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Assessment of potential predictors of calretinin and mesothelin to improve the diagnostic performance to detect malignant mesothelioma: results from a population-based cohort study

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ABSTRACT

Objectives Mesothelin and calretinin are blood-based markers for malignant mesothelioma. The objective of this study was to analyse the markers in plasma samples from cancer-free men and to identify factors influencing their concentrations to minimise false-positive test results when using these markers for the early detection of malignant mesothelioma.

Setting The present analyses used data and archived blood samples of the population-based Heinz Nixdorf Recall Study among elderly people collected from 2011 to 2014.

Participants A total of 569 men (median age 70 years) without a malignant disease at the time of blood sampling were selected for these analyses.

Primary and secondary outcome Mesothelin and calretinin concentration in plasma samples.

Results We observed 24 mesothelin concentrations ≥1.5 nM (specificity 95.8%, 95% CI 93.8% to 97.2%) and 34 calretinin concentrations ≥1.0 ng/mL (specificity 94.0%, 95% CI 91.7% to 95.7%). Only five men had both markers above these cut-offs. Renal dysfunction and hypertension were major predictors of elevated mesothelin in addition to age. Regarding calretinin, the effect of renal dysfunction was slightly weaker and hypertension was not associated with increased concentrations. However, a diagnosis of cancer after blood collection and bronchial asthma were associated with positive calretinin results.

Conclusions The combined determination of mesothelin and calretinin results in only few false-positive marker tests. Both markers are mainly influenced by renal dysfunction. The determination of cystatin C concentrations may be informative when interpreting the test results.

Strengths and limitations of this study

- This is the first study that extensively examines potential predictors associated with positive test results of blood-based markers for mesothelioma in plasma samples of cancer-free participants in a large population-based cohort study.
- Strong determinants of elevated marker concentrations were identified in order to improve the specificity of calretinin and mesothelin for the early detection of malignant mesothelioma.
- Due to very high overall specificities of mesothelin and calretinin of 95%, the statistical analyses on predictors of false-positive tests were based on small numbers.
- The study population was enrolled from the general population and not a cohort of asbestos workers with a higher prevalence of asbestosis.
- The study focused on men only because the incidence of mesothelioma is significantly higher in men than in women. Hence, possible gender differences could not be investigated.

INTRODUCTION

Malignant mesothelioma is an aggressive cancer of the serous membranes with an increasing incidence worldwide.[1] Mesothelioma shows a latency period up to 40 years and median survival after diagnosis is between 9 and 13 months, depending on treatment,[2] because symptoms commonly occur only at late stages of this cancer. Thus, the diagnosis of mesothelioma at early stages might be a promising opportunity to improve therapy.

Up to date, the most prominent blood-based marker for mesothelioma is mesothelin. As recently shown in a meta-analysis, mesothelin as individual marker is characterised by a specificity of 89% (95% CI 86% to 91%) and a sensitivity of 58% (95% CI 54% to 62%) for the discrimination of patients with mesothelioma from asbestos-exposed subjects.[3] One of the best immunohistochemical markers for mesothelioma is calretinin.[4] Furthermore, it was shown that calretinin concentrations were elevated in the blood

of patients with diagnosed mesothelioma compared with control groups.[5] Calretinin as individual blood-based marker reached 71% sensitivity at a fixed specificity of 95% for the discrimination of patients with mesothelioma from asbestos-exposed subjects.[6]

However, in screening with tumour markers a very high specificity is desirable to avoid false-positive tests in cancer-free subjects.[7] Thus, there is an urgent need to identify parameters influencing the marker concentrations in subjects without the malignant disease. For mesothelin it is well known that renal failure could lead to increased marker concentrations,[8–10] but less is known about the effect on calretinin. To improve the use of markers as screening tools for the early detection of cancer, strong determinants of false-positive tests in a cancer-free population have to be considered.[10]

The aim of this study was to analyse mesothelin and calretinin in plasma samples from a large group of cancer-free men with a comprehensive set of auxiliary data in order to identify predictors of positive tests to improve the specificity of the markers for the early detection of malignant mesothelioma.

METHODS

Ethics statement

The study was approved by the ethical commission of the Medical Faculty of the University Duisburg-Essen (approval number 11-4678). All participants gave their written informed consent.

Study population

The study population consisted of 569 men from the second follow-up survey (2011–2014) of the Heinz Nixdorf Recall Study (HNRS) within the framework of AeKo (‘Arbeitsmedizinische Forschung in epidemiologischen Kohortenstudien’—Occupational medical research in epidemiological cohort studies).[11] HNRS is a prospective population-based cohort study in the Ruhr area, an urban conglomeration of industrial cities in Germany. Its rationale, design and conduct have been previously described.[12] For this analysis, subjects did not suffer from a malignant disease at the time of blood collection.

Determination of mesothelin and calretinin

For measurement of mesothelin and calretinin peripheral blood was collected from each participant in 9.0 mL S-Monovette EDTA gel tubes (Sarstedt, Nümbrecht, Germany) and centrifuged at 2000 x g for 10 min. Plasma was separated from the cellular fraction and frozen immediately.

Plasma mesothelin (ng/mL) was measured using the Human Mesothelin Quantikine ELISA kit (R&D Systems, Wiesbaden-Nordenstadt, Germany) according to the manufacturers’ instructions. Results were converted to nanomolar using the equation nM=x ng/mL/32.175. Marker results were marked as positive if mesothelin concentrations were ≥1.5 nM (cut-off). The intra-assay coefficient of variation (CV) was 3.6% and the inter-assay CV was 5.7%.

Plasma calretinin (ng/mL) was measured as described previously.[5] Marker results were marked as positive if calretinin concentrations were ≥1.0 ng/mL (cut-off). For the calretinin assay the intra-assay CV was 6.3% and the inter-assay CV was 6.4%.

A high stability of mesothelin and calretinin in plasma and serum was revealed regarding frozen storage as well as repeated freeze/thaw cycles.[5 6 8 13]

Potential predictors of positive marker tests

We used information on potential predictors of positive tests from the questionnaire (age, smoking status, hypertension, chronic diseases such as asthma and diabetes mellitus, and medication). We further included follow-up

Table 1 Distribution of selected blood parameters in 569 men of the Heinz Nixdorf Recall Study

Blood parameter	Standard value	n	Median	IQR
Brain natriuretic peptide (pg/mL)	<200	569	26.7	13.8–54.9
C-reactive protein (mg/dL)	0–0.3	564*	0.13	0.07–0.29
Creatinine (mg/dL)	0.9–1.3	569	1.16	1.06–1.27
Cystatin C (mg/L)	19–65 years: 0.53–0.95 66–74 years: 0.6–1.3 ≥75 years: 0.7–1.47	568*	0.86	0.75–0.99
Fibrinogen (mg/dL)	210–400	567*	328	281–380
Hemoglobin A1c (%)	4–6	567*	5.9	5.6–6.2
Haemoglobin (g/dL)	16–70 years: 13.7–17.2 71–75 years: 12.1–17.6 76–81 years: 11.8–17.5	569	14.9	14.1–15.5
Lactate dehydrogenase (U/L)	100–247	568*	177	160–196
Red blood cells (x 10^{12}L)	4.5–5.6	569	4.8	4.55–5.05

*Blood parameters could not be determined for all 569 subjects.

Table 2 Characteristics of 569 men of the Heinz Nixdorf Recall Study

			n	%
Personal	Age (years)	Median	70	
		Range	56–84	
	Smoking status	Current	70	12.3
		Former	321	56.4
		Never	176	30.9
Diseases (Self-assessed)	Cancer (past blood collection)	>1 year	11	1.9
		≤1 year	9	1.6
	Hypertension		365	64.1
	Bronchial asthma		18	3.2
	Bronchitis		11	1.9
	Chronic obstructive pulmonary disease		32	5.6
	Pneumoconiosis		10	1.8
	Pneumonia		12	2.1
	Pulmonary emphysema		9	1.6
	Tuberculosis		25	4.4
	Arthritis		10	1.8
	Diabetes mellitus		120	21.1
	Hepatitis		51	9.0
Medication	Angiotensin-converting-enzyme inhibitors		297	52.2
	Antihypertensive drugs		350	61.5
	Beta blockers		200	35.1
	Cardiac glycoside		8	1.4
	Antidiabetic medication		120	21.1
Marker concentrations	Mesothelin (nM)	Median	0.67	
		IQR	0.52–0.93	
	Calretinin (ng/mL)	Median	0.23	
		IQR	0.16–0.35	

information on the development of cancer and data on miscellaneous blood parameters at time of blood sampling. The distribution of blood parameters in the study population and standard values are given in table 1. All blood analyses were performed in the central laboratory of the University Hospital Essen with standard methods as formerly described.[14]

Statistical analysis

All calculations were done using SAS V.9.4 (SAS Institute). Median and inter-quartile range (IQR) were used to describe the distribution of continuous variables. Spearman's correlation coefficients (r_s) and p values were used to describe rank correlations between variables.

Prevalence ORs (PORs) with 95% CIs were estimated to assess the risk of marker concentrations being above the cut-off. Potential predictors were age, smoking status (never, former current), 11 different diseases (yes, no), development of cancer after blood sampling, intake of five different medications within the last 7 days before examination (yes, no) and 36 laboratory parameters (within, below or above standard values). First, we estimated PORs with univariate logistic regression models to identify potential influencing variables. Based on knowledge from literature and preliminary work, variables were assorted to corresponding disease groups, that is, malignant (diagnosis after blood collection), renal and heart diseases. Subsequently, for each marker separately, multiple regression models with one parameter from each disease group based on the univariate models were developed. As representative of the disease group the parameter with the strongest effect was selected.

RESULTS

Study population

Table 2 depicts the characteristics and marker results of 569 men from HNRS. The median age of the study population was 70 years (range: 56–84 years). A large fraction of the participants were former smokers (56.4%), 176

participants (30.9%) were non-smokers and 70 participants (12.3%) were current smokers.

After blood collection cancer was diagnosed in 20 participants, where 9 cases received a diagnosis within 12 months. The most frequent disease was hypertension with 365 cases (64.1%), followed by diabetes mellitus with 120 cases (21.1%). Hence, antihypertensive drugs were commonly used (n=350, 61.5%), and there was a very high correlation between hypertension and the intake of heart medications. Nearly every participant with hypertension took antihypertensive drugs (95.9%), and only every other took beta blockers (54.2%).

Blood parameters

The median concentrations of all blood parameters were within the standard values (table 1). Median mesothelin concentration was 0.67 nM (IQR 0.52–0.93 nM) and median calretinin concentration 0.23 ng/mL (IQR 0.16–0.35 ng/mL) (table 2). Positive mesothelin results (≥1.5 nM) were observed for 24 subjects (4.2%) and positive calretinin results (≥1.0 ng/mL) for 34 subjects (6.0%). Hence, the specificities for mesothelin and calretinin were 95.8% (95% CI 93.8% to 97.2%) and 94.0% (95% CI 91.7% to 95.7%) for the whole study population, respectively. Five men had elevated concentrations of both markers, resulting in a specificity of 99.1% (95% CI 97.9% to 99.7%).

The Spearman correlations between mesothelin, calretinin, age and blood parameters are presented in (table 3). Calretinin and mesothelin were positively correlated (r_S=0.18, p<0.001). The strongest correlation with r_S=0.55 (p<0.001) was observed between cystatin C and creatinine, both being markers of renal function. The concentrations of both markers increased by age, but age was also positively correlated with most of the blood parameters.

Potential predictors of positive test results for mesothelin and calretinin

Table 4 depicts the results of the univariate logistic regression models for predictors of mesothelin and calretinin concentrations above the cut-off. Cystatin C, creatinine, brain natriuretic peptide (BNP) and haemoglobin are associated with renal dysfunction. Cystatin C showed a POR of 20.8 (95% CI 8.48 to 50.8) for mesothelin and 3.48 (95% CI 1.34 to 9.02) for calretinin. Creatinine, BNP, haemoglobin, haematocrit, and the amount of red blood cells showed an impact only on mesothelin. Furthermore, hypertension and the intake of heart medication had an impact on increased POR only for mesothelin (13.7 (95% CI 1.83 to 101.9) and 15.3 (95% CI 2.06 to 114.4), respectively). The intake of beta blockers was associated with both markers.

Age showed a significantly increased POR (1.09, 95% CI 1.02 to 1.17) for mesothelin but not for calretinin. Cancer diagnosed after blood collection showed an POR of 4.33 (95% CI 1.36 to 13.7) for calretinin. In contrast, mesothelin was not increased in participants with a diagnosis of cancer after blood collection. Furthermore, C reactive protein and fibrinogen were associated with mesothelin but not calretinin. Bronchial asthma was a predictor of elevated calretinin. Bronchitis and increased hemoglobin A1c (HbA1c), a marker associated with diabetes mellitus, showed an impact on mesothelin. Several other parameters showed an increased POR for mesothelin (eg, alkaline phosphatase) but were based on very small numbers, whereas calretinin was not affected by other parameters (data not shown).

Table 5 shows the results of the multiple logistic regression models in the whole study population. Regarding the risk estimates for a positive mesothelin test, the PORs for cystatin C, hypertension and age were 11.0 (95% CI 4.02 to 30.0), 8.41 (95% CI 1.07 to 66.0) and 1.08 (95% CI 1.00 to 1.17), respectively. Regarding calretinin, cystatin C, cancer diagnosed after blood collection and bronchial asthma showed an impact on the marker concentration with a POR of 4.03 (95% CI 1.49 to 10.9), 4.98 (95% CI 1.51 to 16.5) and 5.19 (95% CI 1.54 to 17.4), respectively. Hypertension did not affect calretinin (POR 1.05, 95% CI 0.49 to 2.27).

DISCUSSION

Commonly, mesothelioma is diagnosed at late stages of the disease when symptoms like coughing, chest pain and difficulty breathing occur. Principally, tumour markers might facilitate the detection of cancer at early stages when clinical symptoms have not yet occurred. Mesothelin and calretinin are two candidates to serve as reliable blood-based markers for the early detection of mesothelioma.[3 4 6] Generally, the diagnosis of cancer at early stages, when clinical symptoms have not yet occurred, appears to be a promising opportunity to improve therapeutic outcome, ideally resulting in a decreased mortality. The treatment of early stages in combination with the new therapies that are on the horizon could lead to improvements in overall survival of patients with mesothelioma.[15–19]

In screening, false-positive tests should be avoided in cancer-free individuals.[7 20] However, knowledge is limited regarding the influence of benign diseases and blood parameters as potential confounders of mesothelin and calretinin, leading to increased marker concentrations in subjects without malignant mesothelioma.

This is the first study that extensively examines potential influencing parameters associated with mesothelin and calretinin concentrations above the cut-off in plasma samples of cancer-free participants in a large population-based cohort study. In this population-based cohort, we observed overall specificities of mesothelin and calretinin of about 95%. Only five men had both markers above the cut-offs, which would indicate a specificity of 99.1% (95% CI 97.7% to 99.7%) in the general population. In a recent study, we were able to demonstrate that a combination of mesothelin and calretinin was able to improve the performance of mesothelin alone.[6] The specificity in the target population of asbestos-exposed

Table 3 Spearman correlations (rS) and p Values of age, mesothelin, calretinin and blood parameters in 569 men of the Heinz Nixdorf Recall Study

	Age (years)		Mesothelin		Calretinin		Cystatin C		Creatinine		Brain natriuretic peptide		Haemoglobin		C-reactive protein		Fibrinogen		Lactate dehydrogenase	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Mesothelin (nM)	**0.29**	**<0.001**																		
Calretinin (ng/mL)	**0.12**	**0.004**	**0.18**	**<0.001**																
Cystatin C (mg/L)	**0.43**	**<0.001**	**0.34**	**<0.001**	**0.31**	**<0.001**														
Creatinine (mg/dL)	**0.13**	**0.002**	**0.28**	**<0.001**	**0.21**	**<0.001**	**0.55**	**<0.001**												
Brain natriuretic peptide (pg/mL)	**0.43**	**<0.001**	0.06	0.127	**0.08**	**0.052**	**0.26**	**<0.001**	0.04	0.295										
Haemoglobin (g/dL)	**−0.16**	**<0.001**	**−0.09**	**0.032**	−0.03	0.509	**−0.10**	**0.013**	−0.06	0.181	**−0.16**	**<0.001**								
C-reactive protein (mg/dL)	**0.11**	**0.009**	**0.10**	**0.022**	**0.14**	**0.001**	**0.29**	**<0.001**	0.06	0.188	**0.15**	**<0.001**	**−0.10**	**0.014**						
Fibrinogen (mg/dL)	**0.10**	**0.014**	**0.09**	**0.027**	**0.11**	**0.012**	**0.16**	**<0.001**	0.03	0.428	**0.11**	**0.007**	**−0.16**	**<0.001**	**0.50**	**<0.001**				
Lactate dehydrogenase (U/L)	**0.17**	**<0.001**	**0.14**	**0.001**	**0.12**	**0.003**	**0.19**	**<0.001**	**0.08**	**0.062**	**0.23**	**<0.001**	−0.05	0.241	**0.15**	**0.001**	0.05	0.275		
Hemoglobin A1c (%)	**0.14**	**0.001**	0.02	0.628	**0.09**	**0.033**	**0.16**	**<0.001**	**0.16**	**<0.001**	**0.09**	**0.024**	**−0.14**	**0.001**	**0.12**	**0.005**	**0.15**	**<0.001**	−0.01	0.780

Statistically significant results are marked in bold.

Table 4 Prevalence ORs (PORs) and 95% CIs for predictors of increased mesothelin and calretinin assessed with univariate logistic regression models in 569 men of the Heinz Nixdorf Recall Study

			Mesothelin ≥1.5 nM				Calretinin ≥1 ng/mL			
Variable	**Effect**	**N***	**n†**	**POR**	**95% CI**		**n†**	**POR**	**95% CI**	
Age (years)		569	24	**1.09**	**1.02**	**1.17**	34	1.01	0.95	1.06
Cancer past blood collection (reference: no)	Yes	20	0	–			4	**4.33**	**1.36**	**13.7**
C-reactive protein (mg/dL) (reference: within standard values)	Greater than standard value‡	137	11	**3.02**	**1.30**	**7.01**	9	1.13	0.51	2.48
Fibrinogen (mg/dL) (reference: less than or equal to standard values)	Greater than standard value‡	98	9	**3.06**	**1.30**	**7.21**	6	1.03	0.41	2.55
Cystatin C (mg/dL) (reference: less than or equal to standard value)	Greater than standard value‡	37	12	**20.8**	**8.48**	**50.8**	6	**3.48**	**1.34**	**9.02**
Creatinine (mg/dL) (reference: less than or equal to standard value)	Greater than standard value‡	112	15	**7.70**	**3.27**	**18.1**	10	1.77	0.82	3.82
Brain natriuretic peptide (pg/mL) (reference: within standard values)	Greater than standard value‡	52	8	**5.69**	**2.31**	**14.0**	6	2.28	0.90	5.79
Haemoglobin (g/dL) (reference: greater than or equal to standard value)	Less than standard value‡	34	9	**12.5**	**4.98**	**31.3**	31	1.57	0.46	5.43
Haematocrit (L/L) (reference: greater than or equal to standard value)	Less than standard value‡	81	11	**5.74**	**2.48**	**13.3**	6	1.32	0.53	3.28
Red blood cells ($x10^{12}$L) (reference: greater than or equal to standard value)	Less than standard value‡	116	12	**4.24**	**1.85**	**9.71**	9	1.44	0.65	3.18
Hemoglobin A1c (%) (reference: less than or equal to standard values)	Greater than standard value‡	196	13	**2.32**	**1.02**	**5.29**	12	1.04	0.50	2.14
Bronchial asthma (reference: no)	Yes	18	1	1.35	0.17	10.6	4	**4.95**	**1.54**	**16.0**
Bronchitis (reference: no)	Yes	11	3	**9.57**	**2.37**	**38.7**	2	3.77	0.78	18.2
Hypertension (reference: no)	Yes	365	23	**13.7**	**1.83**	**101.9**	23	1.18	0.56	2.47
Antihypertensive intake (reference: no)	Yes	350	23	**15.3**	**2.06**	**114.4**	23	1.33	0.64	2.79
Beta blocker intake (reference: no)	Yes	200	16	**3.92**	**1.65**	**9.34**	18	**2.18**	**1.09**	**4.38**

Statistically significant results are marked in bold
*Men with effect.
†Men with positive marker result and effect.
‡The standard values of all blood parameters are given in table 1.

Table 5 Prevalence ORs (PORs) and 95% CIs for predictors of increased mesothelin and calretinin assessed with multiple logistic regression models

Variable	Effect	Mesothelin ≥1.5nM			Calretinin ≥1ng/mL		
		n*	POR	95% CI	n*	POR	95% CI
Age (years)		23	1.08	1.00 1.17	–	–	
Cancer past blood collection (reference: no)	Yes	–	–		4	4.98	1.51 16.5
Cystatin C (mg/dL) (reference: less than or equal to standard value)	Greater than standard value†	11	11.0	4.02 30.0	6	4.03	1.49 10.9
C reactive protein (mg/dL) (reference: less than or equal to standard value)	Greater than standard value†	11	2.27	0.87 5.93	–	–	
HbA1c (%) (reference: less than or equal to standard values)	Greater than standard value†	13	1.14	0.43 3.00	–	–	
Hypertension (reference: no)	Yes	22	8.41	1.07 66.0	23	1.05	0.49 2.27
Bronchitis (reference: no)	Yes	3	5.80	0.99 33.9	–	–	
Bronchial asthma (reference: no)	Yes	–	v		4	5.19	1.54 17.4

Statistically significant results are marked in bold
*Men with positive marker result and effect.
†The standard values of all blood parameters are given in table 1.

workers, including cases with asbestosis, is likely lower as shown in a meta-analysis.[3]

An advantage of this population-based cohort is the wealth of data acquired, which cannot be assembled in clinical routine.[7] However, the study focused on men because in general the reported incidences of mesothelioma are significantly higher for men than women.[21 22] This might be a result of an increased occupational exposure to asbestos among men.[23] Hence, it was not the aim of this study to determine whether gender differences exist regarding the association between calretinin, mesothelin and the potential confounding factors investigated. Furthermore, despite the large study population the statistical analyses on predictors of false-positive tests were based on small numbers which resulted from the high overall specificities of mesothelin and calretinin of 95%.

In this study, renal dysfunction as a well-known confounder was confirmed for mesothelin. An influence of renal dysfunction was also identified for calretinin although weaker. The glomerular filtration rate was shown to be a confounder of mesothelin in serum[9] and similar results were observed for the association of creatinine and mesothelin.[10] Here, we showed that increased cystatin C values correlated with increased mesothelin and calretinin concentrations in plasma. Notably, the impact of cystatin C on mesothelin seems to be stronger than on calretinin. For the assessment of renal dysfunction, cystatin C might be superior to creatinine because it is not influenced by age, gender and muscle mass.[24 25] For the use of mesothelin and calretinin in diagnostics or early detection, the additional assessment of cystatin C might be meaningful to improve specificity.

Hypertension is a common cause but also a consequence of kidney failure[26] and was already mentioned as potential confounder of mesothelin.[8] In this study, men with hypertension showed an eightfold higher chance of increased mesothelin concentrations in comparison to non-hypertensive men. This association was weaker when estimating prevalence ratios (PRs) assessed with Poisson regression (PR 7.31, 95% CI 0.96 to 55.6, data not shown). Nevertheless, the prevalence of hypertension is high in elderly men, 74.2% of German men between 70 and 79 years and 59.8% between 60 and 69 years suffer from hypertension or take antihypertensive drugs.[27] However, men with prevalent conditions such as hypertension cannot be excluded from medical surveillance. This supports the use of calretinin as an additional marker, as it was not affected by hypertension.

No influence of age could be observed for calretinin, confirming the initial results by Raiko *et al*.[5] In contrast, a weak influence of age on mesothelin could be observed but was only marginally significant when estimating PRs (data not shown). Published results are inconsistent: whereas Pass *et al* noted no differences of mesothelin concentrations regarding age,[28] more recent studies suggested that age was a statistical predictor of mesothelin.[10 29] In this study, we confirm these results analysing

mesothelin concentration in plasma samples of a large number of people from the general population. Thus, for the assessment of mesothelin results age needs to be considered in diagnostics, for example, using an age-depended cut-off. As calretinin is not influenced by age, this also supports calretinin as an appropriate additional marker in early detection.

It was suggested that the release of mesothelin into serum results from malignant mesothelium and not from other inflammatory or malignant pulmonary or pleural diseases.[30 31] In this study, bronchitis was marginally associated with increased mesothelin concentrations. However, bronchial asthma was not associated with increased mesothelin but calretinin concentrations. The connection between inflammatory diseases and increased mesothelin and calretinin values in human plasma remains unclear. However, these findings are based on small numbers with 11 men with diagnosed bronchitis and 18 men with diagnosed bronchial asthma. Additional studies comprising higher numbers of these diseases are needed to assess the influence on the marker concentrations in more detail. However, in screening inflammatory indices should be recorded during medical examinations.[20]

Cancer was diagnosed in 20 participants after blood collection, for nine subjects already within 12 months. A sensitivity analysis revealed that cancer closer to blood collection had a stronger impact on calretinin concentrations than cancers detected later than 1-year past blood collection (data not shown). This is in agreement with a study on bladder cancer[32] and on ovarian cancer,[33] where cancer-related biomarkers showed more frequently elevated concentrations exceeding cut-offs 1 year prior to diagnosis. Four participants with cancer past blood collection showed increased calretinin concentrations above the cut-off at time of blood collection. As none of the identified predictors (renal dysfunction or bronchial asthma) seemed to be responsible for the elevated calretinin concentration, the later developed cancer might be responsible for the increased calretinin concentrations in the plasma of these men. However, as the types of cancers were diverse (prostate adenocarcinoma, renal cell carcinoma, urothelial tumour of the renal pelvis and basal cell carcinoma) prospective studies are needed to analyse the feasibility of calretinin as marker for the early detection of cancers other than mesothelioma.

CONCLUSIONS

Mesothelin and calretinin showed high specificities in this cohort of cancer-free elderly men. Mesothelin was strongly affected by renal dysfunction and at a lower degree by hypertension, where cystatin C may serve as an informative parameter to improve specificity. Calretinin positivity was also affected by renal dysfunction—but to a lesser extent—and showed no association with hypertension. Calretinin might be a useful adjunct to mesothelin for the early detection of mesothelioma, with an increase of the specificity to 99.1% (95% CI 97.9% to 99.7%) for this marker panel.

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Competing interests None declared.

Patient consent Obtained.

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