the reason for correlation with TNF- $\alpha$ . Cancer and lung cancer correlated significantly with progression and severity of asbestosis. A correlation was found between cancer, lung cancer and TNF- $\alpha$  concentrations in serum. By contrast, no correlation was found between serum concentrations of TNF- $\alpha$  and severity or progression of asbestosis. This finding indicates that the serum concentrations of TNF- $\alpha$  relate to cancer rather than to ongoing pneumoconiosis. The correlation between cancer and progression or severity of asbestosis may indicate that a high intake of asbestos is a risk factor both for progression of asbestosis and cancer, or that progressive forms of asbestosis are special risk factors for cancer.

As the TNF- $\alpha$  assay was not positive in a high percentage of people who developed cancer, the method is not suitable for cancer screening. Our results suggest a possible use for the determination of TNF- $\alpha$  in serum as a complementary diagnostic tool for the clinical monitoring of patients with asbestosis who have a high risk of cancer.

The study was supported by the Swedish Medical Research Council.

- 1 Tumour necrosis factor- $\alpha$  (<sup>125</sup>I) assay system with Amerlex-M magnetic separation. *Amersham users guide*. Little Chalfont, Buckinghamshire: Amersham, 1994.

- 2 Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino Jr MA, Shepard HM. Recombinant human necrosis factor- $\alpha$ : effects on proliferation of normal and transformed cells in vitro. *Science* 1985;230:943-5.
- 3 Smith MR, Munger WE, Kung H-F, Takacs L, Durum SK. Direct evidence for an intracellular role for tumour necrosis factor- $\alpha$ . *J Immunol* 1990;144:162-9.
- 4 Davis JM, Narachi MA, Alton NK, Arakawa T. Structure of human tumour necrosis factor- $\alpha$  derived from recombinant DNA. *Biochemistry* 1987;26:1322-6.
- 5 Beutler B, Greenwald D, Hulmes JD, Chang M, Pan Y-CE, Mathison J, et al. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature* 1985;316:552-4.
- 6 Beutler B, Cerami A. The biology of cachectin/TNF—A primary mediator of the host response. *Ann Rev Immunol* 1989;7:625-55.
- 7 Aggarwal BB, Kohr WJ, Hass PE, Moffat B, Spencer SA, Henzel WJ, et al. Human tumour necrosis factor. Production, purification, and characterization. *J Biol Chem* 1985;260:2345-54.
- 8 Wu S, Boyer CM, Whitaker RS, Berchuck A, Wiener JR, Weinberg JB, Bast Jr RC. Tumour necrosis factor  $\alpha$  as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumour cell proliferation and tumour necrosis factor  $\alpha$  expression. *Cancer Res* 1993;53:1939-44.
- 9 Aderka D, Fisher S, Levo Y, Holtmann H, Hahn T, Wallach D. Cachectin/tumour necrosis factor production by cancer patients. *Lancet* 1985;ii:1190.
- 10 Balkwill F, Burke F, Talbot D, Tavernier J, Osborne R, Naylor S et al. Evidence for tumour necrosis factor/cachectin production in cancer. *Lancet* 1987;ii:1229-32.
- 11 Shenouda G, Labateya M, Souhami L, Donath D, Vuong T, Joseph L. Elevated serum cytokines in patients with malignancy and weight loss. *International Journal of Oncology* 1993;2:115-8.
- 12 Feinman R, Henriksen-DeStefano D, Tsujimoto M, Vilcek J. Tumour necrosis factor is an important mediator of tumour cell killing by human monocytes. *J Immunol* 1987;138:635-40.
- 13 Piquet PF, Collart MA, Grau GE, Sappino A-P, Vasalli P. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* 1990;344:245-7.
- 14 Partanen R, Hemminki K, Brandt-Rauf P, Jin C-G, Koskinen H. Serum levels of growth factor receptors, EGFR and Neu in asbestosis patients: a follow-up study. *International Journal of Oncology* 1994;4:1025-8.
- 15 Saarinen UM, Koskelo EK, Teppo AM, Siimes MA. Tumour necrosis factor in children with malignancies. *Cancer Res* 1990;50:592-5.