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Serum epidermal growth factor receptor and p53 as predictors of lung cancer risk in the ATBC study

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Serum samples from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study were used in a nested case control study to identify the possible association between the serum level of epidermal growth factor receptor and p53 in respect to lung cancer. The proteins were assayed for by commercial immunoassays that showed uneven, often unacceptable, quality. For EGFR there was no relationship to lung cancer. Two physiological variables appeared to modify the serum level of EGFR, age by decreasing it annually by about 4 fmol ml^{-1}, and stroke by increasing it by 150 fmol ml^{-1}. For p53, myocardial infarction appeared to cause an increase in serum levels of this protein. While the serum levels of p53 were only moderately increased in lung cancer patients, particularly those with squamous cell carcinoma, the intriguing findings related to the high frequency of p53-positive patients among those belonging to the group of patients being treated by surgery and those belonging to clinical stages 1 and 2 as compared with higher clinical stages. An untested rationalization of these results was that patients with advanced lung cancer, stages 3 and higher, develop autoantibodies against the mutant p53 and thus mask the serum levels of the mutant p53 protein.

Keywords: oncoproteins, p53, lung cancer, immunoassay, prevention, treatment.

Introduction

In cancer cells one or more steps of the growth signalling pathways may be malfunctional leading to overproduction of a protein by an amplified gene or by other mechanisms (e.g. epidermal growth factor receptor, EGFR, Cadena and Gill 1992), production of a non-functional mutant protein (e.g. p53), or production of an overactive mutant protein (e.g. ras). Epidermal growth factor and EGFR have been shown to be overexpressed in many epithelial tumours including squamous cell lung cancer in studies applying tissue immunohistochemistry and serum or urine immunoassay (McKenzie 1991, Partanen *et al*. 1994a,b). EGFR is being used as a prognostic marker in breast cancer but its significance in lung cancer remains unclear (DeVita *et al*. 1997). Immunohistochemical detection of the p53 protein in

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tumour tissue is being evaluated as a prognostic marker in a number of cancers (Brandt-Rauf *et al.* 1995, DeVita *et al.* 1997). The mutant protein can be detected in serum in several forms of cancer, including those of lung and colon (Brandt-Rauf *et al.* 1992, Fontanini *et al.* 1994, Greco *et al.* 1994). Conceptually, serodiagnosis of cancer by means of growth factors and oncoproteins (jointly called oncoproteins in this paper) provides attractive possibilities for screening and early diagnosis of cancer. Oncoproteins are an essential and usually an early mechanistic part of the carcinogenic process and some oncoproteins are derived from mutated genes and thus are new proteins in the body. These properties should guarantee specificity and a chance for early detection. However, appraisal of the usefulness of these proteins as early diagnostic markers has awaited large well-controlled follow-up studies.

Serum samples from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study Group 1994), in which 29133 50–69-year old Finnish men were recruited in 1985–88 are used here in a nested case control sampling to identify the possible association between the serum level of oncoproteins in respect to lung cancer. This cancer type was selected because of its high incidence, frequent mutations in the p53 gene, and previous indications of an elevatedation of oncoproteins in this cancer form (Brandt-Rauf *et al.* 1995); moreover lung cancer was the primary target site of the ATBC study. The special advantage of this study as compared with others carried out in this field is the large, well characterized study population, which enables the assessment of the oncoproteins years before clinical diagnosis. The available interview data enable the control of confounding variables. Together this should allow a rigorous assessment of the applicability of serum oncoprotein analysis in early diagnosis of cancer and provide data on variables affecting the normal levels of oncoproteins.

Materials and methods

The ATBC Study recruited 29133 smoking 50–69-year old Finnish men in the years 1985–88, and these were followed till the end of April 1993. When entering the study each participant provided interview information on health indicators and smoking history and a serum sample was collected and stored. During follow-up information on cancers and deaths were obtained from the Finnish Cancer Registry. The ATBC Study group collected new serum samples from diagnosed cancer cases and also on an annual basis from a randomly chosen subgroup of participants free of cancer.

Patient samples

A matched nested case-control study design was used for assessing the association between p53 and EGFR levels and lung cancer within this cohort. Two substudies were carried out and they are reported as the prevalence and incidence substudies, respectively. The prevalence study focused on serum oncoprotein levels for cancers diagnosed at the start of the ATBC study and during the first year. Only the baseline serum sample was analysed. The incidence substudy focused on new cases identified after the first year of follow-up and both the baseline serum sample and the follow-up sample taken at the time of diagnosis of cancer were analysed. For most assays the two studies gave identical results and the results were given combined.

All samples were transported from the National Public Health Institute in Helsinki to the Center for Nutrition and Toxicology in Huddinge for the analysis of oncoproteins.

Prevalence substudy. Ninety-three (93) prevalent lung cancers were identified in the baseline screen and an additional 71 cases were diagnosed with lung cancer during the first year of study. These cases were distributed between the intervention groups as follows: 16 in placebo, 26 in alpha-tocopherol (AT), 15 in betacarotene (BC), and 14 in combination ATBC, respectively. The whole group of prevalent cases totalled 164 lung cancers of the following histological types: 66 squamous cell, 30 small cell, 28 adenocarcinoma and 40 unknown histologies.

Controls for the prevalent cases were selected among those who were free of lung cancer until April

30 1993. The controls were matched to the cases with respect to date of the baseline serum sample (within 1 week), study area and age at baseline (as close as possible).

Incidence substudy. For the incidence substudy the cases were selected from the placebo group having squamous cell lung cancer diagnosed before April 30 1993. This histology was selected in order to reduce possible sources of variation. A total of 88 cases were found. The controls were selected among the annual controls free of lung cancer until April 30 1993 matching at the intervention group (i.e. placebo), time on study (within 90 days) and age at baseline (as close as possible).

Measurements

Serum samples were analysed for the level of oncoproteins using commercial ELISA assays that are based on the sandwich-type of immunoassays (Oncogene Science). The results were read colorimetrically and quantified with standard protein solutions. We have previously used the assays in several studies (Partanen *et al*. 1994a,b, Hemminki *et al*. 1996). The EGFR assay utilized a mouse monoclonal capture antibody and a rabbit detector antibody (Waterfield *et al*. 1982). The detector antibody was then reacted with a goat anti-rabbit antibody linked to horseradish peroxidase which catalysed the conversion of the substrate, *o*-phenylenediamine to a coloured product. The product was measured spectrophotometrically at 490 nm. EGFR was measured on a quantitative scale (fmol ml^{-1}). From each serum sample a maximum of three assays was performed.

For p53 the mutant epitope-specific PAb 240 antibody was used (QIA03, Oncogene Science). Originally, p53 serum levels were measured on a quantitative scale, but because of variability between the ELISA plates, even within shipments, it was not possible to use quantitative values in analysing the whole material. The original readings from the assays were thus categorized into three groups: 0, 1 and 2 depending on the particular assay as giving no, weak or strong positivity. From each serum sample a maximum of three assays were performed. Aggregate codes from the repeated measurements are given in table 1. In the aggregation two equal repeat measurements overruled any third measurement; in other cases the mean of the repeat measurements was used. The aggregate code 2 was present rarely and thus a dichotomic classification 0 vs 1+2 was used in most analyses. No advantage was noted when using p53 levels in three categories. Analysis was also attempted with quantitative data for the samples where such were available.

Table 1. A scheme used to transform the results from repeated p53 measurements to codes used in the analysis.

Results from p53 analyses	Code used in analysis
000	0
00N	0
010	0
011	1
01N	1
0NN	0
100	0
101	1
10N	1
110	1
11N	1
200	0
201	1
22N	2
N0N	0
N10	1
N11	1
N1N	1
N21	2
N2N	2
NNN	NA

0 = negative, 1 = weak positive, 2 = strong positive, N = not measured, NA = not available. As an example, three measurements 110, weak positive, weak positive, negative, were aggregated to code 1, positive.

ATBC database and statistical methods

For oncoproteins recorded at two levels (i.e. P53) the crude odds ratio estimates and their standard errors were computed from the distribution of discordant matched pairs (Breslow and Day 1980). For oncoproteins recorded on a continuous scale (i.e. EGFR) the crude case-control differences were computed. For both p53 and EGFR the adjusted odds ratio estimates were computed from a conditional logistic regression model which takes matching into account by stratification with respect to case-control pairs. For assessment of oncoprotein variation among controls unconditional logistic regression was used for p53 and a random effects model for EGFR (Laird and Ware 1982).

Possible confounding effects by a number of risk factors were considered when analysing the association of oncoprotein levels and lung cancer risk. The following risk factors were included: myocardial infarction at baseline, angina pectoris at baseline, claudication at baseline, stroke at baseline, diabetes mellitus at baseline, smoking years at baseline, age at start of smoking, number of cigarettes per day, radical operation of tumour, alcohol consumption in grams per day (0, < 20 and > 20), and asbestos exposure at work.

Results

p53 and EGFR were analysed by commercial immunoassays which performed well in the beginning of the study but in the course of the experiments the qualities of the assays deteriorated. For this reason p53 results could not be given in the quantitative scale. Some two-thirds of the samples were successfully analysed for EGFR but when the immunoassay failed the analyses had to be stopped.

p53 assays

The characteristics of the study populations are shown in table 2. Cases and controls resembled each other very closely. Additionally, the populations in the prevalence and incidence study were much alike. The p53 protein was assayed for in serum samples and the results were dichotomized as 0, when no mutant protein was detected, or as 1 or 2 when some protein was detected (see table 1). The unadjusted odds ratio for the presence of p53 was marginally increased in lung cancer cases in the two substudies (table 3). The pooled odds was 1·22. A similar analysis was carried out with the quantitative values obtained from the p53 assay, which were however deemed unreliable. The odds ratio was 1·44 (95 % CI: 0·76 and

Table 2. Characteristics of the populations studied.

	Prevalence Substudy						Incidence Substudy					
	Controls			Cases			Controls			Cases		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Age	60·6	4·8	164	60·7	4·8	164	60·4	4·4	88	60·4	4·5	88
Smoking age	19·6	4·5	164	18·1	3·8	163	19·7	5·8	88	18·0	3·5	88
Smoking years	38·2	8·9	164	41·1	7·1	163	38·3	8·5	88	41·4	5·8	88
Cigarettes per day	19·7	9·0	164	19·4	10·1	163	18·2	7·9	88	21·6	8·9	88
Alcohol, g per day	14·3	19·8	154	19·5	24·2	95	13·4	12·7	84	12·8	17·1	79
Myocardial infarction			12			15			8			5
Angina			15			16			4			9
Claudication			5			18			7			7
Stroke			5			4			2			2
Diabetes			8			8			5			3
Asbestos			2			5			3			3
Operated (cases)						16						20
Stage (cases) (I,II,IIIA,IIIB,IV,OCC):			48/11/16/25/34/1						16/16/18/20/16/2			

Table 3. Crude odds ratios for serum p53 levels in lung cancer patients.

	Control		
	Not elevated	Elevated	Odds ratio (95 % CI)
Prevalence substudy			
Case			
Not elevated	98	15	
Elevated	19	3	1·27 (0·64, 2·49)
Incidence substudy			
Case			
Not elevated	45	8	
Elevated	9	9	1·12 (0·43, 2·92)
Pooled data			
Case			
Not elevated	143	23	
Elevated	28	12	1·22 (0·70, 2·09)

2·72) and 1·67 (95 % CI: 0·40 and 6·79) in the prevalence and incidence study, respectively (data not presented). This will be the only reference to quantitative data on the p53 levels and any data to follow are on a two-level (qualitative) scale.

In the prevalence study data were available on histological types of lung cancer whereas in the incidence study only squamous cell carcinoma was considered. The odds ratios were 2 (number of discordant pairs 8/4) for squamous cell carcinoma, 0·75 (3/4) for small cell carcinoma, and 0·67 (2/3) for adenocarcinoma, none of these reaching statistical significance. In the incidence study there was a possibility to follow the changes in the p53 levels as repeated samples were available for some patients. However, the number of annual discordant case-control pairs was so small that no conclusion was attempted (data not shown).

An interesting observation, reproduced in the two substudies, was that p53 levels were detectable most frequently in the cases that were to be operated (table 4). The discordant pairs for p53 in the pooled data were 7/0 and 6/13 in the operated vs non-operated patients, respectively. It should be noted that in the prevalence substudy data on operation were not available for all individuals. When searching for the explanation we found that this matched with the clinical stage data from the patients (table 5). The ratio of odds ratios was 4·90 (95 % CI 1·30-18·5) when p53 levels were compared in the patients of stages 1 and 2 with those of stage 3 and higher in the pooled data.

The variation in the levels of p53 was analysed jointly for the controls in the two substudies. Unconditional logistic regression was used to assess the effect of covariates on the the proportion of samples with elevatedated p53. Among the variables analysed only myocardial infarction was associated with an increased odds ratio of detectable p53 levels in serum (table 6).

EGFR

For EGFR continuous quantitative data were available for the analyses. The mean serum levels were about 330 and 350 fmol ml^{-1} in the prevalence and incidence study, respectively (table 7). Almost all (except two) the cases were squamous cell cancers. The uniformity of serum levels of EGFR in both

Table 4. Serum p53 levels and radical operation of lung cancer.

	Operated case		Not-operated case	
Prevalence substudy	Not elevated	Elevated	Not elevated	Elevated
Control				
Not elevated	8	3	33	1
Elevated	0	0	5	2
Incidence substudy				
Control				
Not elevated	9	4	36	5
Elevated	0	2	8	7
Pooled data				
Control				
Not elevated	17	7	69	6
Elevated	0	2	13	9

Note: Evaluation of the interaction (ratio of odds-ratios) omitted due to zero cells in the table.

Table 5. Serum p53 levels and tumour stage in lung cancer.

	Stage I or II case		Stage III OR Higher case		
Prevalence substudy	Not elevated	Elevated	Not elevated	Elevated	Ratio of odds ratios (95 % CI)
Control					
Not elevated	35	10	50	3	
Elevated	4	0	7	2	5·83 (0·98, 34·60)
Incidence substudy					
Control					
Not elevated	16	4	29	5	
Elevated	1	4	7	5	5·60 (0·47, 66·44)
Pooled data					
Control					
Not elevated	51	14	79	8	
Elevated	5	4	14	7	4·90 (1·30, 18·50)

Table 6. Analysis of variation of in serum levels of the p53 protein ($N = 244$).

	Odds ratio[a]	95 % CI
Model 1		
Intercept	0·009	0·000 0·791
Age	1·053	0·978 1·133
Model 2[b]		
Intercept	0·025	0·000 2·675
Age	1·042	0·967 1·124
Myocardial	1·732	1·043 2·876
Model 3[b]		
Intercept	0·007	0·000 0·756
Age	1·055	0·979 1·136
Angina	0·901	0·471 1·722
Model 4[b]		
Intercept	0·005	0·000 0·522
Age	1·055	0·981 1·136
Claudication	0·633	0·225 1·784
Model 5[b]		
Intercept	0·013	0·000 1·498
Age	1·050	0·975 1·131
Stroke	1·315	0·564 3·069
Model 6[b]		
Intercept	0·009	0·000 0·826
Age	1·053	0·978 1·133
Diabetes	0·998	0·457 2·177
Model 7[b]		
Intercept	0·014	0·000 1·983
Age	1·081	0·989 1·182
Smoking years	0·965	0·920 1·012
Smoking age	0·972	0·899 1·052
Cigarettes per day	0·997	0·956 1·039
Alcohol	0·998	0·977 1·019

[a] Change in odds ratio when the variable changes one unit.
[b] Effects adjusted for all other covariates in the model.

Table 7. Serum levels of EGFR (fmol ml^{-1}) in cases and controls.

	Prevalence substudy		Incidence substudy	
	Mean	SD	Mean	SD
Cases	333·7	89·3	347·1	69·7
Controls	333·4	97·7	355·2	66·0
Difference	0·3	111·1	–6·7	85·7
Odds ratio(95 % CI)	1·000 (0·995, 1·010)		0·998 (0·993, 1·000)	

Means of the repeated EGFR measurements at baseline used in the analysis.

substudies is illustrated in figure 1. No effect of clinical stage or treatment by operation was noted.

Variation in EGFR levels was analysed by the random effects model (table 8). Two variables showed a statistically significant effect on the EGFR levels: age and stroke. Ageing 1 year appeared to cause a decrease of EGFR at about 3·8 fmol ml^{-1},

Table 8. Analysis of variation[a] of in serum levels of EGFR ($N = 153$).

		Effect[b]	95 % CI	
Model 1	Intercept	579·5	416·1	742·9
	Age	−3·8	−6·5	−1·1
Model 2	Intercept	599·5	433·2	765·7
	Age	−3·9	−6·6	−1·2
	Claudication	15·7	−9·4	41·0
Model 3	Intercept	664·3	495·4	833·2
	Age	−4·0	−6·6	−1·4
	Stroke	75·0	25·0	125·0
Model 4	Intercept	579·0	411·3	746·7
	Age	−3·8	−6·5	−1·1
	Myocardial	−0·2	−21·2	20·6
Model 5	Intercept	577·0	406·7	747·4
	Age	−3·8	−6·5	−1·0
	Angina	−1·2	−23·9	21·5
Model 6	Intercept	592·2	427·9	756·5
	Age	−3·8	−6·5	−1·1
	Diabetes	15·6	−9·7	41·0
Model 7	Intercept	606·3	408·2	804·4
	Age	−4·6	−9·4	0·0
	Smoking years	0·2	−2·8	3·37
	Smoking age	1·2	−2·6	5·1
	Cigarettes per day	−1·0	−2·4	0·4
	Alcohol	0·5	−0·2	1·4
	Asbestos	−8·8	−95·4	77·7

[a] Repeated values measured at baseline used in a random intercept model and fixed effects for all other covariates.

[b] Effects adjusted for all other variables in the model.

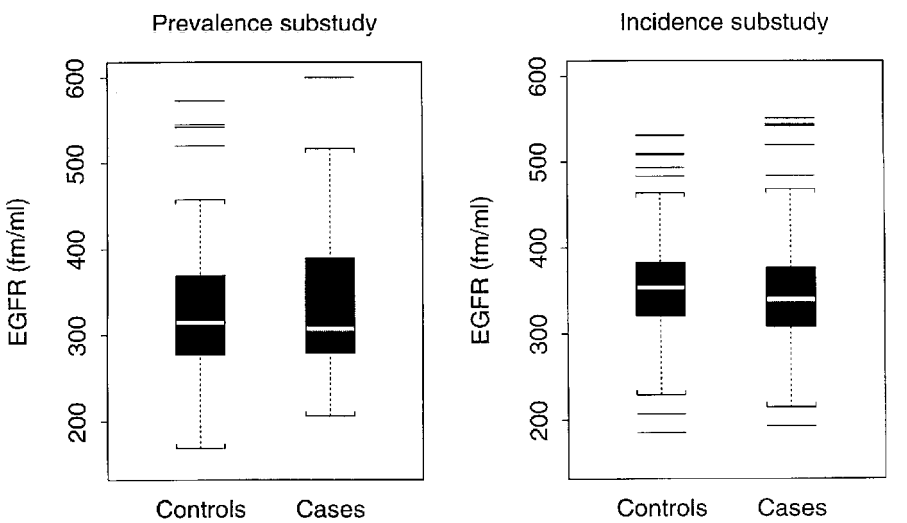

Figure 1. Serum EGFR levels in lung cancer cases and controls in the prevalence and incidence study. The line in the box is the mean, the box represents 50 % of the observations and the whiskers all the data, except for the outliers, marked by the vertical bars.

a magnitude that remained stable in the different models tested. The relationship of age and EGFR is shown in figure 2 on control individuals from the two substudies. The effect of stroke was an increase of 150 fmol ml^{-1} and it was independent of age.

There were 35 control individuals in the incidence substudy who had repeated samples, and the effect of ageing was tested in these samples (table 9). The cross-sectional effect of ageing caused an annual decrease of EGFR at 2·0 fmol ml^{-1} whereas the longitudinal effect was stronger 4·6 fmol ml^{-1} per year.

Discussion

The mechanism of appearance of oncoproteins in serum is not known but overproduction in transformed cells and cell destruction is likely to contribute to this (Brandt-Rauf *et al.* 1995). For EGFR there appears to be a physiological level because this protein can be detected in all subjects. Serodiagnosis of cancer by means of oncoproteins provides attractive possibilities for early and specific diagnosis of cancer. Oncoproteins are an essential and usually an early mechanistic part of the carcinogenic process; some oncoproteins such as p53 are derived from

Table 9. Effect of age on serum levels of EGFR evaluated from the incidence substudy using controls with follow-up measurements ($N = 35$).

	Effect (fmol ml^{-1})	SE	*P*-value
Mean level of EGFR	470·9	165·9	
Cross sectional change/year	−2·0	2·8	0·465
Longitudinal change/year	−4·6	1·1	< 0·001
Difference[a]	2·6	2·9	0·376

[a] Difference between cross sectional and longitudinal effects.

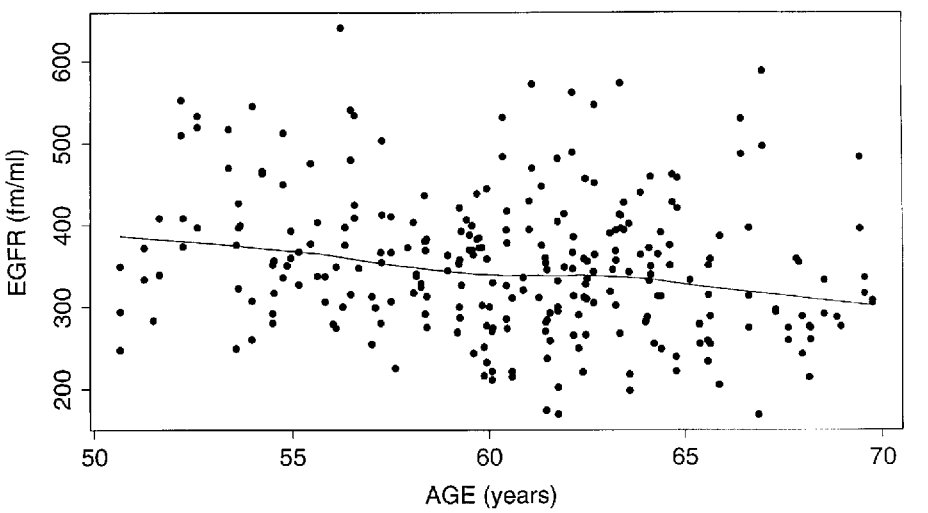

Figure 2. Age-dependence of serum EGFR in all the controls at the baseline. The smoothed curve is the loess graph.

mutated genes and thus are new proteins in the body. Although studies have been published suggesting some predictive value for p53 in lung (Fontanini *et al.* 1994, Partanen *et al.* 1995, Hemminki *et al.* 1996, Husgafvel-Pursiainen *et al.* 1997, Segawa *et al.* 1997), colon (Greco *et al.* 1994), pancreatic (Suwa *et al.* 1997) and haematopoietic malignancies (Trumper *et al.* 1994, Lehtinen *et al.* 1996) the patient series are small. For EGFR the serodiagnostic evidence on lung cancer is weak (Partanen *et al.* 1994a,b). Thus, appraisal of the usefulness of these proteins as early diagnostic markers has awaited large follow-up studies.

Here the primary objective was to analyse the applicability of oncoproteins in early diagnosis of cancer, which could be used among persons seeking medical care for their symptoms or being at a special risk of cancer for other reasons. Others can belong to cancer families, or are tobacco smokers or other heavily exposed populations. In such cases, the stringent criteria of population screening do not hold and the tolerance in predictivity can be lower (Adami and Baron 1994). The direction of false diagnosis largely depends on the individual situation. Yet, it is important that diagnostic efficiency would be maintained, e.g. cost-benefit is reasonable. Oncoproteins may also have prognostic value in early diagnosis, as both p53 and EGFR are already being used for prognostic purposes in the treatment of cancer (McKenzie 1991, DeVita *et al.* 1997).

Given such clinical prospects and the number of previous studies in the area, it was surprising that our study was handicapped because of the variable quality of the commercial ELISA kits, provided by the only supplier known to us. In the course of the measurements from the middle of 1995 to the early part of 1997 the standard curves obtained for the p53 plates varied unacceptably and a large proportion of the kits were not usable. We thus opted for qualitative analysis, first reading in three classes but in final analysis in two classes, detectable or non-detectable. A total of 325 samples were read at least twice using classification 0, 1, 2. Agreement in the repeat samples was observed in 274 cases, 84 % , considered quite satisfactory.

We have used the ELISA kits for EGFR determination since 1993 with reproducible standard curves (Partanen *et al.* 1994a). The present assays performed well till about two-thirds of the EGFR analyses were completed. In early 1996 the standard curves became unacceptable and the assays had to be stopped. The supplier was unable to provide new plates until early 1997. A new assay was introduced by the supplier and we discontinued the analysis because it was not considered justifiable to change the method in the middle of the series. Thus some samples remained unanalysed and some had only a single determination. The coefficient of variation in the EGFR assays was 14·5 %; interindividual variation accounted for 64 % and intraindividual variation and measurement error for 36 % of the total variation noted.

In spite of these difficulties, a number of conclusions could be reached. For EGFR there was no relationship to lung cancer of any histological type. Prior positive results relating EGFR to lung cancer in individuals with asbestosis may have been due to asbestosis rather than lung cancer (Partanen *et al.* 1994a,b). Two physiological variables appeared to modify the serum level of EGFR, age by decreasing it annually by about 4 fmol ml^{-1}, and stroke by increasing it by 150 fmol ml^{-1}. The effect of age appears solid while the effect of stroke was based on 13 individuals and, although physiologically plausible, awaits confirmation in an independent study.

In the case of p53, myocardial infarction appeared to cause an increase in serum levels of this protein. Whether in fact the mutant protein was increased cannot be confirmed by the present data; any reference to clonal and mutational origin of arterial plaques remains highly speculative (Penn and Snyder 1996). Alternatively, this finding may relate to the detection of denatured wild-type p53 released from infarcted tissue, since the PAb 240 antibody used will identify wild-type protein when it is denatured. While the serum levels of p53 were only moderately increased in lung cancer patients, particularly those with squamous cell carcinoma, the intriguing findings related to the high frequency of p53 positive patients among those belonging to the group of patients being treated by surgery and those belonging to clinical stages 1 and 2 as compared with higher clinical stages. Both of these findings were significant statistically but the data correlated in that patients with clinical stages 1 and 2 were those undergoing operation. An untested rationalization of these results is that patients with advanced lung cancer, stages 3 and higher, develop autoantibodies against the mutant p53 and thus mask the serum levels of the mutant p53 protein. In a follow-up study of three asbestos patients developing lung cancer, there was a tendency for decreasing p53 serum levels towards diagnosis, supporting this scenario (Hemminki *et al.* 1996). Futhermore, in studies on small cell lung and pancreatic cancer a correlation was found between the expression of p53 in tumours and the presence of the protein in serum, whereas serum levels only marginally reflected the clinical stage (Segawa *et al.* 1997, Suwa *et al.* 1997). Although there is ample evidence for the presence of anti-p53 antibodies in serum of lung and other cancer patients, usually correlating with the presence of p53 mutations in the tumour, the relationship to stage and other clinical data is not unambiguously established (Lubin *et al.* 1995, Angelopoulou *et al.* 1996, Wollenberg *et al.* 1997). However in a follow-up study of two patients who developed lung cancer, the anti-p53 antibody titres started to increase 1 year before diagnosis (Lubin *et al.* 1995). Also many non-cancerous conditions, such as infections, are known to cause an increase in autoantibodies against p53 (Raedle *et al.* 1996).

In summary, the data of the present study provide evidence that serum EGFR levels have no relationship to lung cancer in smoking men. However, EGFR levels decrease with age and may increase as a result of stroke. p53 levels in serum may be related to squamous cell lung cancer but the relationship is possibly masked by autoantibodies in more advanced disease. This is a testable hypothesis. Myocardial infarction may be related to increased serum levels of p53. Our experience with commercial ELISA kits for p53 and EGFR was frustrating.

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