Research paper

Endotrophin is associated with chronic multimorbidity and all-cause mortality in a cohort of elderly women

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ABSTRACT

Background: The signalling peptide endotrophin is derived through proteolytic cleavage of the carboxyl-terminal during formation of type VI collagen. It is expressed by most descendants of the mesenchymal stem cells lineage, including adipocytes and fibroblasts, and have been proposed to be a central extracellular matrix hormone associated with several age-related diseases. We aimed to assess the association of endotrophin with chronic disease incidence and death in older women.

Methods: 5,602 elderly Danish women from the observational, prospective cohort: The Prospective Epidemiological Risk Factor (PERF) study were included in the analysis which covered baseline (BL) and follow-up (FU) 14 years later. An elastic net was used to investigate the relative importance of 58 variables to serum endotrophin-levels. 20 chronic diseases were defined on the basis of clinical variables available along with diagnoses extracted from both the National Patient Register, the National Diabetes Register and the Danish Cancer Registry. The cross-sectional associations between endotrophin-levels and these 17 chronic age-related diseases were investigated using logistic regression and a set-analysis explored disease-combinations within multimorbidity. The association of endotrophin with mortality was assessed by Cox proportional hazard models.

Findings: Formation of type III collagen (PRO-C3), age and creatine-levels were the most influential variables of endotrophin-levels. Several chronic diseases were significantly associated with endotrophin-levels independent of age and BMI including chronic kidney disease (BL OR=3.7, $p < 0.001$; FU OR = 7.9 $p < 0.001$), diabetes (BL OR = 1.5, $p = 0.0015$, FU OR=1.6, $p = 0.004$) and peripheral arterial disease (BL OR = 1.3, $p = 0.029$; FU OR=2.4, $p < 0.001$). Lastly, endotrophin-levels were significantly rising with number of morbidities ($p < 0.001$) and a predictor of death after adjusting for age and BMI (BL HR=1.95; FU HR = 2.00).

Interpretation: Endotrophin was associated with death and increased with number of morbidities. Endotrophin may be a central hormone of fibroblast that warrant investigation and possible targeted intervention in several chronic diseases.

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Introduction

There are more than 40 age-related chronic diseases associated with elevated levels of extracellular matrix (ECM) turnover [1]. This account for more than 50% of deaths in the western world and include cardiovascular, respiratory, dermatological, nephrological and gastrointestinal diseases [2]. Fibroblasts and activated myo-fibroblasts are central components in the progression of these diseases, where they lead propagation of ECM expansion, the interstitial fibrosis, in all organs [3].

Fibroblasts and other mesenchymal descendants produce ECM proteins that are altered during progression of most chronic diseases. Recently, it has been discovered that the altered ECM is not only a consequence but also a driver of some diseases [4]. During remodelling of the ECM, fragmented peptides are released into the circulation. Some of these peptides harbours cryptic sites with novel function such as regulation of cell growth, differentiation, and angiogenesis [5,6]. Endostatin and tumstatin, fragments of type XVIII and IV collagen, are examples of such peptides.

Research in Context

Evidence before this study

We searched Web of Science on May 1, 2020, for clinical articles evaluating associations between endotrophin and disease in humans. Search terms used were TOPIC: (endotrophin OR PRO-C6) *AND* TOPIC: (disease OR diabetes OR tumor OR cancer OR Alzheimer OR dementia OR hypertension OR heart OR osteoarthritis OR hypertension) *AND* TOPIC: (cohort OR patients OR clinical OR participants OR subjects). We identified 30 peer-reviewed journal articles. Six studies investigated cancer, five studies reported on diabetes and also sclerosis was turning point of four papers. The rest included chronic liver disease, COPD and mortality. Endotrophin is a hormone that has received little attention. As the link between disease and the ECM substantiates by an increasing number of studies the interest in endotrophin is also growing. Almost all studies up until now have focused on single diseases when investigating the functions of endotrophin. No studies that investigated endotrophin's association with multiple different age-relating diseases and multimorbidity were identified.

Added value of this study

This study provides a comprehensive overview of the association between endotrophin and a wide range of chronic diseases in old women. We also showed that endotrophin is associated with increased multimorbidity-severity in human and death. These findings highlight the importance of endotrophin as a chronic disease burden marker and suggest that endotrophin may be used to monitor chronic disease state and thereby help guide health strategies in the old population. To our knowledge this is the first large study investigating the association of endotrophin with multiple chronic diseases.

Implications of all the available evidence

In animal models, endotrophin have previously been shown to be involved in several biological processes, such as fibrosis, inflammation, angiogenesis, insulin resistance and epithelial-mesenchymal transition and in humans endotrophin has previously been linked with several chronic diseases including chronic kidney disease, breast cancer diabetes. Our study provides novel evidence that endotrophin is not only associated with single chronic diseases but also with the degree of chronic multimorbidity and death. Future studies should focus on investigating if endotrophin is a driver of chronic multimorbidity and death.

Another example of a signalling peptide is endotrophin which is located in the pro-peptide of the type VI alpha 3 chain [7]. During formation of type VI collagen, the carboxyl-terminal (C5 domain) is cleaved of. Further processing of the C5 domain generates the signalling fragment endotrophin that can be released into the circulation and quantified by the PRO-C6 assay [8]. Endotrophin is expressed by most cells from mesenchymal stem cells lineage, including adipocytes and fibroblasts [9], and thus, may possibly be a central ECM hormone associated with many chronic fibro-inflammatory disorders.

In animal models, endotrophin has been shown to be involved in several biological processes, such as fibrosis, inflammation, angiogenesis, insulin resistance and epithelial-mesenchymal transition (EMT) [10,11]. In humans, endotrophin has been linked to chronic kidney disease (CKD)[12,13], breast cancer [14], chronic obstructive pulmonary disease (COPD)[15,16], diabetes [17,18] and death [19].

However, the exact functions of endotrophin in humans and the complete picture of the chronic diseases linked to endotrophin remain unknown.

Using a data-driven approach we aimed to get a better understanding of endotrophin's role in chronic age-related diseases in the Prospective Epidemiological Risk Factor (PERF) study (*n* = 5602). The PERF cohort is a study of elderly women that was followed for 14 years and has been linked to several Danish health registries. Accordingly, we examined the association of endotrophin with 58 clinical parameters, 17 chronic age-related diseases, number of morbidities and death at baseline and 14 years later at follow-up.

Methods

Study design

The Prospective Epidemiological Risk Factor (PERF) study, established in 1999−2001 (baseline), took aim at developing the understanding of age-related diseases in elderly women [20]. Women who previously participated in clinical randomized prevention trials or had been screened for inclusion in clinical prevention trials (*n* = 8875) at the Center for Clinical and Basic Research (CCBR) were invited to participate in the PERF study. A total of 5855 Danish elderly women (avg. age 71 yrs.) were enrolled in PERF at baseline and approximately 14 years later (yr. 2013−2014) a follow-up study was established when 2103 women (avg. age 81 yrs.) were re-examined. The study has been described in detail elsewhere [21].

Baseline and follow-up investigations

The participants completed interviews with a doctor or a nurse covering questions related to demographics, lifestyle, and medical history both at baseline and follow-up. A medical physical exam included DXA-scanning at baseline and standard body shape measures at follow-up. Furthermore, a cognitive test resulting in a range of cognitive scores was performed at both time points.

Endotrophin measurements

All subjects provided fasting blood samples both at baseline and follow-up and serum was stored at −80 °C before the biomarkers were measured blinded in a CAP-certified laboratory. Serum endotrophin was measured in November-December 2016 after confirming the stability of the samples by running seven consecutive freeze-thaw cycles with no significant variation detected. Further, a three-year stability test was performed by measuring the same sample with a one-year interval. A complete description of all biomarkers including measuring methods and variables used in the analysis is shown in table S1.

Linkage to disease and death registries

In Denmark, every citizen has a unique personal subject identification number (CPR-number) which enabled matching of the women to various electronic health- and death-registries at the level of individuals. Health information used for this study was derived from: The Danish National Patient Register [22], the National Danish Causes of Death Registry [23], the Danish Adult Diabetes Registry [24], and the Danish Cancer Registry [25] covering the time span of the PERF study and time before baseline (pre-baseline). The national registries used in this analysis have previously been validated [26−29].

Disease phenotypes and multimorbidity

Based on electronic health records, relevant biomarkers, medical work-up and questionnaire-information, 17 different disease
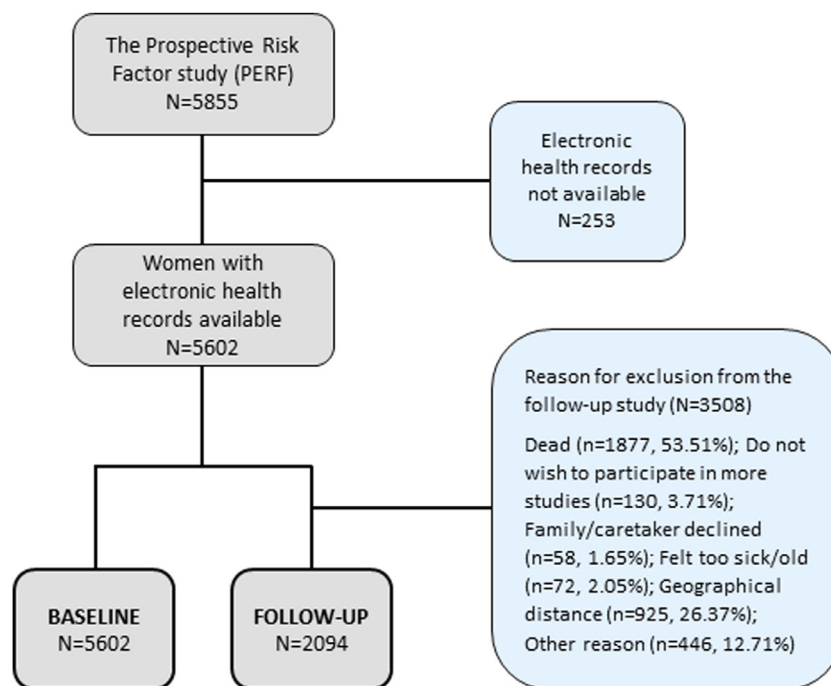
Fig. 1. Flow chart showing the inclusion process and categorization of the postmenopausal women included in the study.

phenotypes were defined (Table 2). The exact definitions used for the phenotypic disease-grouping is available in table S2. These 17 diseases were selected with the aim of covering a wide range of chronic fibro-inflammatory disorders in the elderly [30].

The disease phenotypes were evaluated at baseline and follow-up for each woman resulting in two binary disease-matrices. The *baseline definition* covers the time before baseline and up until 1 year after baseline, and *follow-up* covers pre-baseline up until 14 years after baseline. Data gathered at baseline was used for the baseline definition, whereas the follow-up definition was based on data collected both at baseline and follow-up.

Multimorbidity was evaluated at each time point as a count of *number of diseases* estimated by adding the disease-phenotypes for each woman.

Inclusion criteria

Women with electronic health records available from the Danish National Patient Register were included in the analysis (Fig. 1).

The prospective design of the PERF study unavoidably results in a healthy bias of the women participating in the follow-up study. A thorough investigation of endotrophin-levels and BMI between the women lost to follow-up and the women participating in the follow-up study has been conducted to ensure no systematic bias. The reason for loss to follow-up was most dominantly death (53.5%) and geographical distance (26.4%) followed by unknown/other reasons (12.7%) (Fig. 1). We found that women not participating in the follow-up study in general were older. We also found in an ANCOVA analysis adjusted for age and analysis-time that neither BMI nor endotrophin levels at baseline were significantly different between the women participating in the follow-up study and the women that were lost to follow-up (independent of reason for exclusion) (Figure S1).

Statistical analyses

Endotrophin and variable importance

The relative importance of each variable to the levels of endotrophin in elderly women was investigated with an elastic net applying

5-fold cross-validation repeated 20 times. Endotrophin level was the outcome of the model and all variables included in the model are listed in table S1. The *caret* R-package was used for the elastic net [31]. We chose to use penalized regression because the number of variables was high with the risk of collinearity. Dummy variables were generated for all categorical variables. Continuous variables were firstly normalized and further standardized. For the normalization, all values higher or lower than 1.5 times the inter quartile range (IQR) were set to this upper/lower limit, respectively. Data was standardized to a mean of 0 and a standard deviation of 1. The coefficients of the final model were extracted and used for variable importance assessment.

Endotrophin and chronic age-related disease

A logistic regression model was fitted to investigate the association between endotrophin and disease phenotypes (Table S2) both at baseline and follow-up. All regressions were adjusted for age and BMI at baseline and follow-up, respectively. Furthermore, at baseline the analysis was adjusted for analysis-time. Endotrophin was log2-transformed prior to analysis. Finally, all p-values were adjusted using the method by Benjamini & Yekutieli to target a false discovery rate of 5%. All odds ratios reported corresponds to a doubling of endotrophin-levels.

Endotrophin and multimorbidity

To investigate the association between endotrophin-levels and multimorbidity an aggregated disease-sum was derived for each woman. This was done by adding the number of diseases observed for each woman. Again, we did this at the two different time points. We excluded the multimorbidity-groups with less than 20 women observed and plotted the median endotrophin level $\pm$ Q1/Q3 for each group. Tests for linear or quadratic trend were performed using linear regression.

To visually represent which disease phenotypes were prevalent within each multimorbidity-group, a stacked barplot showing the percentage distribution of each disease phenotype was generated.

The specific disease-combinations within each multimorbidity-group was further investigated with a set analysis. All sets presenting with more than 20 women were visualized in an UpSet plot using the

Table 1
Baseline and follow-up characteristics for the studied women. IQR: Interquartile range.

		Baseline	Follow-up
Characteristics			
	(N = 5602)	*(N = 2094)*	
Age (years)	*median ± IQR (n)*	71.0 ± 10.0 (5602)	81.0 ± 9.2 (2094)
BMI (kg/m^2)	*median ± IQR (n)*	26.0 ± 5.5 (5396)	26.0 ± 6.0 (1950)
Fat % (from DXA)	*median ± IQR (n)*	41.0 ± 9.5 (5060)	-
Waist Circumference	*median ± IQR (n)*	-	86.0 ± 16 (1951)
HOMAIR	*median ± IQR (n)*	82.0 ± 74.0 (5370)	92.0 ± 75.0 (2044)
Systolic blood pressure (mm HG)	*median ± IQR (n)*	150.0 ± 33.0 (5440)	140.0 ± 27.0 (1973)
Diastolic Blood pressure (mm HG)	*median ± IQR (n)*	81.0 ± 16.0 (5442)	80.0 ± 14.0 (1973)
Education	*High school/ University, n/ total (%)*	1594/5594 (28%)	Same as baseline
	School, n/ total (%)	4000/5594 (71%)	Same as baseline
Smoking	*Current/Former, n/ total (%)*	2957/5597 (53%)	1035/2094 (49%)
	Never, n/ total (%)	2640/5597 (47%)	1059/2094 (51%)
Alcohol	*< 7 units/week, n/ total (%)*	2491/5594 (44%)	85/1980 (4%)
	≥ 7 units/week, n/ total (%)	3103/5594 (55%)	1895/1980 (90%)
Recreational walking (min. 10 min)	*≥5 times/week, n/total (%)*	4006/5597 (72%)	1087/1971 (52%)
	<5 times/week, n/ total (%)	1591/5597 (28%)	884/1971 (42%)
Exercise habits	*≥1 time/week, n (%)*	3838/5596 (69%)	1295/1969 (62%)
	<1 time/week, n (%)	1758/5596 (31%)	674/1969 (32%)
Endotrophin (ng/ml)	*median ± IQR (n)*	8.3 ± 2.5 (5371)	10.1 ± 3.9 (2044)
Lymphocytes (%)	*median ± IQR (n)*	33.0 ± 11.0 (5140)	31.0 ± 12.0 (2082)
Neutrophils (%)	*median ± IQR (n)*	59.0 ± 11.0 (5140)	56.0 ± 12.0 (2082)
Cholesterol (mmol/l)	*median ± IQR (n)*	6.3 ± 1.4 (5428)	5.5 ± 1.5 (2085)
Triglycerid (mmol/l)	*median ± IQR (n)*	1.2 ± 0.7 (5422)	1.1 ± 0.6 (2085)

ComplexHeatmap library in R. The endotrophin-levels observed for each disease-combination was visualized with a barplot showing the median ± Q1/Q3.

Endotrophin and all-cause mortality

Cox proportional hazards models were used to evaluate the relationship between endotrophin and all-cause mortality up to 1 year after the endotrophin measurement at baseline and follow-up. Both models were adjusted for BMI and age for the two time points respectively. Endotrophin-levels were log2-transformed prior to analysis. The *cph*-function of the *rms* R-package [32] was used for the Cox-models. An investigation of the same models applying restricted cubic splines showed that the data was linearly associated.

All analyses were performed using R (V. 3.6.2) [33].

Ethics

The PERF study was carried out in accordance with the protocol approved by the Research Ethics Committee of Copenhagen County, Viborg and Northern Jutland, Denmark (KA 99070gm) and in conformance with Good Clinical Practice and the Helsinki Declaration II. Written informed consent was obtained from all participants.

Funding

The funder of the PERF study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Cohort characteristics

There were 5602 elderly women at baseline with available electronic health records, and 2094 women in the follow-up study

(Fig. 1). No systematic bias was found between the women lost to follow-up and the women participating in the follow-up study. Both baseline and follow-up characteristics are summarized in Table 1. On average the women were 70±6.6 years old at baseline and 81± 6.1 years old at follow-up. The mean BMI of 26 did not change between baseline and follow-up. The median endotrophin levels rose from 8.3 ng/ml to 10.1 ng/m from baseline to follow-up. These levels of endotrophin are in accordance with what have been observed in other studies [34,35], and the relatively small delta between the timepoints is expected as endotrophin seems to impact the microenvironment locally.

Disease phenotypes

For this study 17 different age-related inflammatory and fibroproliferative chronic diseases were defined (Table 2) based on electronic health records (specific ICD10 codes), relevant biomarkers, and questionnaire-information. The prevalence of the chronic diseases is shown in Table 2. Generally, the percentage of women with diseases increased from baseline to follow-up, most likely due to higher age. The disease phenotype observed with the highest prevalence, both at baseline and follow-up, was *hypertension* with 37.99% and 66.57% women affected at the respective time-points. The rarest observed disease phenotype was *respiratory cancer* with 0.43% of the women at baseline being sick and 1% at follow-up. These percentages are in line with what would be expected for an elderly population [36−38].

Ranking of variable importance to endotrophin

A variable importance analysis using an elastic net was conducted with the aim of exploring which variables were associated with endotrophin. This was done both for endotrophin measured at baseline (58 variables) and at follow-up (46 variables) (Table S1). The top 20 variables for each analysis are shown in Fig. 2. The analysis implicated the fibrotic biomarker PRO-C3, creatinine and age as the variables with largest impact on endotrophin levels. Further, MMP

Table 2
Prevalence of women with chronic disease phenotypes stratified by disease-group and timepoint.

		Baseline		Follow-up	
Group	Disease phenotypes	N = 5602	(%)	N = 2094	(%)
Brain	Alzheimer's/ Dementia	106	(1.89%)	179	(8.55%)
Cancer	Gastrointestinal Cancer	91	(1.62%)	114	(5.44%)
	Respiratory Cancer	24	(0.43%)	21	(1%)
	Breast Cancer	213	(3.80%)	165	(7.83%)
	Female genital organ Cancer	128	(2.28%)	72	(3.44%)
Cardiovascular	Hypertension	2128	(37.99%)	1394	(66.57%)
	Ischemic Heart Disease	310	(5.53%)	277	(13.23%)
	Peripheral Arterial Disease	444	(7.93%)	149	(7.12%)
	Congestive Heart Failure	116	(2.07%)	188	(8.98%)
	Cerebrovascular Diseases	293	(5.23%)	351	(16.76%)
	Atrial Fibrillation	204	(3.64%)	292	(13.94%)
Musculoskeletal	Rheumatic Disease	278	(4.96%)	227	(10.84%)
	Osteoarthritis	1934	(34.52%)	1262	(60.27%)
	Osteoporosis	1291	(23.05%)	615	(29.37%)
Digestive	Inflammatory Bowel Disease	77	(1.37%)	96	(4.58%)
	Chronic Kidney Disease	1054	(18.81%)	481	(22.97%)
	Chronic Liver Disease	121	(2.16%)	90	(4.3%)
Lung	Chronic Pulmonary Disease	326	(5.82%)	387	(18.48%)
Metabolic	Hyperlipidaemia	1168	(20.85%)	618	(29.51%)
	Diabetes	401	(7.16%)	361	(17.24%)

degraded type VI collagen (C6M), triglyceride, haemoglobin, albumin, total fat and waist circumference ranked high.

Disease phenotypes associated with endotrophin

To quantify the association between endotrophin and different chronic age-related diseases a logistic regression was run for each of the disease phenotypes, both at baseline and follow-up (Fig. 3). All analyses were adjusted for age and BMI and further FDR-corrected. Chronic kidney disease showed to be the phenotype with the strongest association to endotrophin both at baseline and follow-up [(BL; $p_{adj} = 4.72*e-55$, OR = 5.7), (FU; $p_{adj} = 3.14*e-39$, OR = 7.9)]. Also, a range of other chronic diseases were significantly associated with endotrophin levels: diabetes, rheumatic disease, peripheral arterial disease, Ischemic heart disease, chronic liver disease and gastrointestinal cancer. Inflammatory bowel disease and chronic pulmonary disease were not significantly associated with endotrophin levels at baseline, but the association became significant at follow-up.

Endotrophin and multimorbidity

The association between multimorbidity and endotrophin was evaluated by plotting the number of diseases for each woman against endotrophin levels at baseline and follow-up, respectively (Fig. 4). We observed rising endotrophin levels with increased number of chronic diseases. This was true both at baseline and follow-up and significant in tests for linear or quadratic trend (all $p < 0.001$) across the number of diseases. Generally, we saw overall higher endotrophin levels when measured at follow-up, where the age on average was 11 years higher. To ensure age not being the underlying reason for higher endotrophin levels in the groups with more multimorbidity, each multimorbidity-group was stratified into age-groups and
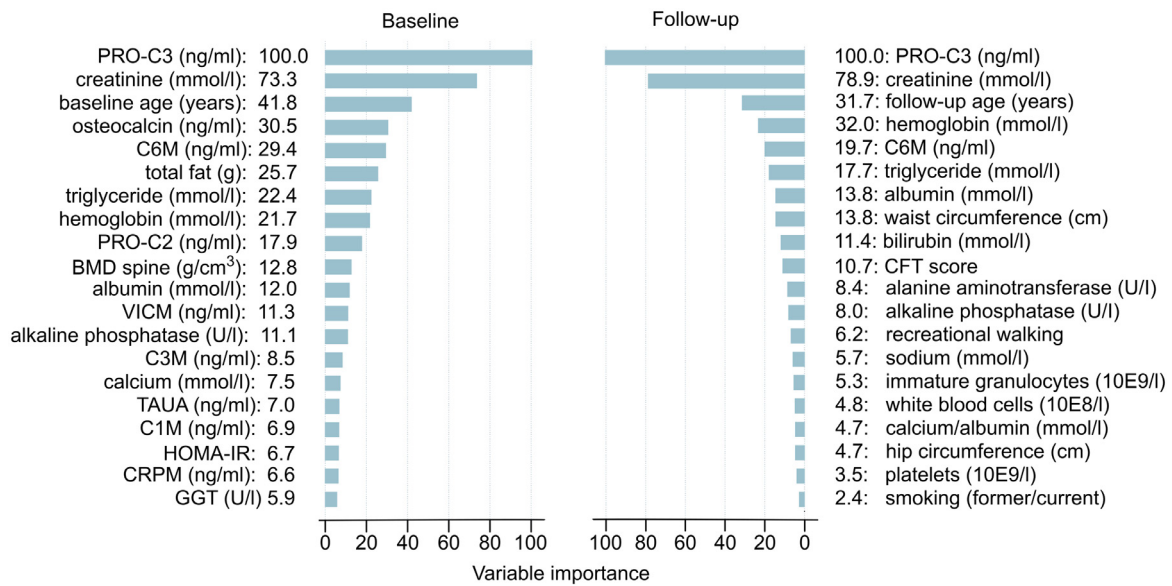

Fig. 2. Variable importance plots showing the top 20 most significant variables in relation to endotrophin. PRO-C2: formation of type II collagen; PRO-C3: formation of type III collagen; C1M: MMP degraded type I collagen; C3M: MMP degraded type III collagen; C6M: MMP degraded type VI collagen; CRPM: MMP degraded CRP (C-reactive protein); BMD: bone mineral density; VICM: MMP degraded and citrullinated vimentin; HOMA-IR: homeostatic model assessment of insulin resistance; mixed cells: monocytes, eosinophils, and basophils; CFT: The category fluency test with animal naming; TAUA: Adam degraded TAU; GGT: Gamma-Glutamyl Transpeptidase.
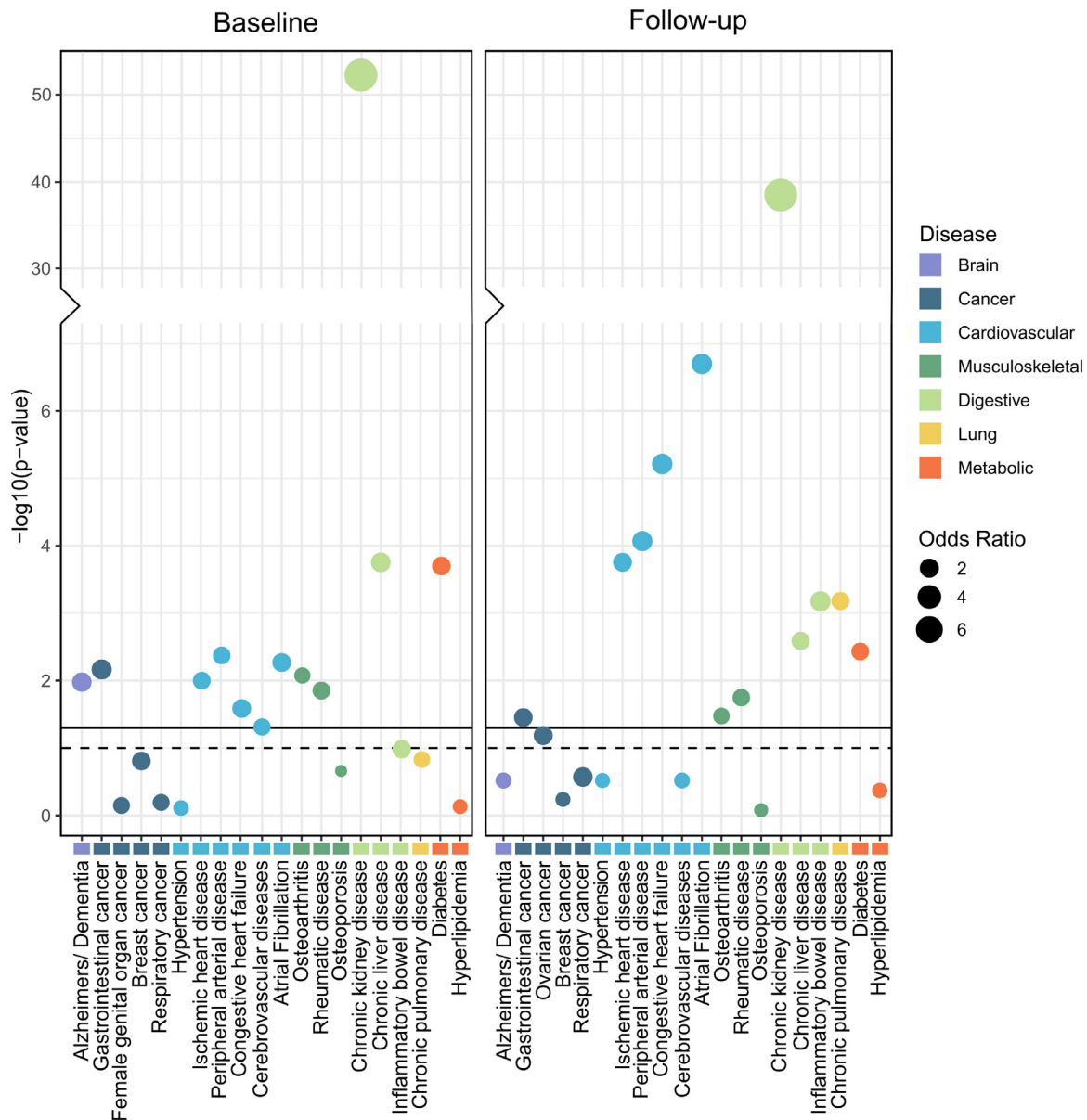
Fig. 3. Association plot for endotrophin and disease phenotypes showing FDR adjusted p-values. The black line depicts the target FDR level of 0.05, dotted line represents a target FDR of 10%.

the endotrophin levels were investigated. We saw that the endotrophin levels were increasing with number of diseases independent of age (Figure S2). The prevalence of diseases within each multimorbidity-group was represented in a stacked barplot, and here we saw that hypertension and osteoarthritis were highly represented in the groups with low multimorbidity.

Hyperlipidaemia and chronic kidney disease maintained a similar percentage prevalence with rising number of morbidities, whereas diabetes, ischemic heart disease, peripheral arterial disease and congestive heart failure rose in the overall prevalence.

To investigate the relationship between multimorbidity and level of endotrophin we performed a set-analysis and examined the disease-combinations presenting with more than 20 women (Fig. 5). We saw that CKD, diabetes and hypertension were among the diseases most represented in the disease-combinations with highest endotrophin-levels. As a limit of 20 women was applied, the disease-combinations presented in Fig. 5 consist predominantly of diseases that has a high prevalence in the population.

All-cause mortality in relation to endotrophin

As endotrophin levels were associated with number of morbidities after adjusting for age and BMI we further hypothesized that endotrophin levels were associated with all-cause death after adjustment for age and BMI. We have previously seen that endotrophin was associated with death up to 15 years after baseline in the PERF cohort [19]. To re-evaluate if the same picture was seen 1 year after baseline and at follow-up we performed a sub-analysis. The association of endotrophin-levels with all-cause mortality after 1 year was evaluated both at baseline and follow-up with a Cox regression analysis with time since the endotrophin measurement as the underlying time scale and adjustment for age and BMI. Endotrophin was a significant predictor of all-cause mortality both at baseline and follow-up (BL: HR = 4.66, 95% CI = 2.35−9.26; $p < 0.0001$; FU: HR = 3.53; 95% CI = 1.88−6.60; $p < 0.0001$). Hazard ratios correspond to a two-fold increase in endotrophin levels. At each time points the model including age, BMI and endotrophin were significantly predicting mortality
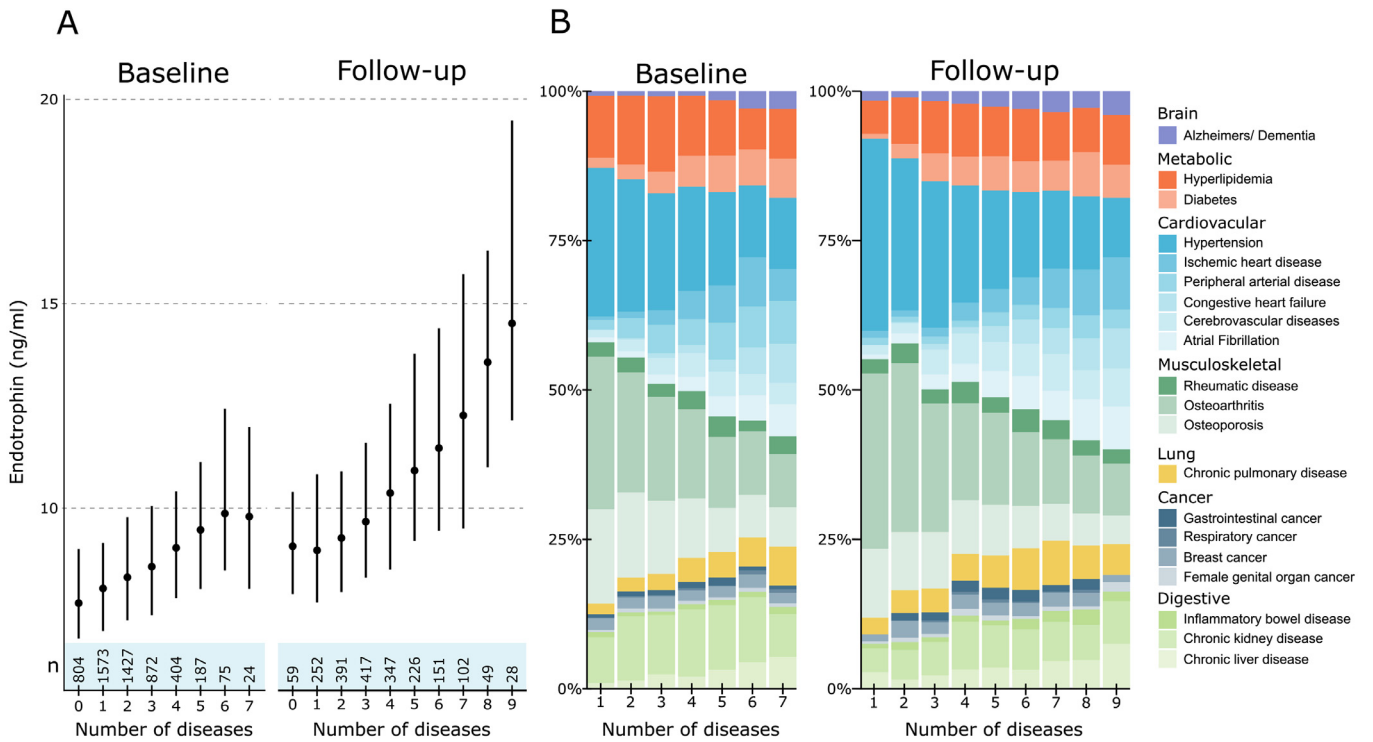
Fig. 4. A: Multimorbidity-plot showing endotrophin levels (median±Q1/Q3) at baseline and follow-up, respectively, stratified by number of diseases. Only multimorbidity-groups with at least 20 women represented is shown. B: Disease-prevalence plot stratified by number of diseases.

with concordance index (c-index) of 0.82 and 0.81, indicating strong predictive models (Fig. 6).

Discussion

The ECM is produced mainly by fibroblasts and was previously thought of only as a structure that added stability and organization to cells and tissue, however it is now recognized that the ECM also provides signalling cues that regulate cell differentiation, growth, and

migration [5,6]. One such example is the procollagen fragment endotrophin (PRO—C6) which is released by proteolysis during formation of type VI collagen. In its cleaved form it has been shown to have signalling abilities in animal models and been linked to development of chronic diseases in humans [10,11,17,39]. In the present study we investigated the association between endotrophin and chronic disease burden in a cohort of 5602 Danish elderly women. To our knowledge, we are the first to investigate the association between endotrophin and disease in a large clinical cohort. Our study found
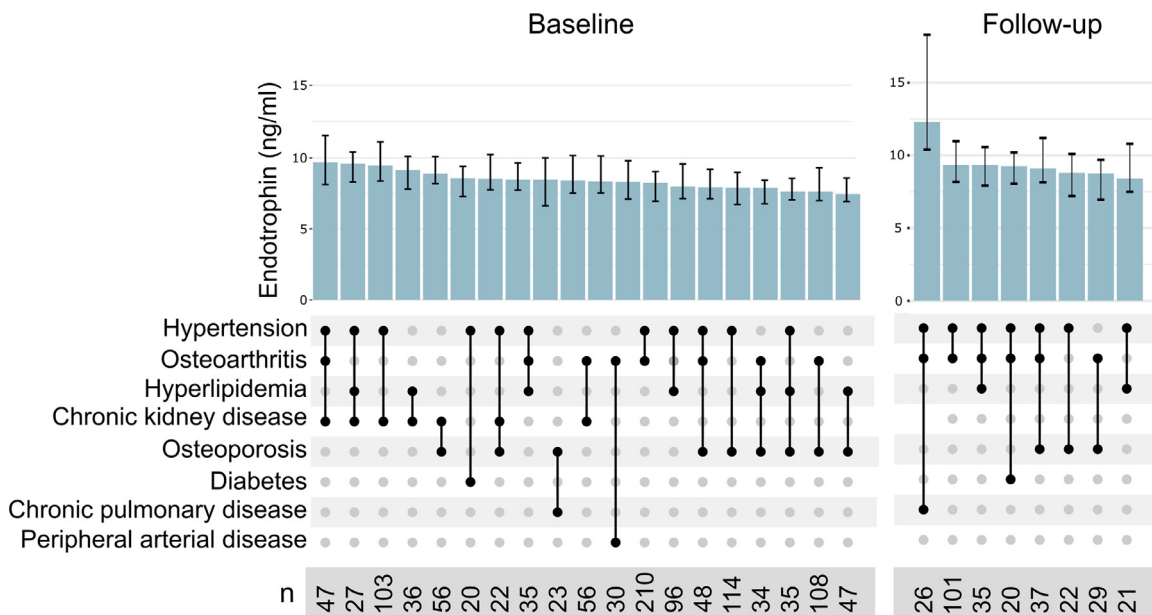

Fig. 5. UpSet-plot: Disease-combinations experienced by at least 20 women at baseline and follow-up. Endotrophin levels (median±Q1/Q3) for each combination of disease shown on top.
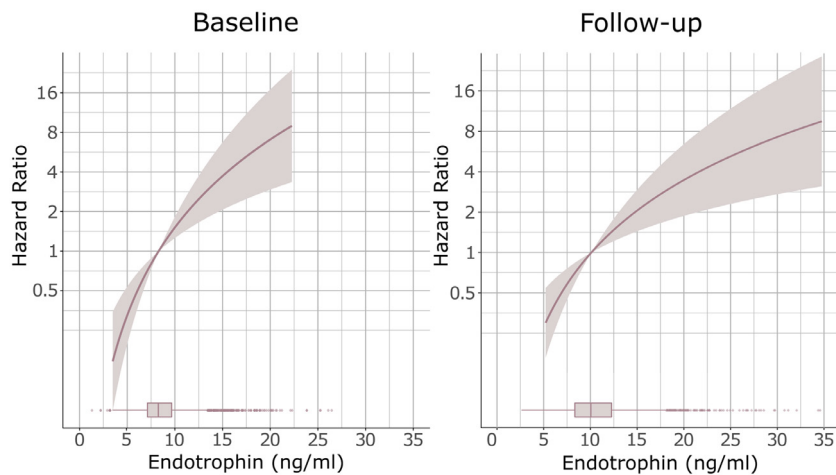
Baseline / Follow-up

Fig. 6. Hazard ratio plots and 95% confidence interval of all-cause mortality for 1 year after baseline (events *n* = 45) and follow-up (events *n* = 52), respectively. Hazard ratios correspond to a two-fold increase in endotrophin levels.

that the variables most associated with endotrophin was the fibrotic fibroblast biomarker PRO-C3 together with creatinine and age. Furthermore, we showed that endotrophin, independent of age and BMI, was significantly associated with 9 age-related chronic diseases out of 17 studied at two different time-points, including chronic kidney disease, diabetes, peripheral arterial disease, and rheumatic disease. Additionally, we saw that endotrophin levels increased significantly with number of morbidities beyond what could be explained by age alone. Lastly, we found endotrophin-levels to be a significant predictor for all-cause mortality both 1 year after baseline and follow-up. Together the findings highlight the role of endotrophin in progression of age-related inflammatory and fibroproliferative diseases.

During aging, the ECM is altered leading to stiffer and mechanically weaker tissues, which compromise the ECM organization and thereby help promote development of chronic age-related diseases [40]. This phenomenon is further accelerated by obesity that can lead to an unbalanced remodelling of the ECM in adipose tissue due to metabolic dysfunction. It has been proposed that systemic insulin resistance induced by obesity is closely related to fibrosis and inflammation in adipose tissues [11,41]. Recent studies in mice have shown that endotrophin is related to a variety of biological processes such as fibrosis, inflammation, and insulin-resistance. Sun et al. showed in high-fat diet mice that overexpression of endotrophin plays a critical role in insulin resistance through stimulation of chronic inflammation and fibrosis under both acute and chronic settings [11]. Further studies in humans have substantiated this link. Yoldemir et al. found endotrophin significantly associated with insulin resistance and upregulated in overweight individual [41] and several clinical studies have found endotrophin elevated in fibrotic conditions where it is produced by fibroblasts [12,13,15,16]. This correlates well with our findings where endotrophin levels were associated with both fibrotic and obesity-related clinical variables and chronic diseases. We found endotrophin levels associated with triglycerides, total fat, HOMA-IR, waist circumference as well as the fibrosis biomarker PRO—C3 that reflect type III collagen deposition [42−44]. We also found endotrophin associated with several chronic conditions associated with obesity and fibrosis such as diabetes, ischemic heart disease, CKD and chronic liver disease. These results highlight the pro-fibrotic properties of endotrophin and underlines its relevance in chronic age-related inflammatory and fibro-proliferate diseases.

The association between endotrophin and several different chronic age-related diseases led to further investigations of the relationship between endotrophin and multimorbidity, defined as the coexistence of multiple chronic diseases within an individual [45].

We found that endotrophin levels rose significantly with the number of co-existing diseases both at baseline and follow-up, even after stratifying by age. Although it is not possible, due to the study-design, to conclude on causality, the results presented in this clinical study, concur with the suggested pro-fibrotic roles in animal models [11], and support that endotrophin is a driving factor for progression of chronic diseases and rising number of morbidities. This is further supported by the association between endotrophin and death that we observed in this study and by a recent publication by Lee et al. showing that endotrophin acts as an accelerator of disease in a mouse model of non-alcoholic liver disease (NAFLD) [46].

As expected, we found, in a set-analysis, that the diseases represented in a disease-combination influenced the endotrophin-levels. Generally, we saw that disease-combinations containing CKD had higher endotrophin levels compared to other combinations. This was not surprising as we found endotrophin significantly associated with CKD both at baseline and follow-up in a logistic regression adjusted for age and BMI. Furthermore, we found creatinine to be the second highest ranked variable impacting endotrophin at both time points. This is in line with several studies that have previously investigated the association between endotrophin and CKD. In a study from 2017 [13] Rasmussen et al. showed that high values of endotrophin were significantly associated with CKD disease progression and the same year, Stribos et al. [47]. showed that endotrophin was able to reflect CKD severity in renal transplant patients. The same conclusion was made in a study with type 1 diabetes patients [39], where endotrophin was found to be an independent predictor of decline in estimated glomerular filtration rate (eGFR).

Understanding the relationship between endotrophin and development of single and multiple chronic age-related diseases might provide the key for a better understanding of how the ECM can promote diseases. Accumulating evidence suggests an imbalance of the ECM to be associated with disease progression. Despite fibroblasts and the altered ECM playing a central role in many chronic diseases, no fibroblast biomarkers are currently used in standard clinical practice. It remains an underappreciated topic of clinical chemistry as to how central fibroblast biological events contribute to multimorbidity and death. Measuring serological endotrophin may therefore provide information of the fibroblast component of chronic diseases and help monitor progression of chronic diseases in the elderly and guide preventive actions to decrease multimorbidity and death.

This study had several strengths and limitations. The limitations include the fact that some diseases were less prevalent causing problems especially when grouping the data by disease-combinations.

This was the primary reason for evaluating multimorbidity as the sum of diseases, though one should be aware that a particular combination of specific diseases may have a greater impact on health compared to others. The chronic diseases in this article were defined using a combination of information from Danish registries, biomarker measures, questionnaire answers and other clinical variables (Table S2) and were thereby not directly validated by a medical doctor. Also, the study-design of this large cohort made it impossible to conclude on causality. Furthermore, the data only included Danish elderly women, and may therefore not be generalizable to the general population. The strengths of this study include the large number of endotrophin measurements in humans at two time points and the possibility to link this with comprehensive data on disease history combined with a large number of clinical variables.

In conclusion, this study suggests that endotrophin is associated with the degree of multimorbidity and death in elderly women. Measuring endotrophin, in addition to standard clinical chemistry, may provide information on fibroblast activity and help monitor multimorbidity and death in the elderly.

Declaration of Competing Interest

CLB, CC, LMS, MK, PF report grants from the Danish Research Foundation (Den Danske Forskningsfond) during the conduct of the study. CLB and MK report personal fees from ProScion A/S. CLB, MK and PF report personal fees from Nordic Bioscience A/S. CLB, CC and MK have stock in Nordic Bioscience A/S. CC reports being a board member of Nordic Bioscience A/S. CC and SB reports being a board member of ProScion A/S. JWH has nothing to disclose. LMS reports being a PhD student at ProScion A/S and Nordic Bioscience A/S. SB reports ownership in Intomics A/S, Hoba Therapeutics A/S and Lundbeck A/S.

Acknowledgments

Contributors

LMS, CLB, JWH, CC and MK contributed to the conception and design of the study. CLB, CC, MK and LMS administrated the project and had responsibility for the management and coordination of the project. PF, LMS, SB and CLB advised on all statistical aspects and interpreted the data. LMS performed the statistical analysis. LMS and CLB drafted the manuscript. LMS, CLB, MK, CC, SB, PF and JWH reviewed the manuscript and approved the final version to be published. LMS, CBL, MK and PF had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. CC and MK conceptualized the PERF cohort and provided the study material. CC, MK, SB and JWH constituted mentorship. LMS generated and preprocessed all plots and visualization.

Data sharing

The original data of the Prospective Epidemiological Risk Factor study and the linkage data from various registries are currently stored at Nordic Bioscience. Access to this database will be granted, on condition that researchers have appropriate ethical permission and sign the appropriate Material Transfer Agreement form.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103391.

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