Is the effect of depression on all-cause mortality mediated by inflammatory and neurohormonal markers? Findings from the Prime Belfast study

Additive effects of depressive symptoms, inflammatory and neurohormonal biomarkers on all-cause mortality, findings from the PRIME Belfast study.

Maria F Hughes (PhD)[1, 2, 3, 4], Christopher C. Patterson (PhD)[1], Katherine M Appleton (PhD)[5], Yolande Esquirol (PhD) [1, 6] Jayne V Woodside (PhD)[1], Michael Donnelly (PhD)[1], Gerard Linden[1], Tanja Zeller (PhD)[2, 3], Stefan Blankenberg (PhD)[2, 3], Frank Kee (PhD)[1]

Affiliations

[1] UK Clinical Research Collaboration Centre of Excellence for Public Health, Queens University of Belfast, Northern Ireland, United Kingdom. [2] University Heart Centre Hamburg, Clinic for General and Interventional Cardiology, Hamburg, Germany. [3] German Centre for Cardiovascular Research (DZHK) Partner Site Hamburg/Lübeck/Kiel, Germany. [4] MRC Epidemiology Unit, University of Cambridge, United Kingdom. [5] Department of Psychology, Bournemouth University, Dorset, United Kingdom. [6] UMR 1027 Université Paul Sabatier – CHU, Toulouse, France.

Address for correspondence

Maria Hughes, CRC Centre of Excellence for Public Health Northern Ireland, School of Medicine, Dentistry and Biomedical Sciences, Institute of Clinical Sciences B, Queens University Belfast, Grosvenor Road, Belfast, United Kingdom, BT126BJ. maria.hughes@qub.ac.uk Telephone number: 0044 2890 635009.

Word Count: 4216 (abstract: 244 words), 5 Tables, 2 Supplementary tables

Conflicts of Interest: None

Source of Funding: This work was supported by a Medical Research Council grant number G0601463, ID no:80983, Biomarkers in the MORGAM populations. Abbott Diagnostics, BRAHMS AG, Diadexus and Roche Diagnostics provided test reagents for biomarker testing.

ABSTRACT

Objective: Depression and socioeconomic disadvantage are linked to all-cause mortality but the potential underlying biological mechanisms are unknown. Inflammatory and neurohormonal biomarkers may mediate the relationship between depressive symptoms and mortality.

Methods: We used Cox proportional hazards modelling to examine the association between depression, measured by the 10-item Welsh Pure Depression Inventory, in 2389 men from the Belfast PRIME prospective cohort aged 50-59 years, and mortality (418 deaths) during 18 years of follow-up. In addition, we examined the potential mediating influence of biomarkers of neurohormonal function {N-terminal Pro-brain natriuretic peptide (NTProBNP), mid-region pro-atrial natriuretic peptide (MRProANP), mid-region Pro-adrenomedullin (MRProADM), c-terminal pro-endothelin-1 (CTProET)} and inflammatory status {C-reactive protein (CRP), Neopterin, Interleukin-1 receptor antagonist (IL1Ra) and Interleukin-18 (IL18)} on any excess mortality associated with depression.

Results: Higher levels of depressive symptoms and lower socioeconomic circumstances (manual occupation, fewer years of education, poorer material conditions), were associated with higher levels of CRP, IL1Ra and CTProET. After adjustment for socioeconomic and lifestyle risk factors, depressive symptoms significantly predicted all-cause mortality, a 1 point higher score on depressive symptoms being associated with hazard ratio of 1.10, (95% confidence interval 1.04-1.16). The proportion of depressive symptoms's effect on mortality explained by CRP was 7.3% suggesting a minimal mediation effect. Individually, CRP, IL1Ra, NTProBNP, MRProANP, MRProADM and CTProET, contributed very marginally to the effect of depression on mortality.

Conclusions: Inflammatory and neurohormonal biomarkers are associated with depression and with increased mortality but may contribute an additive effect rather than mediating this relationship.

Keywords: depression, biomarkers, all-cause mortality, mediators

Abbreviations:
NTProBNP: N-terminal Pro-brain natriuretic peptide
MRProANP: mid-region pro-atrial natriuretic peptide
MRProADM: mid-region Pro-adrenomedullin
CTProET: c-terminal pro-endothelin-1 (CTProET)
CRP C-reactive protein

IL1Ra Interleukin-1 receptor antagonist
IL18: Interleukin-18
SES: socioeconomic status
HDL cholesterol: High density lipoprotein cholesterol
HPA axis: hypothalamic pituitary adrenal axis
BMI: body mass index
HR: hazard ratio

INTRODUCTION

In population based studies, depression has been related to a 1.4 fold excess risk of all-cause mortality.[1] Symptoms of depression and anxiety (psychological distress) occur in around 7.5% of the general population [2] and range along a continuum of severity. In one study, even participants with minor levels of symptoms (as indicated by low scores on the General Health Questionnaire scale of psychiatric morbidity) were at increased risk of mortality from all causes including cardiovascular disease.[2] This suggests that an association between depression and mortality exists well below the threshold that would lead to a diagnosis of depression that would require specific treatment. It is important to note that there are difficulties in capturing depressive symptoms in population based studies and the associated methodological challenges can affect estimates of prevalence and risk.[3]

Disparities in income, occupation and education are associated with psychopathologies including mood disorder, and with unhealthy behaviours such as smoking and excess alcohol intake. In turn, these phenomena have been shown to associate with variation in all-cause and disease specific mortality.[2, 4] Several plausible mechanisms in the biological domain may explain, in part, how depression may lead directly or indirectly to poor health and higher mortality risk. Adverse metabolic changes may be a mediating mechanism for long term psychosocial stress and depression which might be expected to manifest in higher cardiovascular and mortality risk.[5] Some research suggests that depression may lead to a dis-regulation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in increased levels of inflammatory cytokines and cortisol release.[5] Such symptoms are associated with an altered autonomic function response to stressors and can also cause changes in neurohormonal markers such as cortisol and epinephrine, though notably they are difficult to measure reliably.[6] Novel blood neurohormonal biomarkers such as N terminal pro-Brain natriuretic peptide (NTProBNP) and Midregion pro-Adrenomedulin (MRProADM) are related to cortisol and ephinephrine [6] and have been previously linked with clinical depression.[7-9] MrProADM is

produced by various cell types and is involved in electrolyte homeostasis and HPA axis regulation [Akpinar et al. 2013; Armstead et al. 2010]. The potential roles of these markers in relation to depression have received only limited research attention. Mid-region pro atrial natriuretic peptide (MRproANP) is closely related to NTProBNP and both are released by cardiac myocytes to promote diuresis and vasodilation. These natriuretic peptides also demonstrate anxiolytic behaviour, animal and human studies suggest that these natriuretic peptides partially surpress the HPA axis [Strohle et al 00, Wiedemann '00, '01 Hermann- Linger '03, Meyer et al. '15]. C-terminal pro Endothelin-1 is a vasoconstrictor produced by endothelial and vascular smooth muscle cells, it can increase pro-inflammatory cytokine levels such as interleukins (Interleukin-18 (IL18) and Interleukin-1 receptor antagonist (IL1Ra) [Hong et al. 2006, Yammine et al. 2014]. Of these neurohormonal biomarkers, only NTProBNP has previously been tested for association with psychosocial/SES factors in a community based context.[16] IL18 and IL1Ra are cytokines which are immunomodulatory. IL1RA decreases the inflammatory response by antagonising the action of proinflammatory cytokines at the receptor level and is reported to be more stable than its derivative IL1. These biomarkers perform similar functions to IL6 and ICAM-1 which were previously examined in French PRIME cohorts.[13] Neopterin is a chemokine released in response to Interferon gamma and can cause a cell mediated immune response. CRP is an acute phase protein released in response to cytokines and has been previously examined for its association with depression in PRIME and more widely in other psychosocial research [5, Howren et al. 2009]. The relationship between CRP, depression and its effect on the risk of chronic disease has been explored [Vaccarino et al. 2007, Hamer et al. 2008, Surtees et al. 2008, Empana et al. 2013.

In this study we investigate the role of biomarkers as mediators in the relationship between depression and mortality. Chronic experience of low socioeconomic status including enduring financial hardship or insecurity, marginalisation or social exclusion may affect people's mood and stress levels and

lead to long term psychological distress which consequently impact on biological health.[10] Chronic long term psychosocial stress may accumulate over a lifetime, and the effects may become more evident in middle aged populations, as their risk of all-cause morbidity and mortality increases.[11] The PRIME Study is a prospective cohort of middle-aged men in three centres across France and a centre in Belfast, Northern Ireland.[12] Depressive symptoms have been evaluated in the entire PRIME cohort.[3, 13] Previous research from the French cohorts has shown a link between depressive symptoms, inflammatory biomarkers C-reactive protein, Interleukin-6 and ICAM-1 and cardiovascular disease.[13] This study extends this research using the Belfast cohort to investigate the relationships between measures of depression and socio-economic status (e.g. work type, level of education and material disadvantage) with inflammatory and novel neurohormonal and vascular function biomarkers and to determine the extent to which the relationship between depression and mortality risk may be mediated by levels of these biomarkers while viewing socioeconomic status as a confounding variable.

METHODS

Study design

PRIME Belfast is one of four cohorts in the PRIME study and consists of 2744 men aged 50-59, recruited from industries and various employment groups with broadly similar social class structures to cohorts in France, who were examined for the presence of a wide range of cardiovascular risk factors at baseline in 1991-93 and followed up for 18 years for cardiovascular disease and all-cause mortality. Cardio-metabolic risk factors (e.g. cholesterol, systolic BP) and medication use were determined at baseline through standardised interviews, self-administered questionnaires and medical examinations,

informed consent was given by all participants.[14] Follow-up to January 2012 was achieved by contacting subjects annually by letter and conducting routine searches of a mortality register (managed by the regional public health service's Business Services Organisation). The study was approved by the Research Ethics Committee, Queens University Belfast. Death certificates were searched for supporting clinical and post-mortem information on cause of death, and all death records were independently evaluated by a medical committee.

Biomarker measurement

A non-fasting blood sample was collected at baseline and serum stored for future use. Biomarkers were measured in 2009 from frozen specimens at the MORGAM Biomarker Laboratory, Mainz, Germany, whose assessment procedures and details have been described elsewhere.[15] Inflammatory markers include high sensitive CRP, IL18, IL1RA and Neopterin. Markers of neurohormonal activity and vascular function include NTProBNP, MRProANP MRProADM and CTProET

Depressive symptoms

Depressive symptoms were assessed using 10 questions from a validated 15 item questionnaire – the Welsh Pure Depression Inventory [17] - completed at baseline. Question responses (true/false scored as 1/0) were summed to a single depression score scaled between 0 and 10, where higher scores denote more symptoms of depression. The Cronbach's alpha for the 10 items is 0.71, the 10 items have not been formally validated but demonstrated high face validity and follow expected relationships with CHD/mortality [Empana et al. 2013,]. For analysis, we used depression as a continuous scale and categorical variable divided into fourths (0, 1, 2-3, 4-10).

Socioeconomic and lifestyle variables

Education level was categorised into low (<12 years), medium (12-14 years) and high (15+ years) according to the number of years of full-time education. Work type was divided into manual or non-manual occupations. Material living conditions were measured by an aggregate of home ownership, the number of household bathrooms and cars divided into three categories [14]. Low material condition was defined by rental accommodation with one or fewer cars, baths/showers or toilets. High material condition was defined as home ownership, 2 or more cars either 2 or more baths/showers or 2 or more toilets, the remaining subjects were classified as living in mid-range material conditions. Lifetime smoking was categorised based on past and present smoking (never smoker, ≤15 pack years, >15, ≤30 pack years or >30 pack years.[18] Alcohol was explored across five categories (non drinkers, 0.1-228ml/week, 129-265ml/week, 266-441ml/week, ≥442ml/week). Body mass index (BMI) can mediate the relationship between depression and mortality [Howren et al. 2009] was defined as normal weight <25kg/m2, overweight 25-30kg/m2, obese >30kg/m2. Physical activity can partially mediate the relationship between depression and mortality [Whooley et al. 2008, Hamer et al. 2011, Win et al. 2011], thus PA was included as a covariate, defined as the total amount of time spent at leisure physical activity and occupational physical activity including travel to and from work expressed in MET hours/week, examined in quartiles (0-62.22, 62.23-116.90, 116.91-528.0), derived from the MOSPA-Q and available for 69.3% of participants [Wagner et al. 2003].

Statistical Analysis

Analysis was restricted to men without cardiovascular disease at baseline (determined by self-report and validated with medical records). Of the 2744 PRIME Belfast men, 355 (12.9%) were excluded that had cardiovascular disease at baseline. Biomarkers had varying proportions of missing values due to sample unavailability or other technical reasons. Missing data was handled through multiple imputation models which included all biomarkers, risk factors,

follow-up time and vital status at the end of follow-up (see supplementary material). Missing biomarker values varied across biomarkers (11.9-25.8% Supplementary Table 1), and 54 men had missing data on the depression score, with this data appearing to be missing at random. There were only small differences in results for complete case and imputed results (presented in supplementary table 2) but results were consistent so multiple imputed results are presented. Quantitative baseline characteristics were summarised as means and standard deviations, all biomarkers were log transformed to approximate normal distributions and summarised using geometric means and interquartile ranges. Biomarker values were compared between depressive symptoms and socieconomic factors by one way analysis of variance and large samples z-tests. As this is an explorative study no adjustment for multiple testing was performed and due to the large number of tests, p-values should be interpreted with caution and in connection with effect estimates. Spearman's rank correlation coefficients were used to assess associations between biomarkers and other variables.

Cox proportional hazards analysis provided hazard ratios (HR) and 95% confidence intervals (CI) associated with depressive symptoms, biomarkers and all-cause mortality. Multivariable model 1 included age and depressive symptoms; model 2 additionally included social risk factors (material condition, education level, work type) while model 3 included also lifestyle related risk factors, lifetime cigarette smoking, alcohol consumption, body mass index (BMI), systolic BP and each single biomarker. The residual percentage contribution of individual biomarkers to depressive symptoms after adjustment for age, social and lifestyle factors was estimated using the expression 100 (b_o-b_1/b_o) where b_o is the coefficient for depression on the log hazard scale in the Cox regression model without biomarkers and b_1 is the coefficient in the corresponding model adjusted for each biomarker.[19] Multiple Imputation was carried out in STATA (release 11 (StataCorp, College Station, TX, USA), 20 rounds were performed and the resulting dataset was imported and analysed in SPSS version 21 (IBM Inc., Armonk, NY, USA). The imputation model incorporated a linear regression for log transformed biomarkers and HDL cholesterol,

an ordered logistic regression for depression, material condition, alcohol, pack years and BMI and a logistic regression for work type, history of diabetes and status at start and end of follow-up. Also included in the imputation equations were the follow-up time and status at the end of follow-up (more information available in supplementary material).

RESULTS

The study comprised 2389 middle-aged men and 418 deaths over 18 years of follow up. The presence of depressive symptoms as categorised by a high baseline depression score (range 4-10) was apparent in 12.9% of men (308/2389), and was more frequently observed in cases (death from any cause) than in non-cases (15.8 versus 11.2% $p<0.001$). Mean values on all risk factors were different between cases and non-cases (Table 1).

Table 1 Descriptive statistics for men free of cardiovascular disease at baseline presented by status at follow-up. Values are mean (SD), geometric mean (first quartile, third quartile) or Numbers (%).

	All (N=2389)	Dead (N=418)	Alive (N=1971)	p value
Age, years	54.7	55.8	54.5	<0.001
Current smokers (%)	545 (22.8)	170 (40.7)	375 (19.1)	<0.001
Alcohol consumption (ml/wk)	157.0 (6.2)	219.8 (20.8)	143.9 (6.0)	<0.001
Diabetes history (%)	40 (1.7)	18 (4.3)	22 (1.1)	<0.001
Low material condition (%)	904 (37.8)	192 (45.9)	712 (36.1)	0.006
Manual worker (%)	1299 (54.4)	270 (64.6)	1029 (52.2)	<0.001
Low education level (%)	669 (28.0)	145 (34.7)	524 (26.6)	<0.001
Depression score (mean, SD)	1.44 (1.7)	1.77 (1.9)	1.38 (1.7)	<0.001
Depression score ≥4 (%)	308 (12.9)	66 (15.8)	221 (11.2)	<0.001
CRP (mg/L)	1.6 (0.8-3.0)	2.19	1.48	<0.001
IL18 (pg/mL)	282 (200-363)	288	282	0.43
IL1Ra (pg/mL)	240 (159-316)	263	234.4	<0.001
Neopterin (nmol/L)	5.89 (4.8-6.9)	6.17	5.75	0.001
NTProBNP (pg/mL)	29.5 (15.8-50.1)	39.8	27.5	<0.001
MRProANP (pmol/L)	63.1 (50.1-79.4)	67.6	63.1	0.013
MRProADM (nmol/L)	0.45 (0.4-0.5)	0.47	0.44	<0.001
CTProET (pmol/L)	49.0 (42.5-54.7)	51.7	48.5	<0.001

Linear correlations between biomarkers, depressive symptoms and lifestyle risk factors are given in Table 2. Inflammatory markers were mutually but not strongly correlated (coefficients ranged from 0.13 to 0.32). Neurohormonal biomarkers were mutually correlated (coefficients ranged from 0.13-0.60) with strongest correlations between NTProBNP and MRProANP (0.60) and MRProADM and CTProET (0.57). A very low but statistically significant correlation was observed between depressive symptoms and inflammatory markers (ranging from 0.04 to 0.08 for CRP, Neopterin and IL1Ra) and neurohormonal markers (ranging from 0.04 to 0.09 for MRProADM, CTProET and NTProBNP) (Table 2).

Higher levels of CRP, IL1Ra, Neopterin, NTProBNP, MRProADM and CTProET were significantly but weakly associated with higher levels of depressive symptoms expressed as continuous and categorical variables (Table 3) while MRProANP and IL18 showed no significant association. Higher levels of CRP, IL1Ra and CTProET were also associated with manual work, low materialconditions and less education. NTProBNP and MRProADM levels were higher for lower material conditions but not for manual work or education. Higher levels of MRProANP and Neopterin were significantly associated with manual work and education levels, while IL18 levels were not significantly associated with any socioeconomic traits. Socioeconomic status, education and work type play a role in the association between depressive symptoms and mortality but are subsequently viewed as a confounding influence used for adjustment rather than considered in terms of their own importance.

In a Cox Proportional hazards model based on 418 deaths, a 1 unit increase in depressive symptoms was associated with a 10% increased risk of all-cause mortality after adjustment for social and lifestyle risk factors (Table 4). When inflammatory or neurohormonal markers were individually added, the HR for depressive symptoms was reduced only marginally from 1.10 to 1.09 for its association with all-cause mortality (first column of Table 4). Six of the eight biomarkers (CRP, IL1Ra, NTProBNP, MRProANP, MRProADM and CTProET) remained significantly predictive of mortality in these models) (second column of

Table 4). For five of these six biomarkers there was a significantly increased mortality risk in the top quarters of the biomarkers distribution compared with the bottom quarter following similar adjustment (third to sixth columns of Table 4). In these models, none of the social risk factors remained significant after adjustment for lifestyle variables (Table 5). BMI may act as a mediator in the association with all-cause mortality and CRP, IL1Ra and MRProADM. For example in the fully adjusted models without CRP, the HR for BMI is 0.85 (95% C.I. 0.73, 0.99, p=0.045) which decreases to HR 0.82 (95% C.I. 0.70, 0.96, p=0.015) with the addition of CRP.

The residual percentage contribution of individual biomarkers to all-cause mortality was small and ranged from 0% (IL18) ADM) to 9.4% (CTProET) (Table 4). The effect of CRP as a mediator explained 7.3% of the relationship between depression and all-cause mortality after adjustment for all other risk factors.

Insulin resistance may dysregulate homeostatic mechanisms affecting associations between these biomarkers and depressive symptoms. A sensitivity analysis excluded 40 participants with a history of diabetes at baseline from the analysis, in addition 18 deaths due to trauma (no inflammatory/neurohormonal basis) and those with CVD at baseline were excluded. Cox models were adjusted for age, depressive symptoms, social and lifestyle risk factors and biomarkers (Supplementary table XXX). Hazard ratios for CRP and IL1Ra marginally increased compared to the larger dataset suggesting that diabetes potentially obscured the effect of these biomarkers, but overall results remained similar.

Table 2 Spearman rank correlation coefficient matrix between continuous biomarkers, depressive symptoms, socioeconomic and lifestyle variables.

	CRP	IL18	IL1Ra	Neopterin	NTProBNP	MRProANP	MRProADM	CTProET	depres sym	SBP	HDLc	BMI
CRP	-											
IL18	0.18***	-										
IL1Ra	0.32***	0.27***	-									
Neopterin	0.20***	0.14***	0.12***	-								
NTProBNP	0.11***	0.02	0.03	0.14***	-							
MRProANP	-0.06**	-0.02	-0.09**	0.14***	0.6***	-						
MRProADM	0.25***	0.11***	0.22***	0.25***	0.19***	0.25***	-					
CTProET	0.21***	0.13***	0.16***	0.23***	0.13***	0.18***	0.58***	-				
depress sym	0.08***	-0.01	0.06**	0.04	0.04	-0.03	0.05	0.09***	-			
systolic BP	0.15***	0.02	0.12***	0.01	0.11***	0.02	0.12***	0.08***	-0.01	-		
HDL cholesterol	-0.19***	-0.16***	-0.23***	-0.11***	0.03	0.11***	-0.07**	-0.06**	-0.02	-0.03	-	
BMI	0.26***	0.09***	0.26***	0.05*	-0.08***	-0.11***	0.24***	0.11***	-0.01	0.28***	0.25***	-
Physical activity	-0.05	0.01	-0.03	-0.02	-0.03	0.02	-0.11***	-0.09***	-0.09***	0.01	0.09***	-0.01

Table 3 Biomarkers compared in depressive and socioeconomic groups using ANOVA tests or ANOVA tests for trend on log transformed values.

Depressive and SES risk factor		CRP		IL18		IL1Ra		Neopterin	
Depression	(N)	geometric mean	p value	geometric mean	p value	geometric mean	p value	geometric mean	p value
Depression score 0	975	1.48		288.4		234.4		5.75	
Depression score 1	522	1.51	<0.001	281.8	0.836	234.4	0.003	5.89	0.012
Depression score 2-3	584	1.62		275.4		245.5		5.89	
Depression score 4-10	308	2.04		288.4		257.0		6.17	
Depression (continuous)	2389	1.60	<0.001	281.8	0.521	239.9	<0.001	5.89	0.005
Work									
manual work	1299	1.82	<0.001	288.4	0.064	257.0	<0.001	6.03	<0.001
non-manual work	1090	1.38		275.4		218.8		5.75	
Material conditions									
low	908	1.82		288.4		251.2		5.89	
medium	521	1.58	<0.001	288.4	0.871	239.9	<0.001	5.89	0.829
high	960	1.41		281.8		229.1		5.89	
Years full time education									
<12 years	669	1.73		288.4		257.0		6.03	
12-14 years	793	1.85	<0.001	281.8	0.174	257.0	<0.001	5.89	0.004
15+ years	927	1.34		281.8		218.8		5.75	

Table 3 continued.

Depressive and SES risk factor		NTProBNP		MRProANP		MRProADM		CTProET	
Depression	(N)	geometric mean	p value	geometric mean	p value	geometric mean	p value	geometric mean	p value
Depression score 0	975	27.5	0.042	64.6	0.254	0.43	0.024	47.4	<0.001
Depression score 1	522	29.5		64.6		0.44		47.6	
Depression score 2-3	584	30.9		64.6		0.44		48.4	
Depression score 4-10	308	30.9		61.7		0.45		50.1	
Depression (continuous)	2389	29.5	0.034	63.1	0.174	0.44	0.013	47.9	<0.001
Work									
manual work	1299	30.2	0.127	63.1	0.025	0.45	0.643	47.9	0.009
non-manual work	1090	28.2		66.1		0.45		46.8	
Material conditions									
low	908	31.6	0.022	63.1	0.105	0.45	0.017	49.0	0.007
medium	521	30.2		63.1		0.44		46.8	
high	960	28.2		64.6		0.44		46.8	
Years full time education									
<12 years	669	30.9	0.273	63.0	0.008	0.45	0.213	49.0	0.003
12-14 years	793	28.2		62.5		0.44		47.9	
15+ years	927	29.5		66.1		0.44		46.8	

Table 4 Multivariate adjusted hazard ratios (95% Confidence Intervals) for all-cause mortality with depressive symptoms with univariate addition of biomarkers. Models are adjusted for social risk factors (work type, material condition, education), lifestyle factors (systolic blood pressure, lifetime smoking, alcohol, history of diabetes at baseline, BMI and HDL cholesterol. Biomarkers given in continuous form represent a 1 unit increase in the logarithm of each biomarker (equivalent to a x10 increase in the untransformed biomarker) and the untransformed biomarker in quartile divisions with quarter 1 the reference category. All-cause mortality based on 418 deaths in 18 years of follow up. Percentage of mediating effect of depressive symptoms explained by biomarker is calculated based on the log transformed continuous form of the biomarker.

		HR (95% C.I.) associated with biomarker					Percentage of age, social, lifestyle adjusted effect of depressive symptoms explained by biomarker
	HR (95% C.I.) associated with 1 unit increase in depressive symptoms	per 1 log unit increase of biomarker	Q 1	Quarter 2	Quarter 3	Quarter 4	
+ age	1.14 (1.08, 1.19)						
+ social risk factors	1.12 (1.06, 1.17)						
+ lifestyle risk factors	1.10 (1.04, 1.16)						
+ CRP	1.09 (1.04, 1.15	1.43 (1.13, 1.82)	ref	0.98 (0.71, 1.37)	1.34 (0.98, 1.82)	1.47 (1.08, 2.01)	7.3%
+ IL18	1.10 (1.04, 1.16)	1.06 (0.55, 2.02)	ref	0.81 (0.60, 1.09)	0.96 (0.72, 1.28)	0.97 (0.73, 1.30)	0.0%
+ IL1Ra	1.09 (1.04, 1.15)	2.10 (1.28, 3.40)	ref	1.03 (0.75, 1.42)	1.23 (0.91, 1.67)	1.40 (1.04, 1.91)	5.2%
+ Neopterin	1.09 (1.04, 1.15)	2.19 (0.93, 5.22)	ref	1.06 (0.75, 1.49)	1.20 (0.88, 1.64)	1.27 (0.91, 1.77)	3.1%
+ NTProBNP	1.09 (1.04, 1.15)	1.70 (1.33, 2.18)	ref	1.08 (0.79, 1.48)	1.18 (0.87, 1.60)	1.71 (1.27, 2.29)	7.3%
+ MRProANP	1.10 (1.04, 1.16)	1.97 (1.03, 3.77)	ref	1.10 (0.80, 1.52)	1.17 (0.84, 1.63)	1.43 (1.04, 1.98)	6.3%
+ MRProADM	1.09 (1.04, 1.15)	5.36 (1.30, 22.1)	ref	1.20 (0.84, 1.71)	1.34 (0.94, 1.90)	1.53 (1.06, 2.22)	7.3%
+ CTProET	1.09 (1.03, 1.15)	5.48 (1.36, 22.0)	ref	0.81 (0.57, 1.15)	1.01 (0.72, 1.42)	1.29 (0.95, 1.77)	9.4%

Table 5 Multivariate adjusted hazard ratios (95% confidence Intervals) describing the association between depressive symptoms and all-cause mortality (418 cases). The model shows the hazard ratios and p values for relationships between socioeconomic, lifestyle and biomarker variables, upon successive adjustment with some variables not reaching significance in the final model.

Variable	HR (95% C.I.)	P value	HR (95% C.I.)	P value	HR (95% C.I.)	P value	HR (95% C.I.)	P value
Age	1.16 (1.12, 1.21)	0.001	1.16 (1.13, 1.22)	0.001	1.16 (1.11, 1.19)	0.001	1.15 (1.11, 1.19)	0.001
depression	1.13 (1.08, 1.19)	0.001	1.11 (1.06, 1.17)	0.001	1.10 (1.04, 1.16)	0.001	1.09 (1.04, 1.15)	0.001
work type			0.71 (0.56, 0.89)	0.004	0.79 (0.62, 0.99)	0.048	0.8 (0.63, 1.008)	0.058
material condition			0.88 (0.78, 0.98)	0.024	0.95 (0.83, 1.06)	0.344	0.95 (0.85, 1.06)	0.412
education			0.92 (0.80, 1.05)	0.193	0.94 (0.83, 1.08)	0.425	0.94 (0.82, 1.07)	0.422
history of diabetes					2.11 (1.30, 3.42)	0.002	1.99 (1.22, 3.23)	0.005
lifetime smoking					1.33 (1.22, 1.44)	0.001	1.30 (1.19, 1.43)	0.001
alcohol					1.07 (1.01, 1.15)	0.043	1.07 (1.01, 1.15)	0.036
systolic BP					1.01 (1.01, 1.02)	0.001	1.01 (1.01, 1.01)	0.001
HDLcholesterol					1.12 (0.82, 1.54)	0.456	1.19 (0.87, 1.64)	0.257
BMI					0.85 (0.72, 0.99)	0.042	0.82 (0.69, 0.96)	0.014

Physical activity		0.98 (0.87, 1.11)	0.755	0.98 (0.87, 1.11)	0.813
CRP				1.43 (1.13, 1.80)	0.003

DISCUSSION

Our study found that depressive symptoms were associated with all-cause mortality, but inflammatory and neurohormonal biomarkers are only weak mediators of this relationship. . The inflammatory markers CRP, IL1Ra and neurohormonal biomarkers NTProBNP, MRProANP, MRProADM and CTProET are associated with depressive symptoms and may play some additive role in the association between depressive symptoms and all-cause mortality after adjustment for age, socioeconomic and lifestyle factors. Our results provide tentative support for the hypothesis that these biomarkers are involved in the biological domain of the pathway to depression, and warrant further study in larger longitudinal cohorts, and with greater levels of depressive symptoms. The discussion will (i) place these findings in context, (ii) identify further research questions suggested by the results, and (iii) highlight possible limitations.

Biomarkers, depression and all-cause mortality

Men in the PRIME Belfast study generally have low levels of depressive symptoms, with 13.8% of middle-aged men with scores in the range 4-10 of the 10-item modified Welsh depression score, which are consistent with previous findings from this cohort.[3, 13] Similar levels of depressive symptoms have been reported using the CES-D scale in older (13.2%) and elderly (21.5%) general populations from Europe [Beekman et al.][1][20]. The method of measuring depressive symptoms and analysing them in relation to endpoints can strongly affect results.[3] CRP and IL1Ra have been reliably associated with depression in prospectively followed cohorts.[13, 20-21] Our estimates of a weak association between CRP, IL1Ra and depressive symptoms are similar to previous estimates [20-21, Vaccarino et al. 2007] and for CRP are comparable to the French PRIME estimates of its effect on depression and coronary heart disease.[13] Despite doubt surrounding the measurement of socioeconomic and occupational factors between the two countries, the relationship between CRP and depressive symptoms on outcomes is comparable in both studies [13]. BMI is a potential mediating factor between CRP, IL1Ra and MRProADM and depressive symptoms. Previous studies on

CRP and depressive symptoms corroborate BMI's mediating link (Howren et al. 2009, Ma et al. 2011, Copeland et al. 2012). IL1 (and its derivative IL1Ra), IL6 and thus CRP and MRProADM may be secreted by adipose tissue/adipocytes potentially leading to an increased inflammatory response (Howren et al 2009). Neopterin has been associated with clinical depression, melancholia and chronic fatigue,[22] but while we observed a dose response relationship between depression and all three biomarkers measured at baseline, neopterin was not significantly related to all-cause mortality in contrast to the other two biomarkers. These biomarkers represent different stages in the inflammatory process, neopterin representing a cell-based immune response that may be related to more specific types of depression, while inflammatory regulators such as CRP and IL1Ra have been associated with atypical depression subtypes related to psychosocial stress [23] which may be more common in our sample. While these biomarkers may not be specific to depression but also related to other cardiovascular disease, recent meta-analysis has demonstrated beneficial effects of anti-inflammatory drug treatment on depressive symptoms [Kohler et al. 2015]. This emphasises the importance of searching for novel biomarkers/underlying cellular mechanisms to support the identification of subgroups which could benefit from this treatment. Neurohormonal biomarkers NTProBNP, MRProADM and CTProET were associated with depressive symptoms and all-cause mortality in the prospective analysis. NTProBNP is reported to be weakly correlated with the neurohormones epinephrine (r^2=0.17) and norepinephrine (r^2=0.33) which are standard markers of psychosocial stress [6]. NTProBNP has previously been associated with major depression [], and correlated with depressive symptoms in healthy populations [Politi et al. 2007, Michel et al. 2014], and those with CVD [van der Broek et al. 2011] and diabetes [9]. Previous research has indicated a possible role of MRProADM with major depression [7] and bipolar disorders [8]. CTProET has not previously been associated with depression but has been linked to low socioeconomic status [24] and psychosocial stress associated with cardiovascular disease.[25] However up to 26% missing data was observed for MRProANP, MRProADM and CTProET which makes our estimates less precise but our study appears to be the first to examine the effect of these biomarkers on depressive symptoms

and mortality. Individually, inflammatory and neurohormonal biomarkers explain only a small proportion (0-9%) of the biological association between depressive symptoms and mortality in our dataset. As these estimates were small and could in part, be accounted for by the large sampling variability, we did not calculate confidence bounds.

Further research questions

The biomarkers represent several stages in the inflammatory and neurohormonal process impacting on the HPA axis. From our data, it is not possible to establish the direction of causation, whether biomarkers have led to depression, or depression leads to increased levels of biomarkers. Wium-Andersen et al. 2014 recently demonstrated that CRP is not causally related to depression using Mendelian randomisation.[26] They showed that while CRP was elevated in those with depression, genetically elevated CRP was not. Duvuis et al. also concluded depression increased CRP unidirectionally.[Duvuis et al. 2011]. Copeland et al. 2012 [27] examined CRP and depression using serial measurements at several time points over 6 years and also concluded that depression resulted in increased CRP levels which bidirectionally activates the stress response system. Depression is a clinically heterogeneous condition which can mask this stress response system and hinder efforts to identify its complex biological basis. Chronic exposure to psychosocial stress results in "wear and tear" on physiological systems, an effect described as allostatic load [5] which is difficult to capture in the general population. Disregulation of stress hormones and cytokines in response to stress can result in gradual overcompensation by other systems (e.g. metabolic) contributing to allostatic load.[28] Chronic psychosocial stress mediated through socioeconomic disadvantage via the putative biological mechanism of allostatic load has been linked to disease.[29] This putative mechanism inferred by our observed associations requires further investigation in larger studies with higher levels of depressive symptoms, repeated measures of depression and biomarkers would allow more detailed insight into the biological trends inferred from this work.

Study strengths and limitations

Prospective studies examining the association between depression, biomarkers and health outcomes are limited, so our study provides useful information on a range of novel biomarkers several of which have never been studied in this context before, and insights into the underlying biological pathway involved between depressive symptoms and mortality. We have excluded men with CVD at baseline, reducing the possibility that pre-existing chronic illness explained the association between depressive symptoms, biomarkers and outcomes. We have adjusted for a wide range of variables which may confound the association between biomarkers and depression. However we are not able to adjust for medication use, and acknowledge that anti-inflammatory medicines, antidepressants and statins may affect inflammatory variables. Limitations of the study include low levels of depressive symptoms, and modest statistical power to detect small effects on outcome events. The single baseline measurement of both depressive symptoms and biomarkers also limits our ability to assess whether depression precipitated or was preceded by an inflammatory or neurohormonal response in addition to a more formal mediation analysis. Further causal mediation analysis would be desirable when suitable methods for survival data are implemented[2]

Conclusions

] In a healthy middle-aged male cohort, inflammatory and neurohormonal biomarkers do not mediate the effect of depression on mortality but may play an additive role with depressive symptoms which could worsen the prognosis of the individual.

Acknowledgements

We would like to thank the study participants of PRIME Belfast. The PRIME MEN/Belfast study was coordinated at Queen's University Belfast, Belfast, Northern Ireland: F. Kee (principal investigator), A. Evans (former principal investigator), J. Yarnell, G. Linden, E. Gardner, A. Scott, C. McCrory and A.

Hall. We would like to thank Dr. Barbora Silarova for helpful comments and discussion on the manuscript.

Reference List

1. Wulsin LR, Evans JC, Vasan RS, Murabito JM, Kelly-Hayes M, Benjamin EJ. Depressive symptoms, coronary heart disease, and overall mortality in the Framingham Heart Study. *Psychosom Med* 2005;67:697-702.
2. Russ TC, Stamatakis E, Hamer M, Starr JM, Kivimäki M, Batty GD. Association between psychological distress and mortality: individual participant pooled analysis of 10 prospective cohort studies. *BMJ* 2012;345:e4933.
3. Appleton K, Woodside JV, Arveiler D,Haas B, Amouyel P, Montaye P, Ferrieres J, Ruidavets JB, JWG Yarnell Kee F, Evans A, Bingham A, P Duchimetiere P, Patterson CC, for the PRIME study. Depression and mortality; artefact of measurement and analysis? *J. Affective Disorders* 2013;151(2):632-8.
4. Singh-Manoux A, Clarke P, Marmot M. Multiple measures of socio-economic position and psychosocial health: proximal and distal measures. *Int J. Epidemiol* 2002;31:1192-1199.
5. Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev* 2010;35:2-16.
6. Richards AM, Nicholls G, Yandle TG Frampton C, Espiner EA, Turner JG, Buttimore RC, Lainchbury JG, Elliot JM, Ikram H, Crozier IG Smyth DW. Plasma n-terminal pro-brain natriuretic peptide and adrenomedullin, new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation* 1998;97:1921-1929.
7. Akpinar A, Yaman GB, Demirdas A,Onal S. Possible role of adrenomedullin and nitric oxide in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;46:120-125.
8. Huang J,Perlis RH, Lee PH Rush JA, Fava M, Sachs GS, Lieberman J,Hamilton SP, Sullivan P, Sklar P, Purcell S, Smoller JW. Cross-disorder genomewide analysis of schiziohrenia, bipolar disorder and depression. *Am J Psychiatry* 2010;167:1254-1263.
9. Feinkohl I, Sattar N, Welsh P, Reynolds RM,Deary IJ,Strachan MWJ,Price JF on behalf of the Edinburgh Type 2 Diabetes Study. Association of N-terminal pro-brain natriuretic peptide

with cognitive function and depression in elderly people with type 2 diabetes. *Plos One* 2012;7;9:e44569.

10. Steptoe A, Kivimäki M, Stress and cardiovascular disease; an update on current knowledge. *Annu Rev Public Health* 2013;34:337-54.
11. Elovainio M, Ferrie JE, Singh-Manoux A, Shipley M, Batty GD, Head J. Hamer M, Jokela M, Virtanen M, Brunner E, Marmot MG, Kivimaki M. Socioeconomic differences in cardiometabolic factors: Social Causation or Health-related Selection? Evidence from the Whitehall II Cohort Study, 1991-2004. *Am J Epidemiol* 2011;3:1-11.
12. Yarnell JWG, on behalf of the PRIME Study group. The PRIME study; classical risk factors do not explain the several-fold differences in risk of coronary heart disease between France and Northern Ireland. *Q J Med* 1998;91:667-676.
13. Empana JP,Sykes DH, Juhan-Vague I, Arveiler D, Ferrieres J,Amouyel P,Bingham A, Montage M,Ruidavets JB, Haas B, Evans A, Jouven X, Duchimetiere P for the PRIME Study group. Contributions of depressive mood and circulating inflammatory markers to coronary heart disease in health European men. The Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation* 2005;111:2299-2305.
14. Yarnell JWG Yu S, McCrum E, Arveiler D, Haas B, Dallongeville J, Montaye M, Amouyel P, Ferrieres J, Ruidadets J-B, Evans A, Bingham A, Ducimetiere P; for the PRIME study group. Education, socioeconomic and lifestyle factors and risk of coronary heart disease; the PRIME study. *Int J Epidemiol* 2005;34:268-275.
15. Blankenberg S, Zeller T, Saarela O, Havulinna AS, Kee F, Tunstall-Pedoe H, Kuulasmaa K, Yarnell J Schnabel RB, Wild P, Munzel TF, Lackner KJ, Tiret L, Evans A, Salomaa S. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts (MORGAM) *Circulation* 2010;121:2388-97.
16. Michal M, Wiltink J, Kirchner Y, Wild PS, Münzel T, Ojeda FM, Zeller T, Schnabel RB, Lackner K, Blettner M, Zweiner I, Beutel ME. Differential associations of depressive symptom

dimensions with cardio-vascular disease in the community; results from the Gutenberg Health Study. *Plos One* 2013 8;8:e72014.

17. Rodda BE, Miller MC, Bruhn JG. Prediction of anxiety and depression patterns among coronary patients using a Markov process analysis. *Behav Sci* 1971;16:482-89.
18. Yarnell JWG, Patterson CC, Arveiler D, Amouyel P, Ferrières J, Woodside JV, Haas B, Montaye M, Ruidavets JB, Kee F, Evans A, Bingham A, Ducimetière. Contribution of lifetime smoking habit in France and Northern Ireland to country and socioeconomic differentials in mortality and cardiovascular incidence: The PRIME Study. *J Epidemiol Community Health* 2012; 66:7:599-604.
19. Hardoon, SL,Whincup, PH, Lennon, LT, Wannamethee, SG, Capewell, S, Morris, RW. How much of the recent decline in the incidence of myocardial infarction in British men can be explained by changes in cardiovascular risk factors? Evidence from a prospective population-based study. *Circulation* 2008;117;598–604.
20. Milaneschi Y, Corsi AM, Penninx BW,Bandinello S,Guralnik JM, Ferrucci L. Interleukin-1 receptor antagonist and incident depressive symptoms over 6 years in older persons; the InChianti Study. *Biol Psychiatry* 2009;65:973-978.
21. Valkanova V,Ebmeier KP, Allan CL. CRP, IL-6 and depression; a systematic review and meta-analysis of longitudinal studies. *J Affective Disorders* 2013;150:736-44.
22. Maes M,Mihaylova I,Kubera M,Ringel K. Activation of cell-mediated immunity in depression; association with inflammation, melancholia, clinical staging and the fatigue and somatic symptom cluster of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2012;36:169-175.
23. Penninx BWJ, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. *BMC Med* 2013;11:129-140.

24. Hong S, Nelesen RA, Krohn PL Mills PJ, Dimsdale JE. The association of social status and blood pressure with markers of vascular inflammation. *Psychosom Med* 2006;68:517-23.
25. Yammine L, Kang DH,Baum MM, Meininger JC. Endothelin-1 and psychosocial risk factors for cardiovascular disease: a systematic review. *Psychosom Med* 2014;76:109-21.
26. Wium-Andersen MK, Dynnes Ørstead D, Nordestgaard BG. Elevated c-reactive protein, depression,somatic diseases and all-cause mortality; a Mendelian randomisation study. *Biol Psychiatry* 2014; pii: S0006-3223(13)00916-5.
27. Copeland WE,Shanahan L,Worthman C, Angold A, Costello EJ. Cumulative depression episodes predict later C-reactive protein levels; a prospective analysis. *Biol Psychiatry* 2012;71:15-21.
28. McEwan BS & Tucker P. Critical biological pathways for chronic psychosocial stress and research opportunities to advance the consideration of stress in chemical risk assessment, J Am *Public Health Assoc* 2011;101:S131-9.
29. McEwan BS Allostasis and allostatic load implications for neuropsychopharmacology. *Neuropsychopharmacology* 2000;22:108.
30. van Zoonen K, Buntrock C, Ebert DD, Smit F, Reynolds CF, Beekman ATF, Cuijpers P. Preventing the onset of major depressive disorder: a meta-analytic review of psychological interventions. Int J. Epidemiol 2013;43:318-329.

[1] Beekman AT, Deeg DJ, Van Limbeek J, et al. Criterion validity of the Center for Epidemiologic Studies Depression scale (CES-D): results from a community-based sample of older subjects in The Netherlands. Psychol Med 1997;27:231–5.
[2] Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. Int J. Epidemiol 2013;42:1511-9.

Politi P, Minoretti P, Piaggi N, Brondino N, Emanuele E. Elevated plasma N-terminal proBNP levels in unmedicated patients with major depressive disorder. Neurosci Lett 2007;417: 322–325.

van den Broek KC, deFilippi CR, Christenson RH, Seliger SL, Gottdiener JS, et al. Predictive value of depressive symptoms and b-type natriuretic peptide for new-onset heart failure and mortality. Am J Cardiol 2011;107: 723.

Ströhle A, Kellner M, Holsboer F, Wiedemann K. Anxiolytic activity of atrial natriuretic peptide in patients with panic disorder. Am J Psychiatry 2001;158(9):1514–1516

Wiedemann K, Jahn H, Kellner M. Effects of natriuretic peptides upon hypothalamo-pituitary-adrenocortical system activity and anxiety behaviour. Exp Clin Endocrinol Diabetes 2000;108(1):5–13

Wiedemann K, Jahn H, Yassouridis A, Kellner M. Anxiolytic-like effects of atrial natriuretic peptide on cholecystokinin tetrapeptide-induced panic attacks: preliminary findings. Arch Gen Psychiatry 2001;58(4):371–377

Herrmann-Lingen C, Binder L, Klinge M, Sander J, Schenker W, Beyermann B, von Lewinski D, Pieske B High plasma levels of N-terminal pro-atrial natriuretic peptide associated with low anxiety in severe heart failure. Psychosom Med 2003;65(4):517–522

Armstead WM, Kiessling JW, Bdeir K, Kofke WA, Vavilala MS. Adrenomedullin prevents sex-dependent impairment of autoregulation during hypotension after piglet brain injury through inhibition of ERK MAPK upregulation. J Neurotrauma 2010;27:391–402.

Meyer T, Herrrmann-Lingen C, Chavanon M-L et al. Higher plasma levels of MR-pro-atrial natriuretic peptide are linked to less anxiety: results from the observational DUAST-CHF study. Clin Res Cardiol; 2015:104:574-581.

Strohle, A., Holsboer, F.. Stress responsive neurohormones in depression and anxiety. Pharmacopsychiatry 2003 36 (Suppl 3), S207–S214.

Köhler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O, Krogh J. Effect of Anti-inflammatory Treatment on Depression, Depressive Symptoms, and Adverse Effects. JAMA Psychiatry, 2014; 1;71:12;1381-91.