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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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ONLINE REPOSITORY

Serum levels of club (Clara) cell secretory protein predict cancer mortality in adults

Stefano Guerra, Monica M Vasquez, Amber Spangenberg, Marilyn Halonen, Fernando D Martinez

1. List of tobacco-related and other types of cancer in TESAOD

Table E1. Number of death events due to "tobacco-related" cancers and other types of cancer in TESAOD.

Tumor primary sites	**N**
Tobacco-related cancers	**88**
Lung	42
Colorectum	12
Pancreas	11
Uterine cervix, ovary	9
Urinary bladder, kidney	7
Esophagus	3
Liver	2
Stomach	1
Oral cavity	1
Other types of cancer	**44**
Breast	17
Prostate	8
Leukemia	6
Lymphoma	3
Skin	3
Others	7
Unspecified	9

2. Summary of results from CC16 measurements on prospective TESAOD samples

Among the 1086 TESAOD participants included in this study, 555 (51%) had serum samples available during the follow-up. For prospective CC16 measurements, the sample collected at the earliest follow-up survey after the baseline assessment was used for each participant. Prospective samples came from the follow-up surveys 2 (N=99), 5 (45), 6 (349), and 7 (62). The mean number of years between the baseline and the prospective CC16 measurement was 6.7 years with a range of 1 to 10 years.

The basic characteristics of the 555 participants with prospective measurements and the 531 participants with no prospective measurements are compared in Table E2. As compared with the latter, at baseline participants with prospective measurements were older ($p < 0.0001$) and less likely to be current smokers ($p = 0.01$). In addition, those with prospective measurements had lower baseline serum CC16 than did those with no prospective measurements ($p = 0.02$). Overall, subjects with prospective measurements were more likely to die by 2011 than those with no prospective measurements (HR: 1.77, 1.51 – 2.08; $p < 0.0001$). However, these differences were mainly due to the older age of the former group.

For the 555 participants with prospective measurements, we calculated the rate of change of serum CC16 between baseline and the prospective measurement in two ways:

Method 1 – as the difference between CC16 levels from prospective measurement and from baseline measurement divided by the years between the baseline and prospective measurement;

Method 2 - as the difference between CC16 residuals from prospective measurement and from baseline measurement divided by the years between the baseline and prospective measurement. The residuals came from random coefficients models fitting CC16 levels against age and age squared to take into account the J-shaped relation of serum CC16 to age.

Table E2. Basic characteristics of the 555 participants with prospective CC16 measurements and the 531 participants with no prospective CC16 measurements.

	Participants with prospective measurements N = 555	Participants with no prospective measurements N = 531	P
Female sex	329 (59%)	309 (58%)	0.72
Age: median in years	54	37	<0.0001
Smoking*			0.01
Never	222 (40%)	208 (39%)	
Former	154 (28%)	113 (21%)	
Current	178 (32%)	210 (40%)	
Serum CC16: geometric mean in ng/ml	7.32	7.90	0.02
Death events by January 2011	403 (73%)	250 (47%)	<0.0001

* Total N = 554 among participants with prospective measurements because of one subject with missing smoking information

Table E3 shows the HRs for all-cause and cancer mortality associated with baseline serum CC16 (inverse standardized values) and with quartiles of CC16 slope calculated according to Method 1. In these models, the estimates of the effects of baseline CC16 were confirmed for both all-cause or cancer-specific mortality risk. We found evidence for a U-shaped association between quartiles of CC16 slope and mortality. Mortality risk was significantly lower in subjects with low-medium rate of change as compared with those with the lowest rate of change, as would be expected based on the hypothesis that CC16 increase over time is protective against mortality. However, mortality increased again with higher rates of change, and was highest among those with high rate of CC16 change. These U-shaped trends were confirmed when analyses were restricted to the 539 subjects who were alive at the time when survey 7 was completed and when we used quartiles of CC16 slope calculated using Method 2.

Table E3. Hazard ratio for all-cause and cancer mortality associated with baseline serum CC16 (inverse standardized values) and with quartiles of the rate of CC16 change (calculated using Method 1).

	ALL-CAUSE MORTALITY Adjusted* Hazard Ratios (95% CI) P value	CANCER MORTALITY Adjusted* Hazard Ratios (95% CI) P value
AdjHR associated with:		
1-SD decrease in log baseline CC16	1.16 (1.03 – 1.30)	1.53 (1.19 – 1.95)
P value	p = 0.01	p = 0.0008
AdjHR associated with		
Lowest rate of CC16 change(ref)	1	1
Low-Medium rate of CC16 change	0.70 (0.51 – 0.95)	0.68 (0.36 – 1.29)
Medium-High rate of CC16 change	0.98 (0.72 – 1.32)	0.70 (0.35 – 1.38)
High rate of CC16 change	1.12 (0.83 – 1.51)	1.05 (0.55 – 1.99)
P value for differences across quartiles	p = 0.02	p = 0.40

* adjusted for sex, age, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, and change in pack-years between baseline and prospective measurements

The results of these prospective analyses should be interpreted with caution because of several limitations. First, subjects with prospective measurements were not representative of the total population in terms of age, CC16 levels, and mortality risk. Second, the sample size of these analyses was relatively small. Third, at older ages serum CC16 levels have been shown to reflect conserved airway epithelial integrity but also decreased glomerular filtration rate (GFR)[1]. Therefore, subjects with rapidly increasing serum CC16 may represent a subgroup with poorer rather than better health outcomes, a scenario in line with the U-shaped curve for mortality risk that we found in association with CC16 change. Of note, this would be particularly relevant in subjects with prospective measurements because they were on average 17 years older than other participants.

Future studies that are specifically designed to address temporal changes of CC16 over time will be required to determine whether the predictive value of this biomarker can be improved by considering both baseline levels and their temporal trajectories.

3. Smoking history sub-analysis

In analyses that took into account smoking history during the follow-up, we found that – among the 381 participants who were current smokers at baseline and had follow-up information on smoking habits – the CC16 effects on cancer appeared stronger in the group of 182 subjects who consistently reported current smoking at all completed follow-up surveys than in the group of 199 subjects who did not (HRs for cancer mortality associated with 1-SD decrease in CC16: 1.51, 1.12 – 2.02, p = 0.006; and 1.12, 0.75 – 1.68, p = 0.58; respectively). The small sample size of these secondary analyses should be taken into account when interpreting these data.

References

1. Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. *Am J Respir Crit Care Med.* Feb 1999;159(2):646-678.