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Exploring the potential role of Allostatic Load Biomarkers in Risk Assessment of Patients Presenting with Depressive Symptoms

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Abstract

Background

Depression is a major health problem worldwide and the majority of patients presenting with depressive symptoms are managed in primary care. Current approaches for assessing depressive symptoms in primary care are not accurate in predicting future clinical outcomes, which may potentially lead to over or under treatment. The Allostatic Load (AL) theory suggests that by measuring multi-system biomarker levels as a proxy of measuring multi-system physiological dysregulation, it is possible to identify individuals at risk of having adverse health outcomes at a prodromal stage. Allostatic Index (AI) score, calculated by applying statistical formulations to different multi-system biomarkers, have been associated with depressive symptoms.

Aims and Objectives

To test the hypothesis, that a combination of allostatic load (AL) biomarkers will form a predictive algorithm in defining clinically meaningful outcomes in a population of patients presenting with depressive symptoms.

The key objectives were:

1. To explore the relationship between various allostatic load biomarkers and prevalence of depressive symptoms in patients, especially in patients diagnosed with three common cardiometabolic diseases (Coronary Heart Disease (CHD), Diabetes and Stroke).

2 To explore whether allostatic load biomarkers predict clinical outcomes in patients with depressive symptoms, especially in patients with three common cardiometabolic diseases (CHD, Diabetes and Stroke).

3 To develop a predictive tool to identify individuals with depressive symptoms at highest risk of adverse clinical outcomes.

Methods

Datasets used: 'DepChron' was a dataset of 35,537 patients with existing cardiometabolic disease collected as a part of routine clinical practice. 'Psobid' was a research data source containing health related information from 666 participants recruited from the general population. The clinical outcomes for

both datasets were studied using electronic data linkage to hospital and mortality health records, undertaken by Information Services Division, Scotland.

Cross-sectional associations between allostatic load biomarkers calculated at baseline, with clinical severity of depression assessed by a symptom score, were assessed using logistic and linear regression models in both datasets. Cox's proportional hazards survival analysis models were used to assess the relationship of allostatic load biomarkers at baseline and the risk of adverse physical health outcomes at follow-up, in patients with depressive symptoms. The possibility of interaction between depressive symptoms and allostatic load biomarkers in risk prediction of adverse clinical outcomes was studied using the analysis of variance (ANOVA) test. Finally, the value of constructing a risk scoring scale using patient demographics and allostatic load biomarkers for predicting adverse outcomes in depressed patients was investigated using clinical risk prediction modelling and Area Under Curve (AUC) statistics.

Key Results

Literature Review Findings
The literature review showed that twelve blood based peripheral biomarkers were statistically significant in predicting six different clinical outcomes in participants with depressive symptoms. Outcomes related to both mental health (depressive symptoms) and physical health were statistically associated with pre-treatment levels of peripheral biomarkers; however only two studies investigated outcomes related to physical health.

Cross-sectional Analysis Findings
In DepChron, dysregulation of individual allostatic biomarkers (mainly cardiometabolic) were found to have a non-linear association with increased probability of co-morbid depressive symptoms (as assessed by Hospital Anxiety and Depression Score HADS-D≥8). A composite Al score constructed using five biomarkers did not lead to any improvement in the observed strength of the association. In Psobid, BMI was found to have a significant cross-sectional association with the probability of depressive symptoms (assessed by General Health Questionnaire GHQ-28≥5). BMI, triglycerides, highly sensitive C - reactive

protein (CRP) and High Density Lipoprotein-HDL cholesterol were found to have a significant cross-sectional relationship with the continuous measure of GHQ-28. A composite AI score constructed using 12 biomarkers did not show a significant association with depressive symptoms among Psobid participants.

Longitudinal Analysis Findings

In DepChron, three clinical outcomes were studied over four years: all-cause death, all-cause hospital admissions and composite major adverse cardiovascular outcome-MACE (cardiovascular death or admission due to MI/stroke/HF). Presence of depressive symptoms and composite AI score calculated using mainly peripheral cardiometabolic biomarkers was found to have a significant association with all three clinical outcomes over the following four years in DepChron patients. There was no evidence of an interaction between AI score and presence of depressive symptoms in risk prediction of any of the three clinical outcomes. There was a statistically significant interaction noted between SBP and depressive symptoms in risk prediction of major adverse cardiovascular outcome, and also between HbA1c and depressive symptoms in risk prediction of all-cause mortality for patients with diabetes. In Psobid, depressive symptoms (assessed by GHQ-28≥5) did not have a statistically significant association with any of the four outcomes under study at seven years: all cause death, all cause hospital admission, MACE and incidence of new cancer. A composite AI score at baseline had a significant association with the risk of MACE at seven years, after adjusting for confounders. A continuous measure of IL-6 observed at baseline had a significant association with the risk of three clinical outcomes- all-cause mortality, all-cause hospital admissions and major adverse cardiovascular event. Raised total cholesterol at baseline was associated with lower risk of all-cause death at seven years while raised waist hip ratio-WHR at baseline was associated with higher risk of MACE at seven years among Psobid participants. There was no significant interaction between depressive symptoms and peripheral biomarkers (individual or combined) in risk prediction of any of the four clinical outcomes under consideration.

Risk Scoring System Development

In the DepChron cohort, a scoring system was constructed based on eight baseline demographic and clinical variables to predict the risk of MACE over four

years. The AUC value for the risk scoring system was modest at 56.7% (95% CI 55.6 to 57.5%). In Psobid, it was not possible to perform this analysis due to the low event rate observed for the clinical outcomes.

Conclusion

Individual peripheral biomarkers were found to have a cross-sectional association with depressive symptoms both in patients with cardiometabolic disease and middle-aged participants recruited from the general population. AI score calculated with different statistical formulations was of no greater benefit in predicting concurrent depressive symptoms or clinical outcomes at follow-up, over and above its individual constituent biomarkers, in either patient cohort.

SBP had a significant interaction with depressive symptoms in predicting cardiovascular events in patients with cardiometabolic disease; HbA1c had a significant interaction with depressive symptoms in predicting all-cause mortality in patients with diabetes. Peripheral biomarkers may have a role in predicting clinical outcomes in patients with depressive symptoms, especially for those with existing cardiometabolic disease, and this merits further investigation.

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Acknowledgement

Author's declaration

I declare that I am the sole author of this thesis and I was responsible for leading all aspects of this project as Principal Investigator.

Mr David Purves, from the Robertson Centre for Biostatistics, helped me in data processing of the raw data received from the NHS Greater Glasgow and Clyde for the DepChron dataset. Mr Charles Boachie, from the Robertson Centre for Biostatistics, helped me in the data processing of the outcomes data received from the Information Services Division of Scotland for the DepChron dataset. I undertook all analysis with support from Dr Sarah Barry, one of my supervisors. I interpreted the data with input from my supervisors and Mr Geoff Der (part of expert advisory panel).

Publications and Presentations

<u>Publications arising from this project</u>

Jani, B. D., McLean, G., Nicholl, B. I., Barry, S. J.E., Sattar, N., Mair, F. S., and Cavanagh, J. (2015) Risk assessment and predicting outcomes in patients with depressive symptoms: a review of potential role of peripheral blood based biomarkers. Frontiers in Human Neuroscience, 9(Art 18). (Appendix 1)

Jani, B., Cavanagh, J., Barry, S. J.E., Der, G., Sattar, N., and Mair, F. S. (2014) Revisiting the J shaped curve, exploring the association between cardiovascular risk factors and concurrent depressive symptoms in patients with cardiometabolic disease: findings from a large cross-sectional study. BMC Cardiovascular Disorders, 14, p. 139. (Appendix 2)

Jani, B. D., Cavanagh, J., Barry, S. J.E., Der, G., Sattar, N., and Mair, F. S. (2016) Relationship between blood pressure values, depressive symptoms and cardiovascular outcomes in patients with cardiometabolic disease. Journal of Clinical Hypertension (Greenwich), 1-9. (Appendix 3)

<u>Presentations arising from this project</u>

1. European Society of Cardiology Congress, London, 2015. (Winner Best Poster)
2. European Researcher Night, Glasgow, 2015 (Public Engagement)
3. North American Primary Care Researchers Conference, New York, 2015.
4. Public Health Science, European Public Health Conference, Glasgow, 2014.
5. National Universities Week Soapbox, London, 2014. (Public Engagement)
6. Society of Academic Primary Care Conference, Edinburgh, 2014.
7. Glasgow Speed Science Competition, 2014 (Winner).
8. Society of Academic Primary Care Conference, Nottingham, 2013.

List of Abbreviations

AL= Allostatic Load
CHD=Coronary Heart Disease
DepChron='Depression in Chronic Disease' dataset
LES=Local Enhanced Services
BP=Blood Pressure
BMI=Body Mass Index
Psobid='Psychological, social and biological determinants of ill health' dataset
AI=Allostatic Index
SIMD=Scottish Index of Multiple Deprivation
MDD=Major Depressive Disorder
GP=General Practitioner
DSM=Diagnostics and Statistics Manual
ICD=International Classification of Diseases
HPA= Hypothalamic-Pituatary-Adrenal
CRP=C-reactive protein
IL= Interleukin
AL= Allostatic Load
HDL= High Density Lipoprotein
ROC= Receiver Operating Characteristic
WHI=Waist Hip Ratio
HbA1c=glycosylated haemoglobin
QOF=Quality Outcomes Framework
HADS-D=Hospital Anxiety and Depression Score-Depressive Subscale
GHQ-28=General Health Questionnaire-28 items
SBP=Systolic Blood Pressure
DBP=Diastolic Blood Pressure
MI=Myocardial Infarction
OR= Odds Ratio
HR=Hazards Ratio
CI=Confidence Intervals
ACS=Acute Coronary Syndrome
NICE=National Institute of Health and Care Excellence
RCT= Randomized controlled trial
SSRI=Selective Serotonin Re-uptake Inhibitors
MACE=Major Adverse Cardiovascular Event
ANOVA= analysis of variance
AUC=Area under curve

Chapter 1 Thesis Overview- Aims, Research Questions and Outline of Chapters

1.1 Chapter Summary

This chapter provides a general overview of the thesis, explaining the context and why this subject was chosen. The overall hypothesis underpinning this thesis and its main objectives are described. A brief outline of the datasets analysed for this thesis is provided and followed by details of the research questions and an outline of the chapters.

1.2 Depressive Symptoms in Primary Care - Current Challenges

This section provides background information about the prevalence estimates of depressive symptoms in primary care, economic costs, and the likely outcomes for patients presenting with depressive symptoms in primary care. The challenges of assessing and managing depressive symptoms in primary care are summarised, thereby setting the context of this thesis.

The prevalence estimate for depressive symptoms is approximately 10%, with some significant variations observed across different geographical locations. The estimates from studies, mainly from North America and Europe, have suggested prevalence rates of 5-10% in primary care (1). In a large international study involving 15 different cities across the world, Sartorius and Üstün reported a prevalence of 10.4% of depressive symptoms, but there were significant geographical variations (2). The prevalence rate varied from 29.5% in Santiago, Chile to 2.6% observed in Nagasaki, Japan (2). A study involving five different European countries and more than 8,000 patients reported an overall prevalence of 8.56% in the general population, with higher prevalence reported among women and in urban populations (3). While, a study across six different countries in Europe reported a prevalence estimate of 4 to 18% for major depressive disorder (MDD) in primary care (4). Similar rates (major depression 4.5% and mild depression 13.3%) have been observed in primary care centres in post-conflict areas of northern Sri Lanka (5). The prevalence of depressive symptoms in patients attending primary care has been usually recorded to be higher than that observed in the general population (6,7).

There is a significant economic burden associated with depressive symptoms as a health problem. Ferrari et al concluded that depressive disorders (MDD and dysthymia) were the leading cause of disability adjusted life years and the second leading cause of years lived with disability globally, in the global burden of disease survey in 2010 (8). Meanwhile, a review in *Nature,* in a special edition on this topic, suggested that depression accounts for the biggest share of the world's burden of disease measured by years lost to disability (see Figure 1-1)(9). The estimated annual health and social care costs (including loss of output) from mental illness was £105.2 billion in England and £8.6 billion in

Scotland, with most of the available evidence of economic burden related to depression (10).

Figure 1-1 Top ten causes of disability globally (9)
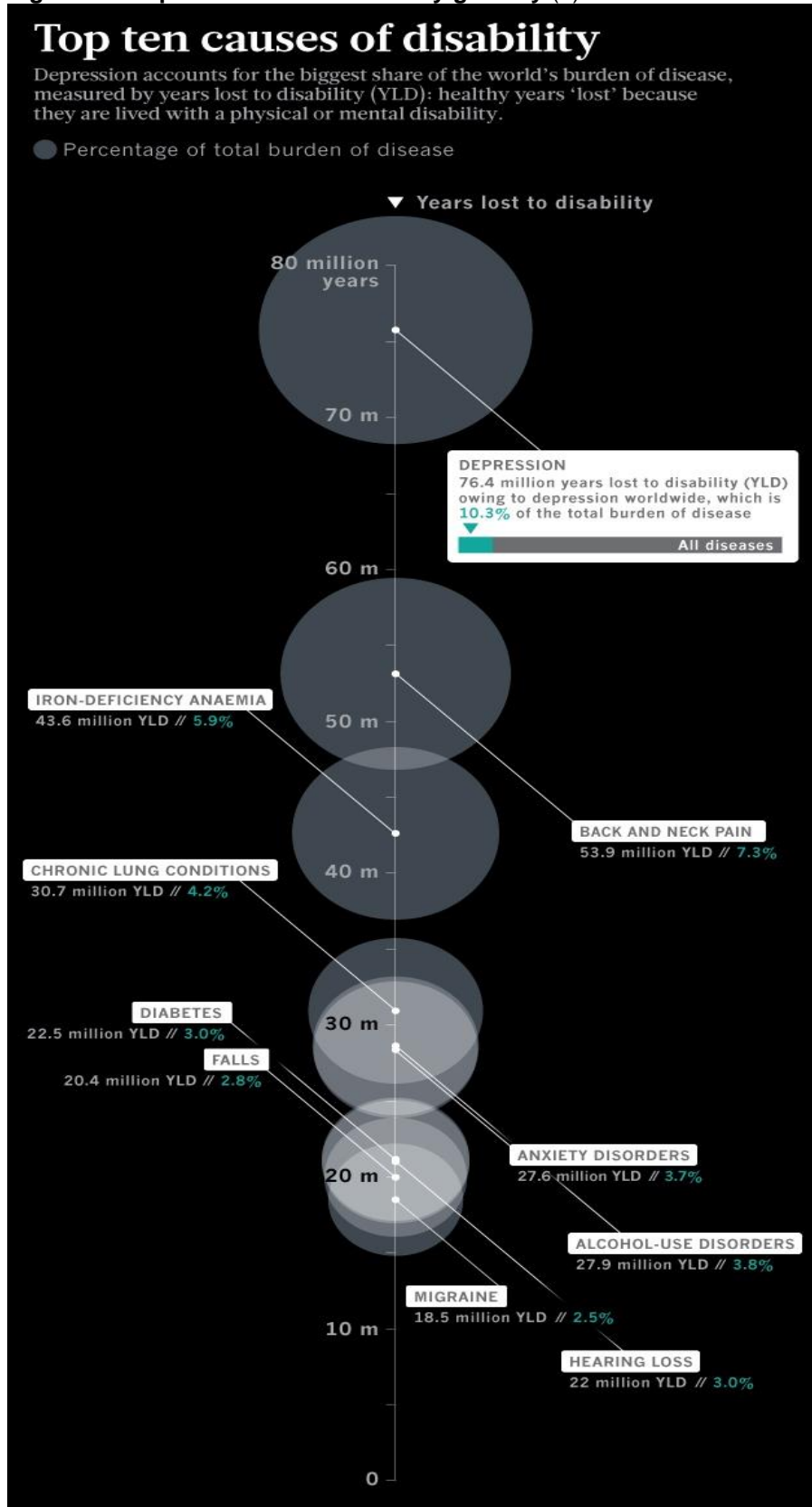
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The likely trajectory and outcome for depressive symptoms in primary care has been studied extensively, with some conflicting results. A systematic review in 2007 found 8 studies investigating recovery for primary care MDD patients and reported recovery rates varying from 32% to 71%, and only one study reporting follow-up longer than 12 months (11). More recently, a study in the Netherlands reported a 43% recovery rate at 39 months for 174 MDD patients in primary care (12). The same study reported that 17% of patients had a chronic and 40% had a fluctuating course of illness. In a Finnish study involving 79 MDD patients, it was found that roughly 25% had achieved full remission, roughly 25% had persistent symptoms and the remaining 49% had a recurring course of illness at 18 months follow-up (13). A review on prognosis of depression in older patients reported a short term(<1 year) persistence rate of 22.7 to 51.3% (14). In summary, depressive symptoms are common in primary care, associated with significant economic costs with some patients experiencing a full recovery (perhaps less than 50%), while the rest may experience a chronic or a relapsing and remitting course.

In my experience as a general practitioner (GP), one of the biggest challenges for a primary care physician in managing patients presenting with depressive symptoms is difficulty in accurate risk stratification owing to heterogeneity in presentation. The two widely used diagnostic criteria for MDD, proposed by the World Health Organisation's (WHO) International Classification of Diseases (ICD-10)(15) and American Psychological Association's (APA) Diagnostic and Statistics Manual-V (DSM-V)(16), have some important differences. In addition, not all patients with depressive symptoms in primary care will meet the diagnostic criteria for MDD and many may have minor or subthreshold depressive symptoms. But there is a significant amount of heterogeneity in defining minor and subthreshold depressive symptoms as well (17). The accuracy of primary care physicians in diagnosing and risk stratifying depressive symptoms has been reported to be less than 50%, when compared to a gold standard diagnostic interview for MDD (18,19). A gold standard diagnostic interview is not feasible in a primary care consultation due to time constraints and lack of training; hence various depression symptom questionnaire tools have been proposed and validated against the gold standard (20). However, it remains unclear if regular

symptom monitoring and structured assessment of depressive symptoms using a validated symptom questionnaire by a primary care physician has any benefit on outcomes, with evidence in favour (21) and against it (22), although higher patient confidence has been reported with the use of such an approach (23). Thus, primary care physicians face difficulties in accurate risk stratification of depressive symptoms and it remains unclear if the use of a structured and validated symptom questionnaire is the correct approach to yield better clinical outcomes.

The difficulties in risk stratification of depressive symptoms are compounded in patients with cardiometabolic disease, due to the increase in prevalence of depressive symptoms, overlap with physical symptoms, and increase in cardiovascular mortality observed with co-occurrence (24). It has been proposed that there are common biological alterations observed with depressive symptoms and cardiometabolic diseases which might mediate the link between the two conditions (25). It remains unclear if the observed relationship between depressive symptoms and various biological alterations (especially in patients with cardiometabolic disease) can be translated in to clinical application by using peripheral biomarkers in risk assessment of depressive symptoms in primary care.

The allostatic load (AL) framework has been put forward as the "price" an individual's body has to pay to maintain internal stability, in response to stress (26,27). The AL theory suggests that by measuring multi-system biomarker levels as a proxy of measuring multi-system physiological dysregulation, it is possible to identify individuals at risk of having adverse health outcomes at a prodromal stage (27). Allostatic Index (AI) score, calculated by applying statistical formulations to different multi-system biomarkers, has been linked with various adverse physical and mental health outcomes, including depressive symptoms (28).

1.3 Project Hypothesis

In this thesis, the hypothesis that a combination of allostatic load (AL) biomarkers will form a predictive algorithm in defining clinically meaningful outcomes in a population of patients presenting with depressive symptoms is tested.

1.4 Objectives

The three main objectives are:

1 To explore the relationship between various allostatic load biomarkers and prevalence of depressive symptoms in patients, especially in patients diagnosed with three common cardiometabolic diseases (Coronary Heart Disease (CHD), Diabetes and Stroke).

2 To explore whether allostatic load biomarkers predict clinical outcomes in patients with depressive symptoms, especially in patients with three common cardiometabolic diseases (CHD, Diabetes and Stroke).

3 To develop a predictive tool to identify individuals with depressive symptoms at highest risk of adverse clinical outcomes.

1.5 Datasets

Two datasets were used to explore the hypothesis and to meet the aforementioned research objectives. The first dataset DepChron ('Depression in Chronic Disease' dataset) comes from the West of Scotland, with a sample size of more than 125,000 patients with a diagnosed cardiometabolic disease. This dataset uses information collected in 2008-09 as part of routine clinical practice within a chronic disease management programme called 'Local Enhanced Services'(LES) in primary care aimed at patients with three cardiometabolic conditions (CHD, diabetes and stroke). LES are contractual arrangements at a local health board level with general practices where incentivisation is offered to primary care practitioners to complete certain indicators of chronic disease management. However, there are no penalties for non-adherence. In the areas under investigation in our study, general practices were paid under the LES scheme to carry out a comprehensive annual health assessment, which included depression screening, for patients with the three common conditions described above. The annual health assessment was usually carried out by a practice nurse and lasted approximately one hour. The protocol for health assessment was specific for each of the three diseases but included monitoring of blood pressure (BP), total cholesterol, body mass index (BMI) and in those with diabetes, HbA1c. The large sample size and data collected from real life clinical practice are major strengths of this dataset.

The second dataset, *Psobid ('Psychological, social and biological determinants of ill health' dataset)* has 666 patients recruited in 2006-07 from the general population in Glasgow, with patients recruited from the most affluent and the most deprived areas based on Scottish Index of Multiple Deprivation(SIMD) (29). Participants aged 35-64 years were invited for two health visits during recruitment and a variety of information ranging from demographics, lifestyle information, medical history, clinical examination, blood tests and brain imaging was collected during these visits. The Psobid dataset has participants with and without a diagnosis of a cardiometabolic condition. The details of information available on behavioural measures for participants and the availability of a wide variety of blood based biomarker results are the key strengths of this dataset.

Data on clinical outcomes for the two cohorts were obtained through the use of data linkage facilities provided by Information Services Division, Scotland. The follow-up data on adverse clinical outcomes such as hospitalization, cardiovascular events and mortality were available over a period of four years for DepChron and seven years for Psobid.

The two datasets were employed in this thesis for three different reasons. Firstly, the two datasets offered distinct advantages with larger sample size in DepChron dataset and better availability of biomarker data in Psobid dataset. Secondly, DepChron was a primary care cohort with existing cardiometabolic disease while Psobid was a younger cohort recruited from general population with relatively better health status. It was hypothesized that the relationship between peripheral biomarkers, depressive symptoms and adverse health outcomes may vary significantly based on underlying health status. Finally, performing the analysis in two datasets had the potential of offering external validity.

1.6 Research Questions

Below are outlined the five research questions to be addressed in this thesis by undertaking analysis of the two datasets, DepChron and Psobid.

1.   Research Question 1 (RQ1):

What is the association, if any, between a composite Allostatic Index (AI) score calculated using available allostatic load biomarkers and depressive symptoms?

2.   Research Question 2 (RQ2):

What is the association, if any, between individual allostatic load biomarkers and depressive symptoms and how does it compare with the relationship between composite AI score and depressive symptoms?

3.   Research Question 3 (RQ3):

What is the association, if any, between a composite AI score at baseline and the risk of future adverse health outcomes such as vascular events, hospitalisation and mortality in patients with depressive symptoms?

4.   Research Question 4 (RQ4):

What is the association, if any, between individual allostatic load biomarkers at baseline and the risk of future adverse health outcomes, such as vascular events, hospitalisation and mortality in patients with depressive symptoms and how does it compare with the relationship between composite AI score at baseline and risk of adverse outcomes?

5.   Research Question 5 (RQ5):

What is the accuracy of a risk scoring system developed using patient demographics, allostatic load biomarker values and severity of depressive symptoms, in predicting adverse health outcomes in patients with depressive symptoms?

1.7 Outline of Chapters

1. Chapter 2 provides a background of the existing literature on the role of peripheral biomarkers in the prediction of clinical outcomes for patients presenting with depressive symptoms.

2. Chapter 3 summarises the current evidence base in the area of depressive symptoms co-morbid with cardiometabolic disease. The prevalence estimates, suggested methods of management, reported clinical outcomes and proposed underlying mechanisms for the relationship between depression and cardiometabolic disease are reviewed.

3. Chapter 4 discusses the allostatic load theory, its relationship with depressive symptoms and other health related outcomes, and the various statistical methods used to calculate the AI score. The characteristics of the two population cohorts-DepChron and Psobid are described in detail.

4. Chapter 5 provides the findings of cross-sectional analysis in the DepChron dataset to address research questions 1 and 2 (RQ 1 and 2).

5. Chapter 6 provides the findings of cross-sectional analysis in the Psobid dataset to address research question 1 and 2 (RQ1 and 2).

6. Chapter 7 describes the findings of longitudinal analysis after following up the cohort in the DepChron dataset for a period of four years to address research questions 3 and 4 (RQ 3 and 4).

7. Chapter 8 describes the findings of longitudinal analysis after following up the cohort in the Psobid dataset for a period of seven years to address research questions 3 and 4 (RQ 3 and4).

8. Chapter 9 presents the findings of risk scoring system development for predicting adverse clinical outcomes in patients in both datasets with depressive symptoms to address research question 5 (RQ5). The presentation of the findings of the analysis were evaluated against the

TRIPOD statement (Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) check list (30).

9. Chapter 10 summarises the findings of this overall programme of work, compares it with the existing literature, and discusses strengths and limitations as well as potential implications of the findings including directions for future research in this area.

Chapter 2 Potential Role of Peripheral Biomarkers in Depression Risk Assessment – A Background

2.1 Chapter Summary

In this chapter, there are five parts including the introduction section. In the introduction section, the heterogeneity of depressive symptoms, the health related hazards associated with subthreshold depressive symptoms and problems in management of depressive symptoms in primary care (owing to drawbacks of currently available diagnostic classification systems) are discussed. This is followed by a brief overview of the proposed mechanisms of pathogenesis of depressive symptoms, the association of depressive symptoms with peripheral biomarkers and the aim of this background chapter. The first section is followed by a methods section describing the literature search and review methodology. The next two sections describe the results from the literature review and discussion of the findings, respectively. Finally, the last part describes the findings after updating the literature search and review.

2.2 Introduction

2.2.1 Heterogeneity in depressive symptoms

Depressive disorders are heterogeneous with a spectrum ranging from minor/sub threshold depression to MDD (31). The methods currently available for risk assessment and stratification of symptom severity for patients presenting with depressive symptoms rely predominantly on counting the absolute number of depressive symptoms present and there are two problems associated with this approach. Firstly, there is no universally accepted standardised definition of MDD or subthreshold depression. The DSM-IV's diagnosis of a MDD requires the presence of at least 5 out of 9 symptoms of depression with significant impairment or distress, while those presenting with at least 2 but fewer than 5 symptoms and no previous history of MDD are stratified as sub threshold or minor depression (32). However, the category of sub threshold depression has been removed from the recently published DSM-V (16). In contrast, the ICD-10 stratifies depressive symptoms on the basis of the number of depressive symptoms present into mild (4 out of 10), moderate (5 or 6 out of 10) and severe (7 or more out of 10) depressive symptoms (15). The problem of lack of consensus in definition also extends to subthreshold depression, with lots of variations in how it is defined based on symptom count and duration (17). Secondly, this approach has also been questioned as it ignores the complexity and diversity observed with different presentations of depressive symptoms (33).

2.2.2 Are all types of depressive symptoms hazardous?

The global burden of disease survey in 2010 attributed significant disability adjusted life years and years lived with disability to subthreshold depression, although these effects were much more significant with MDD (8). Subthreshold depression has been associated with severe deficits in psychological well-being, quality of life, and increased mortality (34–36). In addition, subthreshold depression has also been found to have low spontaneous remission rate and higher risk of converting into MDD, especially in older adults where it is more prevalent (37,38).

2.2.3 Management of depressive symptoms- usefulness of currently available diagnostic classification

The majority of patients reporting depressive symptoms are managed in primary care (20,39). The following section examines the utility of the currently available diagnostic classification against two criteria- ease of use in primary care and deciding appropriate management. The methods of classifying depressive symptoms based on current diagnostic classification systems can be broadly categorized into two main types- 'gold standard' diagnostic interviews and psychometric depression symptom questionnaire tools. Diagnostic interviews are not practical to implement in primary care, especially in the U.K. where average consultation duration is 10 minutes and often involves dealing with multiple health problems (40). On the other hand, GPs have often found the use of psychometric tools 'not consultation friendly', while some GPs have questioned the validity of using these tools (23,41). Furthermore, if GPs do not use either of the above two methods, their diagnostic accuracy has been reported to be about 50% compared to a gold standard diagnostic interview, based on a meta-analysis of 41 studies involving more than 50,000 patients (19).

The uncertainty in stratifying depression severity based on symptom count affects subsequent management. A review of treatment guidelines for depression across North America and Europe revealed that "mild MDD and sub threshold depression has the most variance in recommendations"; with suggested approaches ranging from watchful waiting to active treatment with antidepressants (42). In the last decade, three separate meta-analyses reported that the efficacy of antidepressants is related to the initial severity of depression and they may not be effective in the treatment of mild depression (43–45). However, this view has been challenged recently with emerging evidence suggesting that the efficacy of antidepressants in depression may not be related to its initial severity (46,47), and that efficacy is not limited to MDD only and may extend to the whole spectrum of depressive symptoms (48). Similarly, psychological therapies have also been found to be effective in the management of mild depression and prevention of MDD (49).

In summary, there are significant health hazards associated with minor depression. The diagnostic tools available to primary care physicians for differentiating minor depression and MDD are either impractical or unpopular among primary care physicians. Most importantly, the two different management approaches used for depressive symptoms (antidepressants and psychological therapies) have similar efficacy for both mild depression and MDD. Thus, the risk stratification of depressive symptoms needs a new approach and cannot merely rely on counting the number of depressive symptoms alone. The focus of the stratification of depressive symptoms should be based on the risk of adverse clinical outcomes (both related to depressive symptoms and physical health). In this context, the advances made so far in the study of pathogenesis of depressive symptoms may have a role.

2.2.4 Pathogenesis of depressive symptoms- an overview

The etiopathogenesis of depression has been extensively studied over the last five decades with various explanatory mechanisms involving different physiological systems, suggesting heterogeneity (50). The 'monoamine hypothesis of depression' was proposed in the 1960s with early work showing increased levels of plasma tryptophan (a serotonin precursor) in patients with major depression (51). Failure to suppress cortisol in response to dexamethasone in patients with depression was the initial finding which supported the role of the hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in the pathophysiology of depression (52). The 'cytokine hypothesis' suggests that depression is triggered, in part, via inflammatory processes in response to various internal and external stressors, following some seminal work in the early 1990s (53). The 'neurogenesis hypothesis' of depression proposes that depression is characterized by neurodegeneration and impaired neurogenesis in the brain, in particular the hippocampus region (54). It is likely that several hypotheses may overlap here. Mössner et al have summarized various pathological mechanisms which could form the basis of depressive symptoms and proposed that there could be a role to play for biomarkers in management of depressive symptoms (55) (see Figure 2-1).

Figure 2-1 Various pathological mechanisms associated with depressive symptoms
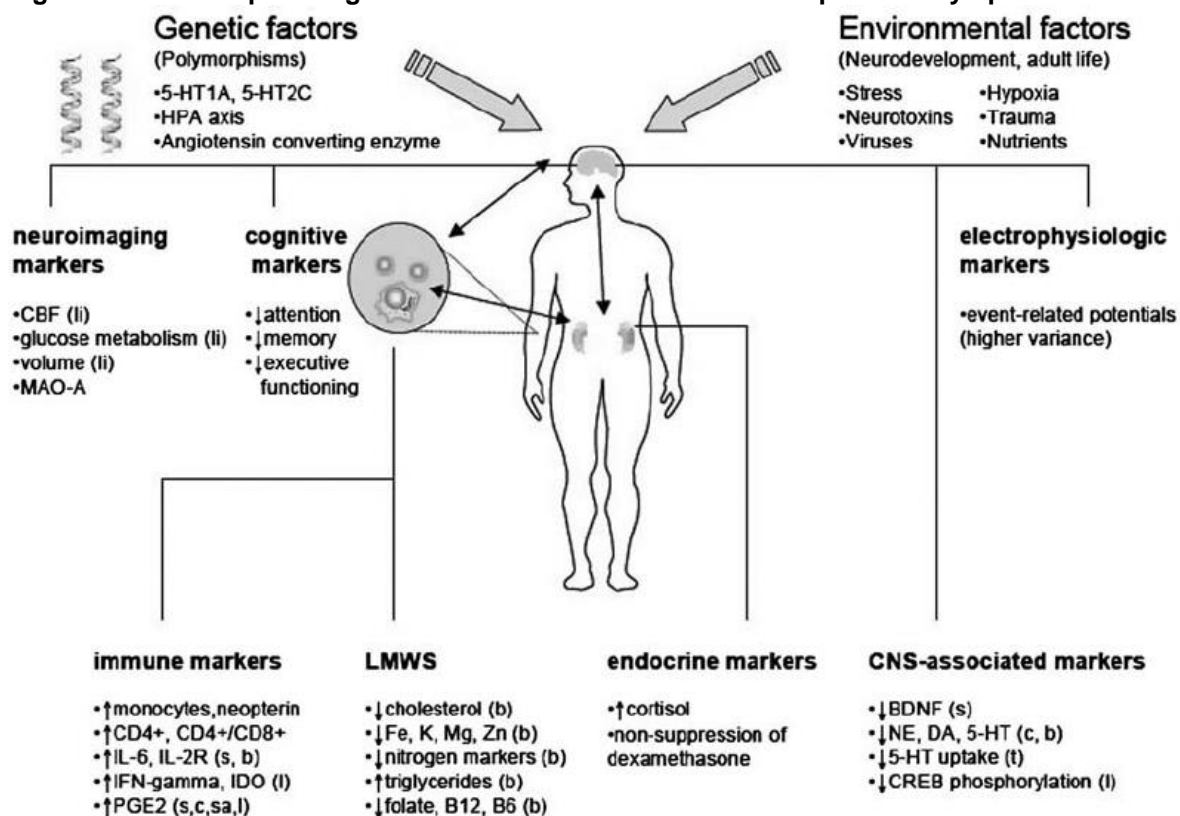
Proposed by Mössner and colleagues(55). Reproduced with permission from Taylor and Francis.

2.2.5 Biomarkers of depression

A biomarker can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001). The research into pathogenesis of depression has led to an extensive evidence base supporting a cross-sectional relationship between depressive symptoms and a number of different biomarkers pertaining to some of the physiological systems described above, but their role, if any, in predicting clinical outcomes in depressive symptoms remains unclear due to lack of a sufficient number of longitudinal studies (56) (57). Peripheral biomarkers (blood based) are relatively non-invasive (other than the need for a blood sample) and easy to measure; hence they have a greater potential for translational application into routine clinical practice, when compared to imaging, genetic and CNS biomarkers. Peripheral biomarkers such as those related to the HPA axis, inflammatory and monoamine systems may have a role in the diagnosis of depression by identifying a 'biological sub-type' of depressive symptoms, and more importantly in prognostication of depressive

symptoms by predicting treatment response, which in turn could help in severity stratification and management (58–60).

2.2.6 Aim of the literature review

The aim of the literature search and review was to examine the current evidence base exploring the potential role of peripheral biomarkers measured at baseline as a risk assessment tool in predicting future outcomes in patients with depressive symptoms.

2.3 Methods

Two electronic databases (Ovid Medline and Embase) for studies published between 1946 and Jan 2013 using the MESH terms "Biological markers" AND "Depression" were searched. All original and review studies using peripheral biomarkers at baseline as a risk assessment tool for predicting future outcomes in patients with depressive symptoms were included. If a meta-analysis was included in the review, findings from that meta-analysis were considered for the review, and not the findings of the primary studies included in that meta-analysis. Clinical outcomes pertaining to both mental health (e.g. depressive symptoms) and physical health (e.g. cardiovascular events) were included. Only studies published in English language were considered for inclusion. Studies related to animal, imaging biomarkers, cerebrospinal fluid biomarkers, and mood disorders other than depression were excluded. Studies which investigated the role of depressive symptoms and peripheral biomarkers independently in predicting adverse physical outcomes but did not examine the interaction between depressive symptoms and peripheral biomarkers, or in other words did not perform a sub-group analysis in patients with depressive symptoms, were excluded. Studies that investigated changes in peripheral biomarker levels following treatment for depressive symptoms were excluded as the aim of this review was to focus on the use of peripheral biomarkers at baseline or pre-treatment as a predictive tool of clinical outcome (both mental and physical), rather than a change in biomarker level itself. The search strategy returned 1096 studies from the two databases after excluding duplicates (see Figure 2-2 for details).

Title, abstract and full text screening followed by reference and citation searching and data extraction were carried out independently by two researchers (myself and Dr Gary McLean). Data extraction comprised of study sample size and country, type of study and setting, details of how a depressive disorder was diagnosed and treated, follow-up duration, biomarkers assessed, clinical outcomes studied and potential bias in the results. The description of methodology used by included studies for biomarker measurement and the source of peripheral biomarker (i.e. serum or plasma or whole blood) was also reviewed in data extraction.

Figure 2-2 Flow chart for the review on the role of peripheral biomarkers predicting outcomes in patients with depressive symptoms (61)
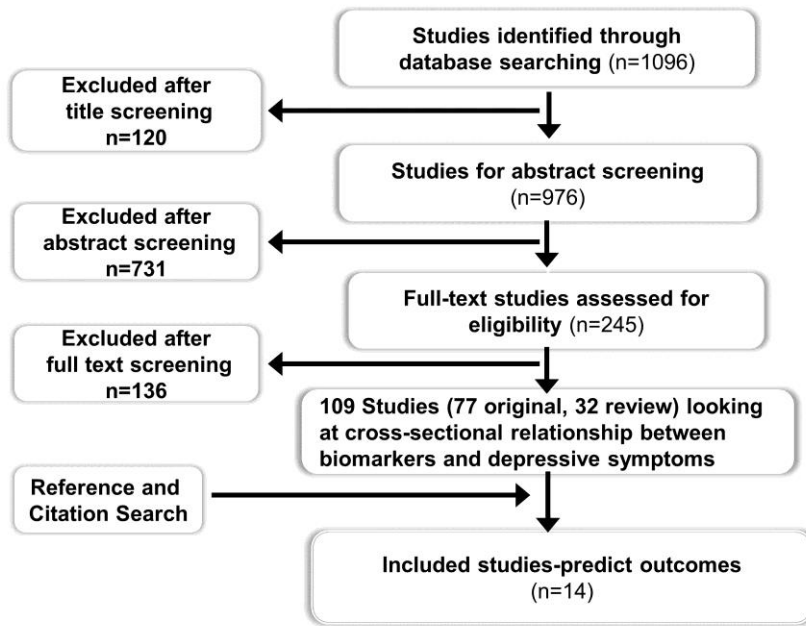
2.4 Results

2.4.1 Included studies and their characteristics

There was extensive evidence (109 studies) exploring and supporting the cross-sectional relationship between depression and different peripheral biomarkers. However, only a minority of studies (n=14) investigated the use of peripheral biomarkers to predict future outcomes in patients with depressive symptoms. Fourteen papers were included for data extraction; which consisted of eight prospective cohort studies (62–69), three case-control studies (70–72), two randomized controlled trials (73,74) and one meta-analysis (75). Full details of included studies are summarized in Table 2-1.

Sample sizes ranged from 8 to 986 with sample sizes of fewer than 50 participants in 6 studies (62,64,65,68,70,71), while three studies had a sample size of 25 or fewer (62,68,70). Follow-up duration ranged from 4 weeks to 18 years with the follow-up duration being less than 6 months in 8 studies (62,64,68–70,72–74), while only five studies followed their subjects for more than 12 months (63,65-67,71). Six studies each used a diagnostic interview technique (64,66,68,70–72), and a depression rating scale (62,63,67,69,73,74); while diagnostic method for depressive disorder was not specified in one of the included studies (65). The nature of the treatment was specified in nine studies (62,64,68–70,72–75); the relationship between outcome and baseline depression severity was only taken into account in five studies (64,66,68,70,71). The included meta-analysis had a variable sample size and follow-up duration depending on the different research questions considered by the study, while the diagnostic methods used were heterogeneous including various symptoms scores and interview techniques (75). The meta-analysis was published in 1993 and none of the original studies included in the meta-analysis were included in this review.

Table 2-1 Summary of studies included in the review(61)**.**

Study and Country	Type of Study and Setting	Sample Size at follow-up	Depression Diagnosis Criteria	Treatment Offered	Follow-up Duration
Alvarez et al (1999); France	Cohort; Psychiatry inpatients	N=8	MADRS ≥ 20	Fluoxetine 20 mg	28 days
Arolt et al (2003); Germany	Case-control; Psychiatry inpatients	N=25 (MDD), N=25 (healthy controls)	Composite International Diagnostic Interview for DSM-IV criteria for MDD	Different groups of anti-depressants	28 days
Baldwin et al (2006); UK	Case-control; Community	N=28 (MDD), N=35 (healthy controls)	SCID for MDD	Not specified	3.1 years
Baune et al (2012); Australia	Cohort; Community	N=73	GDS ≥6	Not specified	23.39 months (average)
Duval et al. (1996); France	Cohort study; Psychiatry Inpatients	N=30	Unstructured interview for DSM-IV for MDD	1.Amitriptyline (n=13) 2.Fluoxetine (n=9) 3.Toloxatone (n=8)	1 month
Jang et al (2008); South Korea	Case-control; Psychiatry Outpatients	N= 59 (MDD), N=34 (healthy controls)	SCID for MDD	Different groups of anti-depressants	6 weeks
Johnston et al (1999); UK	Cohort; Psychiatry Outpatients & Inpatients	N=34	SCID for MDD	Not specified	8 years (average)
Jokinen et al (2009); Sweeden	Cohort; Psychiatry Inpatients	N=346	DSM- IV criteria for all mood disorders, diagnostic method unspecified	Not specified	18 years (average)
Kin et al (1997); Multi-centre	RCT with 3 arms; not specified	N=70 randomized into 3 arms	HDRS ≥ 18	3 arms: 1. Nortriptyline 75 mg 2.Moclobemide 400 mg 3. Placebo	7 weeks
Ladwig et al (2005); Germany	Cohort; Community	N=975 (only males)	von Zerssen affective symptom check list with a score ≥11	Not specified	7.7 years (average)

Lanquillon et al. (2000); Germany	Cohort; Psychiatry Inpatients	N=24	SCID for MDD	Amitriptyline in increasing dose	6 weeks
Perez et al. (1998); Spain	Cohort; Psychiatry Inpatients	N=83	HDRS ≥ 17	Different groups of anti-depressants	6 weeks
Raison et al. (2013); US	RCT with 2 arms; Community	N=60 randomized into two arms	Treatment resistance Depression diagnosed using Massachusetts General Hospital Staging method for treatment resistance ≥ 2	2 arms: 1.Infliximab infusions ×3 2. Placebo	12 weeks
Ribeiro et al. (1993); US	Meta-analysis with 3 different research questions (RQ1-3)	RQ-1 N=127	Heterogeneous, including different symptoms scores and interview techniques	Various	Not specified
		RQ-2 N=412	Heterogeneous, including different symptoms scores and interview techniques	Various	1-7 weeks
		RQ-3 N=411	Heterogeneous, including different symptoms scores and interview techniques	Various	1-60 months

Legend: MADRS: Montgomery Asperg Depression Rating Scale, HDRS: Hamilton Depression Rating Scale, DSM-IV: Diagnostic and Statistical Manual IV, MDD: Major Depressive Disorder, SCID: Structured Diagnostic Interview for DSM-IV, GDS: Geriatric Depression Scale, RCT: Randomized Controlled Trial, 5-HIAA: 5-Hydroxyindoleacetic acid, RQ: Research Question.

2.4.2 Biomarkers studied and method of collection

The included studies assessed 36 different peripheral biomarkers at baseline as a predictor of clinical outcomes. These biomarkers were measured in serum or plasma and could be broadly classified as pertaining to inflammatory (n=14), neurotransmitter metabolism (n=9), neuroendocrine (n=8), metabolic (n=4) and neurotrophic (n=1) systems. The peripheral biomarkers and clinical outcomes considered by the included studies are summarized in Figure 2-3. All included studies assessed statistical significance based on the criteria of having a p-value less than 0.05. Twelve biomarkers were found to be statistically significant in predicting outcomes (summarised in Figure 2-3), while 24 biomarkers were found to have no association with the clinical outcomes studied. Inflammatory (63,67,68,71,74) and neuroendocrine (64-66,73,75) biomarkers were each assessed in five of the included studies, followed by neurotransmitter biomarkers in three studies (62,66,69), neurotrophic biomarkers in two studies (70,72), while metabolic biomarkers were assessed in only one study (71).

The source of peripheral biomarker measurement was plasma in half of the included studies (n=7) (64–66,69,70,73,74); serum in two studies (63,67,72); whole blood (68) and mixed (both serum and plasma) (62) in one study each; and not reported in two of the included studies (71,75). Four of the included studies did not describe the procedures of measuring peripheral biomarker in detail (64,65,71,75). Four of the included studies describe the anticoagulant used for collecting plasma samples, with ethylenediaminetetraacetic acid (EDTA) used by two studies (69,74); and heparin (70) and sodium citrate (62) used by one study each.

2.4.3 Types of clinical outcomes studied

The majority of included studies (n=12) considered outcomes pertaining to mental health or depressive symptoms (62–64,66,68–75), with only two studies assessing physical health outcomes (65,67) (see Table 2-2).   The commonest outcome was author defined positive treatment response to anti-depressants with improvement in depressive symptoms (e.g. 50% reduction in depression rating scale Hamilton Depression Rating Scale (HDRS) from baseline) being

considered by nine included studies (62,64,68–70,72–75). This was followed by other mental health outcomes such as pre-defined criteria for poor outcome of depressive symptoms (n=3)(66,71,75), for example Lee and Murray operational criteria for outcome in depression. Remission of depression symptoms was used in three studies(63,64,74), for example HDRS <8 at follow-up.

Physical health outcomes measured were: cardiovascular deaths-two studies (65,67), myocardial infarction (67) and death due to natural causes (65) by one study each. Biomarkers were shown to be statistically significant in predicting all of the six outcomes considered, including mental and physical outcomes. Figure 2-3 summarizes the six different mental and physical health outcomes studied, the number of studies which examined each outcome, the 12 peripheral biomarkers which were noted to be statistically significant in predicting each outcome and the direction of the relationship between the biomarker and the outcome.

2.4.4 Statistical methods used and their limitations

There were some limitations of the statistical methods used in the included studies. The Area Under Curve (AUC) statistic was presented only by one study, with AUC statistic for Dexamethasone suppression test (DST) reported as 0.65 for predicting increased incidence of cardiovascular deaths, only for the male subset (n=126/382) of their sample (65). DST was found to have a significant impact in predicting three different outcomes in two different studies; which included adverse outcomes such as increased incidence of all-cause mortality and cardiovascular deaths (65), and the favourable outcome of positive treatment response (as measured by 50% reduction in HDRS) to anti-depressants (73). In the included meta-analysis, there was no evidence of an association between DST at baseline and a 50% improvement in HDRS in the group taking anti-depressants, though there was evidence of an association between baseline DST and a 50% improvement in HDRS in the group taking placebo (75). Elevated levels of serum S100B was the only biomarker which was found to have a statistically significant association with the same clinical outcome (positive treatment response to anti-depressants) in more than one included study (70,72).

Figure 2-3 Different outcomes in depression and their statistically significant predictors: findings from review(61)
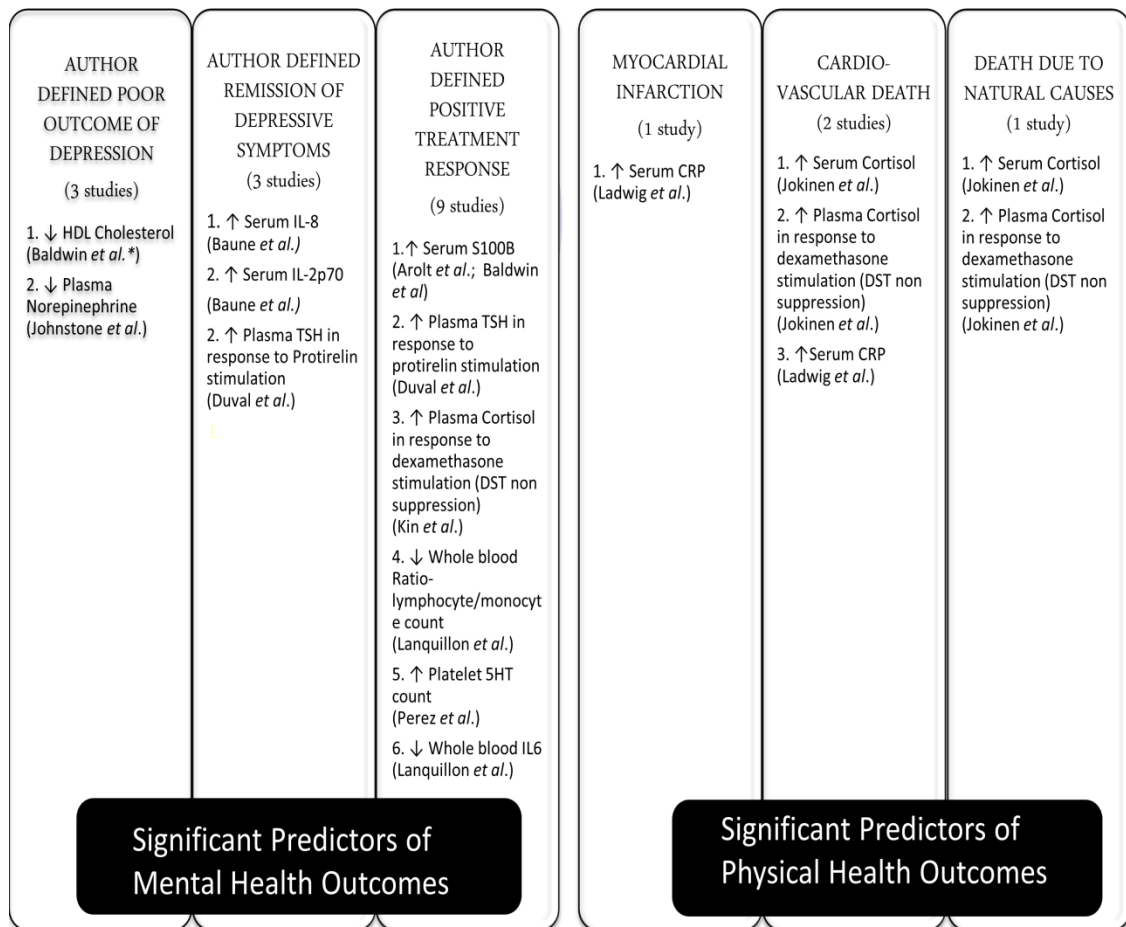
Legend: The figure describes the various mental and physical health outcomes considered by included studies and the number of studies which examined each outcome. The figure includes only those peripheral biomarkers which were found to have a <u>statistically significant </u>impact in predicting each outcome and the direction of the relationship. DST: Dexamethasone Suppression Test, CRP: C Reactive Protein, IL: Interleukin, 5HT: 5 Hydroxytryptamine, TSH: Thyroid Stimulating Hormone, ↑: higher, ↓: lower. * The study did not specify the source of the biomarker studied (i.e. serum or plasma).

Table 2-2 Summary of peripheral biomarkers and clinical outcomes considered by included studies

Study and Country	Source of Biomarker (Serum/Plasma/whole blood), Biomarkers Assessed (and type of biomarker) * implies statistically significant (p-value<0.05)	Outcomes studied
Alvarez et al (1999); France	a. Serum 5 plasma fluoxetine (Neurotransmitter metabolism) b. Plasma norfluoxetine (Neurotransmitter metabolism) c. Plasma fluoxetine plus norfluoxetine (Neurotransmitter metabolism) d. Plasma 5 HT (Neurotransmitter metabolism) e. Serum 5HT (Neurotransmitter metabolism)	Treatment Response defined as 50% reduction in MADRS scores from baseline
Arolt et al (2003); Germany	a. Plasma S100 B protein* (Neurotrophic)	Treatment Response defined as 50% reduction in HDRS from baseline
Baldwin et al (2006); UK	a. HDL cholesterol* (Metabolic) b. LDL cholesterol (Metabolic) c. BMI (Metabolic) d. ESR (Inflammatory) e. Pre-prandial glucose (Metabolic) Source: Serum/Plasma/Whole blood was not specified	Poor Outcome of depression based on author described criteria assessed by SCID
Baune et al (2012); Australia	a. Serum IL1β (Inflammatory) b. Serum IL6 (Inflammatory) c. Serum IL8 * (Inflammatory) d. Serum IL10 (Inflammatory) e. Serum IL 12p70 * (Inflammatory) f. sVCAM-1 (Inflammatory) g. Serum PAI-1 (Inflammatory) h. SAA (Inflammatory) i. Serum TNF-α (Inflammatory) j. Serum CRP (Inflammatory)	Remission of depression symptoms defined as GDS <6
Duval et al. (1996); France	a. Plasma TSH (Neuroendocrine) b. Plasma Free T3 (Neuroendocrine) c. Plasma Free T4 (Neuroendocrine) d. Plasma TSH response to Protirelin stimulation* (*for outcomes 1 and 2*) (Neuroendocrine) e. Plasma Free T3 response to Protirelin stimulation (Neuroendocrine) f. Plasma Free T4 response to Protirelin stimulation (Neuroendocrine)	1. Remission of depression symptoms defined as HDRS<8 2. "Partial Response" (treatment response) defined as HDRS 8-15
Jang et al (2008); South Korea	a. Serum S100B protein * (Neurotrophic)	Treatment Response defined as 50% reduction in HDRS from baseline

Johnston et al (1999); UK	a. Plasma Norepinephrine* (Neuroendocrine) b. Plasma Cortisol (Neuroendocrine)	Poor Outcome defined by Depression Outcome Scale & Lee and Murray criteria
Jokinen et al (2009); Sweeden	a. Plasma Cortisol* **(for outcomes 1 and 2)** (Neuroendocrine) b. Plasma Dexamethasone non-suppression* **(for outcomes 1 and 2)** (Neuroendocrine)	1.Death due to natural causes 2.Cardiovascular deaths
Kin et al (1997); Multi-centre	a. Plasma Dexamethasone non-suppression* **(only in Nortriptyline arm)** (Neuroendocrine)	Treatment Response defined as 50% reduction in HDRS from baseline
Ladwig et al (2005); Germany	a. Serum Highly sensitive CRP high risk group > 3 mg/ml* (Inflammatory)	1.Myocardial Infarction 2.Sudden cardiac death
Lanquillon et al. (2000); Germany	a. Whole blood Lymphocyte count (Inflammatory) b. Whole blood Monocyte count (Inflammatory) c. Whole blood Ratio lymphocyte/monocyte* (Inflammatory) d. Whole blood CRP (Inflammatory) e. Whole blood ESR (Inflammatory) f. Whole blood IL-6 * (Inflammatory) g. Whole blood TNF-alpha (Inflammatory)	Treatment Response defined as 50% reduction in HDRS and MADRS from baseline
Perez et al. (1998); Spain	a. Plasma 5HIAA (Neurotransmitter) b. Plasma Total Tryptophan (Neurotransmitter) c. Plasma 5 HT (Neurotransmitter) d. Platelet 5 HT with high concentration 800 ng/10^9 platelets* **(stronger relationship in SSRI sub-group)** (Neurotransmitter)	Treatment Response defined as 50% reduction in HDRS from baseline
Raison et al. (2013); US	a. Plasma Highly sensitive CRP high risk group > 5 mg/ml (Inflammatory)	1.Treatment Response defined as 50% reduction in HDRS from baseline 2. Remission of depression symptoms defined as HDRS<8
Ribeiro et al. (1993); US	a. Dexamethasone non-suppression* **(for RQ2 only)** Source: Serum/Plasma/Whole blood was not specified for the included studies	1.(RQ1) "Treatment Response" 2.(RQ2) Response to Placebo 3. Long term outcome of depression based on predefined author criteria
Legend: 5HT: 5 Hydroxytryptamine, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, ESR: Erythrocyte Sedimentation Rate, BMI: Body Mass Index, IL: Interleukin, sVCAM-1: Serum Vascular Cell Adhesion Molecule -1, PAI-1: Plasminogen Activator Inhibitor-1, SAA: Serum Amyloid A, TNF-α: Tumour Necrotic Factor-alpha, CRP: C Reactive Protein, TSH: Thyroid Stimulating Hormone, RQ: Research Question.		

2.4.5 Patient demographics and attrition rate

Description of patient demographics, co-morbid conditions and attrition at follow-up for the 14 included studies is provided in Table 2-3. Details of the age of participants were not described by three studies (63,73,75); while information on gender distribution was missing from five studies (63,70,71,73,75). The socio-economic status of participants was very poorly described with only two studies (67,75) characterizing it and only one study (67) including socio-economic status in their statistical analysis. Patients with pre-existing chronic disease were excluded by the majority of the included studies (n=8)(64–70,72) and chronic disease status was not considered or described by four of the included studies (62,71,73,75). Of the two studies which explicitly included patients with co-existing chronic disease (63,74), only one study accounted for the number of co-morbidities in their statistical analysis (74). The reported participant attrition rate at follow-up varied from 0 to 44%; with two included studies not specifying the details of attrition (63,75).

Table 2-3 Patient population and attrition rates for the included studies

Study	Mean Age in years (Standard Deviation, if available) and Sex F=Females M=Males	Co-morbid Medical Conditions	Participant numbers Number of participants at baseline (B) and follow-up(FU); attrition in percentage
Alvarez et al (1999)	45 (13.8) 6F, 2M	Not described	10 B 8 FU 20% attrition
Arolt et al (2003)	46.4 (9.8) Not described	Patients with co-morbid conditions excluded from study	25 B 25 FU No attrition
Baldwin et al (2006)	73.9 Not described	Not described	50 B 28 FU 44% attrition
Baune et al (2012)	Not described	Presence/absence of a list of medical conditions noted and entered into statistical analysis	73 B Sample size at follow-up not specified
Duval et al (1996)	39.8 (12.9); 19M, 11F	Patients with co-morbid conditions excluded from study	30 B 30 FU No attrition
Jang et al (2008)	60.3; 43F, 16M	Patients with co-morbid conditions excluded from study	59 B 59 FU No attrition
Johnston et al (1999)	47; 24F, 10M	Patients with co-morbid conditions excluded from study	47 B 34 FU 27.6% attrition
Jokinen et al (2009)	52 (16.4); 256F, 126M	Patients with co-morbid conditions excluded from study	382 B 346 FU 9.4% attrition
Kin et al (1997)	Not described	Not described	95 B 70 FU 26.3% attrition
Ladwig et al (2005)	57.75 (7.8); 975M	Patients with co-morbid conditions excluded from study	986 B 975 FU 1.1% attrition
Lanquillon et al (2000)	53.5; 15F, 9M	Patients with co-morbid conditions excluded from study	35 B 24 FU 30.5% attrition
Perez et al. (1998)	M 45 (2.9), F 44.9 (2.0); 59F, 24M	Patients with co-morbid conditions excluded from study	89 B 83 FU 6.7% attrition
Raison et al. (2013)	42.5 (8.2) placebo group, 44.3 (9.4) intervention group; 40F, 20M	Notable exclusions- previous history of cancer, history of unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (determined by Physical examination and laboratory testing). Number of co-morbid medical conditions noted and entered into statistical analysis	60 B 60 FU No attrition
Ribeiro et al. (1993)	Not described	Not described	Not described

2.5 Discussion

2.5.1 Summary of Findings

This review shows that blood based peripheral biomarkers were statistically significant in predicting six different clinical outcomes in participants with depressive symptoms. Outcomes related to both mental health (depressive symptoms) and physical health were statistically associated with pre-treatment levels of peripheral biomarkers; however only two studies investigated outcomes related to physical health. Twelve different biomarkers related to five different biological systems (inflammatory, neuroendocrine, neurotransmitter metabolism, neurotrophic and metabolic) were found to have a statistically significant association with clinical outcomes in patients with depressive symptoms, while 24 biomarkers were found to have no association with clinical outcomes studied. Despite extensive research on the biomarkers of etiopathogenesis of depressive symptoms, there is limited published research exploring its translational application in clinical practice. Furthermore, the research is of generally limited quality and lacks clinical utility.

2.5.2 Limitations of the included studies

The included studies have several methodological problems. The study sample size was small and follow-up duration was short in the majority of included studies. In addition, most studies used questionnaire scores that relied on symptom counts for diagnosing depression at baseline, while the gold standard interview technique for depression diagnosis was used by only a minority. There was lot of heterogeneity in the included study in terms of methods used for assessing depression, the follow-up duration and antidepressants offered for study participants. Baseline severity of depressive symptoms assessed using symptom count is associated with higher rate of relapse in patients with depressive symptoms (76), but accounting for the baseline severity of depressive symptoms was only undertaken by a minority of studies. There is a strong evidence base suggesting that depression is two to three time more prevalent in patients with co-existing chronic disease as compared to the general population (77–79), but the effect of co-morbidity on clinical outcomes was examined by

only one study and most studies excluded patients with existing co-morbidities. The information on socio-economic status was either missing or was not accounted for as a confounder in statistical modelling for the majority of the studies.

Importantly, the clinical implications of the observed statistical relationships in the included studies were not well explained. The area under receiver operating characteristic (ROC) curve (80), which is regarded as one of the standard methods for evaluating clinical discriminating power of a biomarker in predicting clinical outcomes, was reported by only one study. The utility of statistical models associating biomarkers with physical outcomes in the included study was not compared against robustly validated and routinely used risk scores, for example the Framingham score for cardiovascular events (81). Finally, some of the biomarkers included in this review are complicated to measure and likely to be expensive, making them impractical for use in routine clinical practice. The source and method of measurement for biomarkers in the included studies were heterogeneous and this may have an influence on assay levels of the biomarkers measured (82–84). The cost implications of doing these tests were not considered in detail in the included studies and this is likely to be a relevant factor when considering their potential use in routine clinical practice.

In summary, there is some evidence that peripheral biomarkers may have a role in stratifying depression severity by means of predicting various physical and mental health outcomes in depression but further and more robust research needs to be done in this area to address the shortcomings of the available evidence.

2.5.3 Outcomes based approach in depression severity stratification

The use of prediction rules and biomarkers to inform clinical decision making is not a novel concept. It has been used in making management decisions in a wide variety of clinical scenarios such as patients presenting with high cholesterol, atrial fibrillation, chest pain, ankle injury and intensive care (85). In psychiatry, this principle has been proposed for predicting inpatient violence (86).

Depression contributes to disease burden not only owing to a reduction in quality of life and functional productivity, but also due to the increased risk of adverse physical outcomes such as hospitalisation and mortality (8). There is strong evidence showing an association of depressive symptoms (MDD and mild depression) with increased risk of adverse physical outcomes such as all-cause mortality, cardiovascular disease, hypertension, stroke, diabetes, Alzeimer's disease, obesity and cancer (69). Physical adverse outcomes associated with depression contribute to a significant amount of morbidity and mortality (8,87). Consequently, it is imperative that the risk of adverse physical outcomes associated with depression should be considered while taking decisions regarding depression severity stratification and subsequent management. Crucially, the clinical utility of biomarkers in predicting physical outcomes in depression, if any, should be compared and validated against some of the established and available risk scoring systems (for example the Framingham score for cardiovascular events (81)) for physical outcomes.

2.5.4 Role of peripheral biomarkers in identifying depression subtypes

The use of peripheral biomarkers in identifying different subtypes of depression has been explored by other studies in the literature. A meta-analysis reviewing the association between HPA axis hyperactivity (Dexamethasone non-suppression) and depression suggested a dose-response relationship, with patients with mild depression showing higher HPA hyperactivity compared to controls but lower than that of patients with MDD (88). Peripheral inflammatory markers such as Tumour necrotic factor (TNF)-α and IL (Interleukin)-6, serum neopterin) have been shown to have an association with melancholic subtypes of MDD (89,90). A review of metabolic and neuroendocrine biomarkers BMI, waist-hip ratio (WHR), fasting glucose, serum adrenocorticotropic hormone (ACTH) in pre-menopausal women with MDD supported their role in identifying three different subtypes of MDD- melancholic, atypical and undifferentiated (91). This suggests that peripheral biomarkers may have a useful role in addressing some of the challenges posed by heterogeneity of depression, with a particular biomarker likely to have a more useful role in a specific subtype of depression.

However, before any decisions are made, much better high quality research is needed.

2.5.5 Novel Biomarkers in Depression

In recent years, novel techniques in proteomics, metabolomics, genetics and epigenetics have led to several new biomarkers being proposed as markers for assessing depressive symptoms depression. Proteomic techniques have been used to identify nine differentiating proteins belonging to lipid metabolism and the immune system from treatment naïve patients with depression, when compared against healthy controls (92). Similarly, metabolomic techniques such as nuclear magnetic response (NMR) based analysis of both urine and plasma have been utilized to identify differentiating proteins related to lipid metabolism and neurotransmitter system with good accuracy in treatment naïve patients with depression, when compared to healthy controls (93,94). Thus, novel techniques may help us identify peripheral biomarkers associated with depressive symptoms, and in turn they may have a role in prognostication of depressive symptoms.

The role of brain-derived neurotrophic gene polymorphisms, glucocorticoid receptor polymorphism and serotonin gene receptor have been studied in the diagnosis and prognostication of depression with some encouraging results(95–97). Although the findings from genome wide association studies (GWAS) to date in depression have failed to make a major breakthrough, they may have a potential role in stratification of depression and further research is ongoing (98,99). Thus, these emerging techniques and biomarkers may have a role in diagnosis, identifying specific subtypes of depression and prognostication in depression (100).

2.5.6 Limitations of review

The review described above has a number of limitations. The search strategy was limited to studies published in the English language due to resource constraints. A variety of other biomarkers such as genetic, imaging and CSF biomarkers may have a role in depression stratification by predicting clinical

outcomes(100). However, this review considered only peripheral or blood-based biomarkers used in current clinical practice due to their comparative non-invasive nature and ease of measurement, as the aim was to identify biomarkers that might be feasible for use in routine clinical practice. The uncertainty surrounding management decisions in patients with depression in current practice is a particular issue at the time of initial presentation(42). Hence, this review was focussed on addressing the issue of the use of peripheral biomarkers at baseline or pre-treatment as a predictive tool of clinical outcome (both mental and physical) and not on assessing changes in a peripheral biomarker level following treatment for depression.

2.5.7 Future research

There is a need for further research in this area, involving large scale studies with longer duration of follow-up, better characterization of patient populations and inclusion of patients with chronic diseases. An 'ideal' scientific process for a biomarker evaluation in clinical risk discrimination has been highlighted in other fields such as cardiovascular disease, and a similar approach can be adopted for biomarkers of depression (101). Further high quality epidemiological studies that minimize potential bias and evaluate clinical utility are urgently needed. Future studies need to incorporate other physical health outcomes such as rate of cardiovascular events, incidence of cancer and all-cause mortality associated with depression and compare validity against established benchmarks, along with mental health outcomes related to depression symptoms. There is some early evidence to suggest that an index comprising multiple biomarkers may exhibit a stronger relationship with depressive symptoms, especially in elderly populations, when compared with examination of individual biomarkers in isolation (102). The role of multiple biomarkers in risk assessment and predicting outcomes in patients with depression needs to be explored and compared against the role of individual biomarkers and is discussed further in Chapter 4.

2.5.8 Conclusion

Pre-treatment levels of 12 different blood based peripheral biomarkers related to five different biological pathways were found to have a statistically

significant relationship with outcomes in patients with depression. Six different outcomes in depression were predicted using these biomarkers, pertaining to both physical and mental health, but the clinical implications remain unclear. It appears likely that peripheral biomarkers may have an important role in helping clinicians to stratify depression severity and to predict clinical outcomes. However, the available evidence has multiple methodological limitations which must be overcome to make any real clinical headway; in particular, interaction between these biomarkers, depressive symptoms and co-morbid physical conditions needs to be explored further. This literature review was revisited prior to thesis submission to ensure the latest findings could be incorporated in this thesis. The following section looks at whether the any further publications have added to the current state of knowledge.

2.6 Literature Search Update

The literature search was updated to include recently published studies. Two electronic databases (Ovid Medline and Embase) for papers published between Jan 2013 and Jan 2016 using the MESH terms "Biological markers" AND "Depression" were searched. The inclusion and exclusion criteria were the same as those described in detail in the methods section, earlier in the chapter. The search results included 186 papers after removing duplicates. Out of these, 143 papers were removed at title screening stage and 29 papers were removed at abstract screening stage. In total, 14 papers were included for full paper screening and only 2 papers met the inclusion criteria. The findings of these 2 studies are discussed below.

The first included study was a secondary data analysis published in the *Journal of Clinical Psychiatry* by Papakostas et al. in 2014 (103). The analysis was based on a sample of 75 patients (originally recruited for a RCT in the US) reported to have SSRI resistant MDD (DSM-IV criteria), with 61 patients completing the follow-up of 60 days. The outcome variable was treatment response to L-Methylfolate 15 mg administered daily, as measured by pooled mean change in HDRS-28 between baseline and at 60 days follow-up. Four biomarker values and 16 genetic marker values at baseline were found to have a statistically significant association with better treatment response to L-Methylfolate at 60 days. The pooled mean change in HDRS was significantly greater in sub-groups of participants with BMI≥30 (vs. BMI<30), hsCRP≥2.25 mg/L sample median value (vs hsCRP level <2.25), S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratio <2.71 (vs. SAM/SAH ratio ≥2.71) and 4-hydroxy-2-nonenal (4-HNE) ≥3.28 ug/ml sample median value (vs. 4-HNE <3.28). SAM/SAH is related to methylation metabolism, while 4-HNE is a metabolite of lipid peroxidation. The main drawbacks of this study were: small sample size, relatively short follow-up and not using complete remission of depressive symptoms as an outcome variable.

The second included study was a meta-analysis published in the *European Neuropsychopharmacology* in 2015 (104). The meta-analysis investigated the

association between baseline inflammatory biomarker values at baseline (IL-6, TNF-alpha, CRP and a composite measure of inflammation) and treatment response in patients with major depression. The authors of this meta-analysis calculated a composite measure of 'inflammation' calculated by applying a statistical formula to a variety of inflammatory biomarker values available in their included studies, based on the consideration that all selected inflammatory biomarkers would measure the same latent construct i.e. 'inflammation'. The details on the statistical formula used for calculating the composite measure of inflammation was not published. Elevated baseline 'inflammation' calculated using a composite measure was not found to have a statistically significant association with treatment response in patients with major depression (number of included studies=13, total number of participants not reported). Similarly, baseline IL-6 values (number of included studies=5, total number of participants not reported), baseline TNF-alpha values (number of included studies=5, total number of participants not reported) and baseline CRP values (number of included studies=4, total number of participants not reported) were not found to have a statistically significant association with treatment response in patients with major depression. The authors concluded that baseline inflammatory markers did not have a significant relationship in predicting treatment response in major depression. However, this meta-analysis had major drawbacks such as: the duration of follow-up in the included studies and the diagnostic criteria or the instrument used for assessing major depression in the included studies were not reported. The original studies included in this meta-analysis were not included in the search strategy employed by the review; this is a limitation of the search strategy employed for the literature review.

Both of these newly included studies did not report information on co-morbidity or socio-economic status of their participants (103,104). Additionally, both these studies did not consider physical clinical outcomes and did not use ROC or cost effectiveness analysis. These limitations are consistent with the limitations observed in the majority of included studies from the original review.

The influence of co-morbid depressive symptoms on prevalence and clinical outcomes in cardiometabolic diseases has been studied extensively, and is discussed in the next chapter- Chapter 3.

Chapter 3 Background Information on Depression in Patients with Cardiometabolic Disease

3.1 Chapter Summary

In this chapter, the following issues are addressed: 1) the reported prevalence estimates for depression in patients with cardiometabolic disease and its associated complications; 2) the current evidence base for assessment methods for depression and for the use of pharmacological and psychological therapies in the management of depression in cardiometabolic disease; and 3) the suggested underlying mechanistic pathways for depression comorbid with cardiometabolic disease.

3.2 Introduction

Depression is up to two to three times more common in patients with cardiometabolic disease as compared to the general population, with prevalence estimates of depression from 15-25% in patients with cardiometabolic diseases such as CHD, diabetes and previous stroke (105–107). Depression, comorbid with these cardiometabolic diseases, has detrimental effects on mortality, clinical outcomes, and functional outcomes such as the ability to carry out activities of daily living (108–111).

The utility of different management approaches have been investigated for patients with depression in cardiometabolic disease. Depression screening has been recommended by some for patients with cardiometabolic disease in view of the high prevalence of depression (112,113); however there is lack of evidence to date that routine depression screening on its own, for patients with cardiometabolic disease, leads to any improvement in depressive symptoms or long term cardiovascular outcomes (114,115). Current evidence shows that the use of antidepressants, psychological therapies and disease management programmes for the management of depression in patients with cardiometabolic disease is associated with short term improvement in depressive symptoms but not associated with improvement in cardiovascular outcomes (116–119).

The exact pathophysiological mechanism explaining the relationship between depression and cardiometabolic diseases remains unclear. Various theories have been hypothesised to explain the association between depression and adverse outcomes in these diseases, for example, increased platelet activation (120), low heart rate variability (121), higher levels of chronic inflammation (122) and insulin resistance (123). This remains an area of ongoing research.

3.3 Prevalence of Depression in Patients with Cardiometabolic Disease and Associated Complications

This section is divided into four parts. The prevalence of depression and associated complications for each of the three cardiometabolic diseases is presented individually in the first three parts; followed by a summary section and a summary table (see Table 3-1) with quality appraisal of evidence using in the final part.

3.3.1 Prevalence of Depression in Patients with Coronary Heart Disease and Associated Complications

Over the last three decades, there have been various studies which have reported an increase in prevalence of depression in patients with CHD (124). The majority of these studies have investigated the prevalence of depressive symptoms in relation to the event of an acute myocardial infarction (MI) (125). Thombs and colleagues published a systematic review of 24 studies and 14,326 patients reporting the prevalence of depressive symptoms hospitalized with acute MI (125). The prevalence estimates varied between 16 to 45% for 8 studies which used a structured clinical interview for diagnosing depression; and 10 to 47% for 17 studies which used a validated questionnaire for diagnosing depression. The weighted prevalence was 20.5% (N=10,785; confidence interval [CI] 19.8% to 21.3%) for studies using a structured interview (125). In addition, this review found that the increase in prevalence of depressive symptoms persisted for up to 12 months following an acute MI. The review noted heterogeneity in study size, timing (in relation to the event of MI) and diagnostic method of depression assessment, and the criteria for symptom duration among the included studies (125). The 'Heart and Soul' study reported a prevalence of 19.6% for depressive symptoms in 1017 patients with stable CHD (105). To summarise, approximately 1 in 5 patients, but possibly as few as 1 in 10 or as many as 1 in 2, with stable CHD or after surviving an acute MI, may suffer from depressive symptoms (105,125).

The increase in risk of mortality and future cardiac events with depression in patients with CHD has been widely reported. Some of the early work in this area was done by Frasure-Smith and colleagues in the early 1990s (126). They followed a group of 222 patients following an acute MI to report that patients who developed depressive symptoms after MI were more likely to die at 6 months of follow-up. There have been several published meta-analyses since the turn of the century that have reported an increase in rate of cardiac events and all cause deaths in patients with depressive symptoms after MI or with CHD (110,127,128). Barth and colleagues reported in a meta-analysis of 20 studies that the risk of death in patients with depressive symptoms and CHD was approximately 1.5 to 3.5 times higher than the risk of death in patients with CHD and no depressive symptoms (Odds Ratio [OR], 2.24; 95% CI 1.37–3.60) (127). In the largest meta-analysis to date based on 16,889 MI patients, Meijer and colleagues report that post-MI depression within 2 years of MI was associated with an increased risk of all-cause mortality (OR, 2.25; 95% CI 1.73–2.93), cardiac mortality (OR, 2.71; 95% CI 1.68–4.36) and further cardiac events (OR, 1.59; 95% CI 1.37–1.85) (110). The same group of authors (Meijer and colleagues) performed another meta-analysis in 2013 which involved time to event survival analysis on 10,175 MI patients from 16 studies. They reported a Hazard Ratio (HR) of 1.32 (95% CI 1.26-1.38) for all-cause mortality and HR of 1.19 (95% CI 1.14-1.24) for cardiovascular events in post MI patients with depression as compared to those without depression (129). Similarly, in the 'Heart and Soul' study of 1017 patients with stable CHD, after adjustment for comorbid conditions and disease severity, depressive symptoms were associated with a 31% higher rate of cardiovascular events (HR 1.31; 95% CI 1.00-1.71) at the end of 4 years (105). In addition to that, depression has been also reported as an important contributory factor in functional impairment in patients with CHD (130,131). There is substantial evidence to suggest that patients with CHD and depressive symptoms are more likely to have poor clinical outcomes, which has led to the American Heart Association identifying depression as a risk factor for poor prognosis in patients who have experienced an acute coronary syndrome (ACS) (109).

The influence of timing of onset of depressive symptoms, in relation to the onset of a cardiac event, and in terms of first ever versus recurrent depressive

episode, on the risk of cardiovascular events has also been studied. A systematic review published in 2011 found that only six studies had investigated these questions (132). The review concluded that there is some evidence to suggest that ACS patients with first and new onset depression are at higher risk of worse prognosis, but that the existing evidence was inconsistent and there were some methodological limitations in the included studies (for e.g. small number of patients with follow-up data in some of the included studies) (132).

3.3.2 Prevalence of Depression in Patients with Diabetes and Associated Complications

The prevalence of depression in patients with diabetes has been extensively studied over the last 30 years. An early meta-analysis of 42 studies published in 2001 by Lustman and colleagues reported that the odds of depression in the diabetic group were twice that of the non-diabetic comparison group (OR 2.0, 95% CI 1.8-2.2) (133). This review did not differentiate between patients with Type 1 and Type 2 diabetes. In the past few years, research has been carried out to differentiate the prevalence estimates of depression between the two types of diabetes. A systematic review published in 2006 reported depression prevalence of 12% for Type 1 diabetes as compared to 3.2% for control subjects (134). The review did not report any odds ratio for comparison, which is a major limitation (134). Another review published in the same year for Type 2 diabetes reported a prevalence estimate of 17.6% vs. 9.8% in the control group (OR 1.6, 95% CI 1.2-2.0) (107). More recently, a review conducted by Roy and his colleagues in 2012 reported a prevalence rate of 12% (range 5.8-43.3%) for Type 1 vs. 3.2% (range 2.7-11.4%) in the control group; and 19.1% (range 6.5-33%) for Type 2 diabetes vs. 10.7% (range 3.8-19.4%) in the control group (135). Again, this systematic review did not report a pooled odds ratio of included studies for comparison of prevalence rates of depression between patients with diabetes and the control group (135), which is a major limitation. Thus, there is evidence from multiple systematic reviews to suggest that the prevalence of depressive symptoms in patients with diabetes, (both types 1 and 2) is two to three times more as compared to those without diabetes (107,133–135).

Several studies have investigated the association of depression co-morbid with diabetes and its potential impact on complications related to diabetes. A meta-analysis done by Lustman and his colleagues of 24 studies of patients with types 1 and 2 diabetes showed that depression was associated with hyperglycaemia in diabetes patients (136). The review found that the effect size of this association was similar for type 1 and 2 diabetes but stronger when depression was diagnosed with a diagnostic interview as compared to a self-reported depression symptoms questionnaire (136). The same group of authors reported an association between co-morbid depression with diabetes and a variety of diabetes complications such as diabetic retinopathy, nephropathy, neuropathy, macrovascular complications, and sexual dysfunction (137). These findings were based on a meta-analysis of 27 studies with more than 5000 patients with diabetes and reported a consistent and moderate effect size of association (overall effect size Pearson correlation co-efficient $r = 0.25$, 95% CI 0.22-0.28) for both types of diabetes (137). Additionally, there is evidence from a review of 47 studies to suggest that depression comorbid with diabetes is associated with increased risk of treatment non-adherence and lack of self-care (Pearson co-efficient $r = 0.21$, 95% CI 0.17-0.25) (138). A large cohort of more than 10,000 patients in the USA with diabetes was followed up for a period of 8 years in another study; and they reported a higher risk of all-cause mortality and CHD related deaths in patients with depression and diabetes as compared to those with diabetes alone (139). A meta-analysis of 42,363 patients with diabetes from 10 studies reported a higher risk of mortality (pooled HR=1.50, 95% CI 1.35-1.66) with co-morbid depressive symptoms (111). Thus, there is consistent evidence to show that depression comorbid with diabetes is associated with increased risk of poor glycaemic control, treatment non-adherence and diabetes related complications and mortality.

3.3.3 Prevalence of Post Stroke Depression and Associated Complications

Depressive disorders are very common among patients who have survived an episode of stroke. Some of the early work in this area was done by Robinson and colleagues, who proposed three distinct types of mood disorders in stroke survivors in 1986 (140). The first type of patients were the ones who met the

criteria for a Major Depressive Disorder, the second group of patients reported symptoms consistent with dysthymia (a mild but chronic form of depression) while the third group of patients presented with a sense of indifference and apathy in their mood (140). The early reported prevalence rates for post stroke depression ranged from 23% to 79%, depending on the clinical setting and instrument chosen to assess depressive symptoms (141). A meta-analysis published in 2005 reported a pooled estimate of 33% (95% CI, 29 to 36%) for depression prevalence in patients with previous stroke (142). These findings were based on a meta-analysis of 51 studies involving patients with stroke in hospital, rehabilitation units and the community (142). A review published in 2010 reported an overall prevalence rate of 21.7% (range 6% to 40%) for post stroke major depression and 19.5% (range 8% to 44%) for post stroke minor depression (143). A multi-national study of 220 stroke patients observed that the prevalence of depression remained as high as 33% for up to a period of 5 years after an episode of stroke (144). The chronic nature of depressive symptoms could be due to realization among stroke survivors that their disability is unlikely to recover. In summary, one in three patients is at risk of developing depressive symptoms for up to a period of 5 years after an acute stroke.

Post stroke depression has been associated with various adverse clinical outcomes. A review assessing post stroke mortality reported increased odds (OR 1.22, 95% CI 1.02 to 1.47) for a period of 2 to 5 years among patients with depressive symptoms based on findings from 13 studies including 59,598 patients with stroke (108). The reported confidence intervals for the pooled effect size are considerably wider and only marginally statistically significant as the lower confidence interval is very close to 1.0. The review also concluded that the risk of mortality after stroke was not significantly different among depressed individuals in the long term follow-up (>5 years) when compared to those with stroke and without depression, however the studies with long term follow-up had small sample size (108). A multi-centre study in China with 1 year follow-up results for 1713 patients with stroke observed a 49% (OR = 1.49, 95%CI: 1.03–2.15) higher odds of recurrent stroke in patients with post stroke depression as compared to patients without it (145). A prospective study in Spain found an association between post stroke depression and cognitive decline at 2 years of follow-up (146). A review reported an association between post stroke

depression and poor functional outcomes and recovery based on 26 included studies; however the review did not report any overall effect sizes and there was a lot of heterogeneity among included studies (106). Thus, there is some evidence that prevalence of depressive symptoms is associated with a higher risk of functional decline and mortality in stroke survivors.

3.3.4 Summary

In this section, a summary of some of the key systematic reviews or meta-analyses for prevalence and associated complications of depressive symptoms in CHD, diabetes and stroke is presented in a table (see Table 3-1). The search strategy was comprehensive for all of these studies and they used multiple databases. Among the reviews on prevalence rates, the majority of the included studies on post MI (125) and post stroke (143) depression were cohort studies. Hence, it was not possible to compare depression prevalence in the study population with a control group, as reported in the review by Roy and colleagues for patients with diabetes (135). Only one review (out of six reviews) used statistical measures for investigating heterogeneity and publication bias. The meta-analysis by Park and colleagues on patients with diabetes found that there was little evidence of heterogeneity (Cochran Q=13.52, p-value=0.20) and no significant publication bias (Egger's regression intercept=0.98, p-value=0.23) (111). The meta-analysis by Bartoli and colleagues on patients with stroke did not report if they had done any adjustments for the effects of potential confounders on their results (108). Overall, these studies used appropriate methodology and their results were based on a large number of primary studies involving large samples of patients. The AMSTAR tool (A Measurement Tool to Assess Systematic Reviews) was used for quality appraisal (147). The AMSTAR is a validated tool which gives a score out of 11, based on 11 pre-defined criteria to assess methodological quality of systematic reviews (http://amstar.ca/Amstar_Checklist.php). The total AMSTAR score for each systematic review is presented in the table while the details on each individual item of the scoring are presented in the appendix (Appendix 6).

Table 3-1 Overview of key studies on prevalence of depression and associated complications in patients with cardiometabolic disease

Lead author; year of Publication	Journal	Subject	Study design	Number of included studies	Patients included in the analysis	Key Findings	AMSTAR score (out of 11)
Thombs et al; 2006	Journal of General Internal Medicine	Prevalence of depression post MI	Systematic Review	24	N=14,326	1. Prevalence estimate varied with different diagnostic techniques for depression. 2. N=8 studies using a diagnostic interview method: prevalence ranged from 16 to 45%; weighted prevalence was 19.8% (CI=19.1% to 20.6%) 3. N=17 studies using a validated questionnaire: prevalence ranged from 10 to 47%; weighted prevalence varied with different questionnaires.	8/11
Roy et al; 2012	Journal of Affective Disorder	Prevalence of depression in diabetes against control	Systematic Review	21	N=170,571	1. Prevalence of depression in Type 1 Diabetes= 12% (range 5.8%-43.3%) against control= 3.2% (range 2.7%-11.4%) 2. Prevalence of depression in Type 2 Diabetes= 19.1% (range 6.5%-33%) against control=10.7% (range 3.8%-19.4%)	7/11
Robinson et al;	Canadian Journal of Psychiatr	Prevalence of depression	Systematic Revie	43	N=7,068	1. Prevalence rate of 21.7% (range 6% to 40%) for post stroke major depression 2. Prevalence rate of 19.5% (range 8% to 44%)for post stroke minor depression	5/11

2010	y	post stroke	w			
Meijer et al; 2013	The British Journal of Psychiatry	Depression after MI and association with outcomes	Meta-analysis	16	N=10,175	1. Hazard Ratio for depression for all-cause mortality in MI patients=1.32 (95% CI=1.21-1.38). Adjusted for: diabetes, smoking, BMI, LV function, age, sex. 2. Hazard Ratio for depression for cardiovascular events in MI patients=1.19 (95% CI 1.14-1.24). Adjusted for: same as above	10/11
Park et al; 2013	General Hospital Psychiatry	Depression co-morbid with diabetes and outcomes	Meta-analysis	10	N=42,363	1. Hazard Ratio for depression for all-cause mortality in patients with diabetes= 1.50 (95% CI 1.35-1.66). Adjusted for age, sex, smoking, physical activity and Charlson comorbidity score.	10/11
Bartoli et al; 2013	Stroke Research and Treatment	Depression after stroke and outcomes	Meta-analysis	13	N=59,598	1. Odds Ratio for depression for all-cause mortality in patients post stroke=1.22 (95% CI 1.02-1.47). Adjusting confounders not stated. 2. Hazard Ratio analysis performed only in 4 studies with N=2075 patients. 3. Hazard Ratio for depression for all-cause mortality in patients post stroke=1.50 (95% CI 1.02-2.26). Adjusting confounders not stated.	9/11
Legend: MI=Myocardial Infarction; CI=Confidence Intervals; LV=Left Ventricle; BMI=body mass index ; AMSTAR= A Measurement Tool to Assess Systematic Reviews (see Appendix 6 for details of the performance of each review on individual items on the score)						

3.4 Assessment and Management of Depression in Patients with Cardiometabolic Disease

This section is divided into two parts. The first part provides an overview on suggested benefits of screening, if any, and discusses various assessment methods of depressive symptoms in patients with cardiometabolic disease; while the second part provides an overview of different management approaches for depression and their effectiveness.

3.4.1 Assessment of Depression in Patients with Cardiometabolic Disease

The role of depression screening in patients with cardiometabolic disease has been controversial. Due to the high prevalence of depression in patients with CHD and its associated complications, the American Heart Association (AHA) recommended depression screening for patients with CHD in their guidelines published in 2008 (112). However, two systematic reviews published subsequently have failed to show any benefit of depression screening in terms of improving outcomes in patients with cardiometabolic disease (114,115). In spite of extensive search strategies, these two systematic reviews did not find a single trial evaluating the efficacy of depression screening as a standalone intervention, in patients with CHD, stroke or diabetes (114,115). Most of the evidence in this area has come from trials evaluating the benefits of depression screening as a part of a wider intervention, which also involved management of depressive symptoms (114,115). These studies have not found any evidence of improvements in mortality or cardiovascular outcomes with comprehensive interventions involving depression screening and its management in patients with cardiometabolic disease (114,115).

In the UK, NICE (National Institute for Health and Care Excellence) recommends that depression screening or 'case finding' in patients with chronic disease should only be targeted towards those who are believed to be 'high risk' (148). The UK Quality and Outcomes Framework (QOF) offered financial incentives to primary care practitioners for routine depression screening for all patients with

CHD and diabetes, between 2006 and 2013 (149). These financial incentives have been withdrawn from the QOF programme since 2013/14 (150). In the UK, NICE has recommended using a short 2 question item scale such as PHQ-2 as a depression screening instrument for 'high risk' patients with chronic disease followed by a detailed depression assessment if the short item scale is found to be positive (148). However, the definition of 'high risk' patients has not been clearly stated in this guideline (148). Similarly in the USA, a working group from the National Heart, Lung and Blood Institute (NHLBI) has recommended a two-step algorithm using PHQ-2 for depression screening in CHD, followed by PHQ-9 for positive cases (151). However, the potential role of universal depression screening for patients with CHD, as a standalone intervention without any specific depression management interventions, in improving cardiovascular outcomes remains unclear.

A number of self-reported depression symptom scores have been used as screening tools in patients with CHD. A systematic review found 13 studies on diagnostic accuracy of depression symptom scores compared against one of the gold standard methods (such as diagnostic interview) for diagnosing depression (114). Different screening instruments assessed in this review were the Hospital Anxiety and Depression Scale-Depressive subscale (HADS-D) (152), PHQ-9 (Patient Health Questionnaire-9) (153), PHQ-2 (Patient Health Quesionnaire-2) (154), Beck Depression Inventory (BDI) (155), Beck Depression Inventory-II (BDI-II) (156), the Centre for Epidemiological Study Depression Scale (CES-D) (157), Geriatric Depression Scale (GDS) (158) and GDS-15 (159). The review did not find any one particular symptom score performing convincingly better over the others, and reported a lack of consistency in results on accuracy from multiple sites.

A variety of symptom scores have been used for diagnosing and screening for depression in patients with diabetes. A systematic review found only 16 studies (7%) evaluating diagnostic accuracy of depression symptom scores out of 235 studies of patients with diabetes (160). BDI- I & II (155,156) and CES-D (157) were by far the two most widely used symptom scores for studying depression in patients with diabetes (160). The review concluded that most of the depression screening tools with available validity results (BDI I and II, CES-D, HADS-D, PHQ-9, Zung's self-rating depression scale (SDS) (161) and World Health Organization

(WHO)-5 (162)) had very high negative predictive value but low positive predictive value with varying prevalence rates (160). The review also did not recommend any one particular depression symptom score as having clear superiority over others for use in diabetes patients (160).

The use of various depression symptom scores has been assessed in detecting post stroke depression. The Post Stroke Depression Rating Scale (PSDS) was developed specifically for assessing depressive symptoms in stroke patients (163), however it has not been widely used compared to some of the aforementioned instruments. The Stroke Aphasic Depression Questionnaire (SADQ) has been developed for assessing depressive symptoms in stroke patients with aphasia and found to have good reliability and validity (164). Similar to CHD and diabetes patients, a review on diagnostic accuracy of depression symptom scores for post stroke depression did not find any supremacy of one particular symptom score. The review concluded that CES-D, HDRS and PHQ-9 were the "most promising options" (165).

In summary, various depression symptom scores have been used for measuring depression in patients with cardiometabolic diseases. There is no single depression symptom score which has significantly better diagnostic accuracy. An overview of the three systematic reviews in this area is presented in Table 3-2. Again, the AMSTAR tool was used for quality appraisal giving a score out of 11, based on 11 pre-defined criteria to assess methodological quality of systematic reviews (147). The total AMSTAR score for each systematic review is presented in the table which shows that the reviews were of high quality, while the details on each individual item of the scoring are presented in the appendix (Appendix 6).

Another important issue is the ease of use of such symptom scores in routine clinical practice. Barriers to utilisation in primary care include: time constraints and GP's perceived doubts about their value and effectiveness which have been discussed in Chapter 2 (40) (23,41). These practical problems may affect use of depression symptom scores for assessing depressive symptoms in patients with cardiometabolic disease in primary care.

Table 3-2 Overview of studies evaluating diagnostic accuracy of depression symptom scores in patients with cardiometabolic disease

Lead author; year of Publication	Journal	Subject	Study design	Number of included studies	Number of patients included in analysis	Key Findings	AMSTAR score (out of 11)
Thombs et al; 2013	Plos One	Accuracy (sensitivity and specificity) of various depression symptom scores in measuring depression for post MI and CHD patients	Systematic Review	N=15	N-5917	1. Best reported sensitivity: BDI and GDS (100% each) 2. Best reported specificity: PHQ-9	10/11
Roy et al; 2012	Diabetic Medicine	Accuracy (sensitivity and specificity) of various depression symptom scores in measuring depression in patients with diabetes	Systematic Review	N=16	N=8297	1. Best reported sensitivity: Zung's rating scale and WHO-5 (100% each) 2. Best reported specificity: GDS (92.3%)	9/11
Meader et al	Journal of Neurology,	Accuracy (sensitivity and specificity) of various	Meta-analys	N=24	N=2097	1. Best reported sensitivity: BDI (86%) and PHQ-9 (86%) 2. Best reported specificity: CES-	9/11

al; 2014	Neurosurgery & Psychiatry	depression symptom scores in measuring post stroke depression	is			D (88%)	
Legend: BDI=Beck's Depression Inventory; GDS=Geriatric Depression Scale; PHQ=Patient Health Questionnaire; WHO=World Health Organization; CES-D: Centre for Epidemiological Studies Depression Scale. AMSTAR= A Measurement Tool to Assess Systematic Reviews (see Appendix 6 for details of the performance of each review on individual items on the score)							

3.4.2 Management of depression in patients with cardiometabolic disease

Current management approaches for depression in cardiometabolic disease can be divided into three categories: psychological therapies; antidepressants; and use of disease management programmes (for e.g. collaborative care) which usually combines the preceding two. The following section provides a summary of the evidence and an overview of some of the key meta-analyses published in this area in a table (see Table 3-3) at the end of this section.

3.4.2.1 CHD Patients

There have been two systematic reviews and meta-analyses on different interventions for management of depression co-morbid with CHD. A Cochrane review on patients with depression with CHD concluded that psychological interventions may have a small but clinically meaningful benefit in short term improvement of depressive symptoms (117). The review found that seven trials investigated the role of psychological therapies in treatment of post CHD depression, but only one trial looked at long term follow-up (>6 months) and remission rates (166). Only one trial (ENRICHD) reported on the rate of cardiac events and deaths in patients with post CHD depression and found no difference between the cognitive behavioural therapy (CBT) group and the usual care group (167). This trial did not find any beneficial effects on mortality rates, or rates of cardiac events and cardiovascular hospitalizations. Overall, results of this review provide some evidence of a small beneficial effect of psychological interventions compared to usual care on depression symptoms and remission rate in the short term, but no evidence of a beneficial effect on cardiac-related mortality or morbidity.

The Cochrane review also analysed the role of pharmacological interventions in the treatment of depression in patients with CHD and found eight trials with 1098 patients in total (117). Baumeister and colleagues conducted a meta-analysis based on the three trials and 707 patients using selective serotonin reuptake inhibitors (SSRI) (117). The meta-analysis showed improvement in depressive symptoms, remission and hospitalization rates in the short term (less

than 6 months follow-up) favouring antidepressants against placebo (168–170). There were no studies investigating the long term benefits on remission rates or improvement of depressive symptoms. The review also found five other trials investigating the role of pharmacological therapies in the short term but concluded that these trials did not report enough information to allow calculation of effect sizes (117). The review concluded that there was not enough evidence to support any beneficial effect of pharmacological interventions on reducing all-cause mortality or future cardiovascular events (117).

A systematic review by Tully et al. published in 2015 concluded that collaborative care (an intervention consisting of a multi-disciplinary disease management programme which includes a structured and regular patient follow-up) led to improvements in depressive symptoms but no improvements in cardiovascular events (171). This review performed a literature search until April 2014 and found 6 RCTs (total n=1284, 655 patients in intervention group) (171). The review reported that collaborative depression care led to a significant reduction in MACE in the short term (RR 0.54; CI 0.31 to 0.95), however the difference was not sustained in the longer term. Subsequent to the publication of this review, Coventry and colleagues published the results of their RCT of collaborative care interventions involving just under 200 patients with diabetes and cardiovascular disease (172). This RCT showed that the patients in the intervention arm had improvements in depressive and anxiety symptoms at 12 months but no improvement in quality of life or disease specific indicators (such as angina symptom score; they did not study cardiovascular outcomes (172).

3.4.2.2 Diabetes Patients

A Cochrane review by Baumeister and colleagues reviewed the role of psychological interventions in the treatment of depression in patients with diabetes in 2012 (116). The meta-analysis showed that treatment of depressive symptoms with psychological interventions (eight trials; 1122 patients) was associated with an improvement in depressive symptoms and remission rates in the short to medium term (less than 6 months) for patients with diabetes (116). The review found that long term effects (more than 6 months) of psychosocial interventions were investigated by only one study. A RCT of 361 patients in the

Netherlands showed that a self-management psychological intervention was associated with improvements in depression symptoms at 9 months of follow-up in diabetes patients (173). The Cochrane review concluded that the evidence on improvements in glycaemic control, medication adherence, quality of life and health care related costs was inconclusive for supporting the use of psychological interventions in the treatment of depression in diabetes patients. Moreover, the review did not find any studies which investigated the benefits of psychological interventions on reducing the rate of diabetes related complications or mortality. In summary, there is evidence to suggest benefits in improving depression related outcomes in the short to medium term but lack of data on improvements in diabetes related or other health outcomes.

The Cochrane review also summarised the role of pharmacological interventions (eight trials; 377 patients) in the treatment of depression for diabetes patients and found very similar results (116). Pharmacological interventions were shown to improve depression symptoms and remission rates in the short to medium term (less than 6 months) in patients with diabetes, but there were no studies investigating long term outcomes (116). Contrary to psychological interventions, treatment with antidepressants was associated with improvements in glycaemic control in the short term (116). The review did not find conclusive evidence on benefits in health related quality of life, medication adherence or health care related costs in diabetes patients (116). Again, there were no trials looking at benefits on diabetes related complications or mortality rates (116).

The authors concluded that both pharmacological and psychological interventions were associated with improvements in depression symptoms and remission rates. However, outcomes such as medication adherence, health related costs and quality of life, diabetes related complications and mortality rates have not been sufficiently studied (116).

A meta-analysis published in 2014 found seven RCTs (with 1895 patients) examining the benefits of collaborative care on patients with diabetes and co-morbid depression (174). The review found that collaborative care led to improvements in depressive symptoms and HbA1c levels at medium to long term follow-up (12 to 52 weeks); the benefits on cardiovascular events were not investigated in the review (174).

3.4.2.3 Stroke Patients

Due to the very high prevalence of depressive symptoms in stroke patients, researchers have tested the benefits of using psychological interventions in prevention as well as treatment of depressive symptoms. In 2008, Hackett and colleagues published two Cochrane reviews that assessed the benefits of psychological interventions in both prevention (175) and treatment of depressive symptoms (176), for patients with stroke. The review for prevention of depressive symptoms found four trials using different psychological interventions involving 902 stroke patients and concluded that these interventions had a small but significant effect in preventing depressive symptoms in stroke patients (175). However, the review for treatment of depressive symptoms using psychological interventions found no benefit in improvement of depressive symptoms, on the basis of three trials and 445 stroke patients (175). Neither of these reviews identified any evidence of benefits of psychological interventions in stroke recovery or improving functional outcome (175,176).

The two Cochrane systematic reviews described above also investigated the benefits of using pharmacological interventions in the prevention and treatment of depressive symptoms for stroke survivors (175,176). The Cochrane review on pharmacological interventions in prevention of depressive symptoms did not find any significant benefit in preventing depressive symptoms in the intervention arm based on results from 11 studies involving 591 patients (175). The review on treatment found 13 trials using 12 different antidepressants and involved 1121 stroke patients (176). The pooled effect for the odds of remission of depressive symptoms at the end of 12 to 26 weeks of follow-up was lower in the control group as compared to the intervention group: OR of 0.47 (95% CI 0.22 to 0.98) (176). Similar to the results with psychological interventions, pharmacological interventions were not shown to have any evidence of beneficial effects in activities of daily living or cognitive function in stroke patients in either review (175,176). Another Cochrane review investigated the benefits of SSRI for various different outcomes in patients with post stroke depression, and included 52 trials with 4059 participants (177). The meta-analysis found evidence of improvements in depressive symptoms (Standardized mean difference SMD= -1.91 (95% CI -2.34 to -1.48) and neurological deficit (SMD= -1.00 (95% CI -1.26 to -0.75) but no evidence of improvement in survival in the intervention group who

received SSRI; and there was high heterogeneity reported among included trials (177). There are some emerging concerns about using antidepressants in stroke survivors. A cohort study of 16770 stroke survivors in Taiwan reported an increase risk of stroke recurrence over 10 years associated with use of antidepressants (HR 1.42; 95% CI 1.24-1.62) (178).

A trial in the USA involving 92 patients with previous stroke found an improvement in depressive symptoms at 12 months of follow-up in the intervention group, who had an 8 week intervention involving a combination of antidepressants and a brief behavioural intervention (OR for remission in intervention group= 2.7 (95% CI 1.1 to 6.6) (179). Another approach for management of post stroke depressive symptoms could be exercise therapy. A meta-analysis of 13 studies and 1022 patients with post stroke depression found a reduction in depressive symptoms at short term follow-up (<6 months) with exercise therapy (SMD = -0.13 (95% CI -0.26 to -0.01) (180). However, the improvement in depressive symptoms was not sustained in the long (12 months or more) (180).

3.4.2.4 Summary

In patients with CHD, there is evidence that psychological, pharmacological and collaborative care interventions can have benefits in the short and medium term (up to 6 months), in terms of reduction in depressive symptoms. There is no evidence to show long term improvements in depressive symptoms or reductions in adverse cardiovascular outcomes. In patients with diabetes, there is evidence of benefit, in terms of reducing depressive symptoms and improving HbA1c control with pharmacotherapy and collaborative care but no benefits on reducing cardiovascular outcomes. In patients with stroke, pharmacotherapy has proven beneficial in terms of improving depressive symptoms and neurological deficits, but there is no evidence of positive effects on survival. Exercise therapy has also been associated with improvement in post stroke depressive symptoms in the short term but not on health care outcomes. Overall, there is lack of evidence on improvements in quality of life or physical health outcomes with any of the interventions across the three disease groups. Please see Table 3-3 for an overview of key studies. Again, the AMSTAR tool was used for quality appraisal

giving a score out of 11, while the details on each individual item of the scoring are presented in the appendix (Appendix 6) (147).

Table 3-3 Overview of studies evaluating different management strategies for depressive symptoms in patients with Cardiometabolic Disease

Lead author; year	Journal	Subject	Number of included studies	Number of patients included in analysis	Key Findings	AMSTAR Score (out of 11)
Baumeister; 2011	Cochrane	Psychological and pharmacological interventions in CHD with depression	Psychological interventions n=7 Pharmacological interventions n=8	n=2858 (psychological) N=1098 (pharmacological)	1. Psychological interventions: SMD improvement at short term (12 weeks): -0.81 (95% CI-1.26 to -0.36) ; SMD improvement at medium term (6 months): -0.19 (95% CI -0.28 to -0.10) 2. Pharmacological interventions: SMD improvement at short term (12 weeks): -0.24 (95% CI -0.38 to -0.09); Odds of remission (12 weeks): OR 1.93 (95% CI 1.14 to 3.25 3. Lack of evidence on long term outcomes, improvement in cardiovascular outcomes	11/11
Tully; 2015	BMJ Open	Collaborative Care in CHD with depression	n=6	n=1284	1. SMD improvement at 12 weeks: -0.31 (95% CI -0.43 to -0.19) 2. No sustained reduction in cardiovascular outcomes	11/11
Baumeister; 2012	Cochrane	Psychological and pharmacological interventions in Diabetes with depression	Psychological interventions n=8 Pharmacological interventions	n=1122 (psychological) n=377 (pharmacological)	1. Psychological interventions: SMD improvement at medium term (6 months): -0.42(95% CI -0.70 to -0.14); Odds of remission at medium term (6 months): OR 2.49 (95% 1.44 to 4.32); no improvements in HbA1c 2. Pharmacological interventions: SMD improvement at short term (12 weeks): -0.61 (95%CI -0.94 to -0.27); Odds of	11/11

			n=8		remission at short term (12 weeks): OR 2.50 (95% CI 1.21 to 5.15); HbA1c improvement at short term (12 weeks): weighted mean difference -0.4% (95% CI -0.6 to -0.1) 3. Lack of evidence on improvement in quality of life, medication adherence and cardiovascular outcomes	
Atlantis; 2014	BMJ Open	Collaborative Care in Depression with diabetes	n=7	n=1895	1. SMD improvement at 12 to 52 weeks: -0.32 (95% CI -0.53 to -0.11) 2. HbA1c improvement at 12 to 52 weeks: weighted mean difference -0.33% (95% CI -0.66 to -0.0)	8/11
Mead; 2012	Cochrane	SSRI for Stroke Recovery	n=52	n=4059	1. SMD improvements in depressive symptoms: -1.91 (95% CI -2.34 to -1.48) 2. SMD improvement in neurological deficit: -1.00 (95% CI -1.26 to -0.75) 3. No evidence of improvement in survival 4. There was with high heterogeneity reported among included trials	10/11
Eng; 2014	Clinical Rehabilitation	Exercise therapy for post stroke depression	n=13	n=1022	1. SMD improvement in depressive symptoms at <6 months: = -0.13 (95% CI -0.26 to -0.01) 2. No improvement in depressive symptoms at longer follow-up	10/11

Cochrane= Cochrane Database of Systematic Reviews; SMD= Standardized mean difference; CI= Confidence intervals; SSRI=Selective Serotonin Reuptake Inhibitors; AMSTAR= A Measurement Tool to Assess Systematic Reviews (see Appendix 6 for details of the performance of each review on individual items on the score)

3.5 Proposed Underlying Mechanisms for Depression Co-morbid with Cardiometabolic Disease

3.5.1 Depression and CHD

There is substantial evidence from various epidemiological studies that presence of depression is an independent risk factor in development of CHD (181,182), and it has been proposed that the relationship between depression and CHD is bidirectional in nature (183). Many researchers have tried to explain why depression is associated with worse outcomes among patients with CHD, and different mechanistic models have been hypothesized (25,184). The mechanistic model which has been most studied is that the adverse effects of depression on cardiovascular outcomes in patients with CHD are mediated through a combination of different biological and behavioural factors. The leading candidates for common causal biological factors are increased platelet activation (120), low heart rate variability (121) and higher levels of chronic inflammation (122). The common behavioural factors which may contribute to adverse outcomes in CHD patients with depression are increased smoking, reduced physical activity and reduced adherence to medications (105,185). The alternative explanation proposed is that those with the most severe CHD are more likely to get depressive symptoms and subsequently more likely to have worst outcomes, hence depression is an epiphenomenon or a non-causal variable risk factor in patients with CHD (186,187). Finally, common genetic factors have been hypothesized as a mechanism to explain the relationship between depression and CHD but the evidence remains inconclusive (188).

3.5.2 Depression and Diabetes

Several possible pathophysiological mechanisms have been proposed to explain the relationship between depression and diabetes (189). Mcintyre and colleagues reported that depression and type 2 diabetes share disturbances in metabolic networks such as glucose-insulin homeostasis, immune-inflammatory processes and glucocorticoid signalling and hence labelled depression as a "neuropsychiatric syndrome" or "metabolic syndrome type II" (190). Based on evidence from animal studies, Wang and colleagues proposed a model that

suggests diabetes impairs hippocampal function via advanced glycation, which in turn impairs hippocampal neurogenesis and contributes to diabetes related depression (191). Another study suggested a lack of brain derived neurotropic factor in the brain as a common pathological factor in depression comorbid with diabetes (192). However, equally, as mentioned in relation to CHD in the preceding section it has been suggested that depression may simply be associated with poor self-care and treatment adherence in diabetic patients; which in turn might explain the relationship between depression and increased risk of diabetes related complications (138).

3.5.3 Depression and Stroke

Researchers studying the relationship between depression and stroke have suggested that biological and psychosocial factors are contributory to this relationship (193). The "vascular depression" hypothesis suggests that post stroke depression was caused by silent cerebral infarcts (194). In addition to that, brain imaging studies have shown a possible role of ischaemic lesions of striato-frontal circuits in post stroke depression (195). On the other hand, the positive association between the degree of disability in stroke and the degree of depressive symptoms have supported the idea that psychosocial factors link depression and stroke (195,196). A meta-analysis of four studies and 260 stroke patients identified 5-HTTLPR genotype as a risk factor for the development of post stroke depression (197). There is a lack of evidence to support the superiority of one of these mechanisms over the other and the most likely explanation is that, for most stroke patients experiencing depressive symptoms, there is overlap and interplay across various biological, psychosocial and genetic factors (193,198).

3.5.4 Summary

It is most likely that a combination of biological, behavioural and genetic factors may contribute towards the observed relationship between depression and cardiometabolic disease. The available evidence currently appears inconclusive for a definitive explanation. The list of potential mechanisms discussed above is not exhaustive and it is not the focus of research in this thesis. Importantly,

there has not been significant translational application of the evidence from mechanistic studies in clinical practice. Certain biomarkers of some of the common biological pathways may have a role to play in assessing and managing depressive symptoms in patients with cardiometabolic disease. For example, two studies included in the review in Chapter 2 found that plasma cortisol and highly sensitive CRP levels at baseline were predictors of which patients with depressive symptoms were likely to experience cardiovascular outcomes (65,67). Unfortunately, these studies specifically excluded patients with existing cardiometabolic disease. There is a need to evaluate the benefits of using peripheral biomarkers in assessing and managing depressive symptoms in patients with cardiometabolic disease.

3.6 Conclusion

Depression is a common co-morbid condition in patients with cardiometabolic diseases such as CHD, diabetes and stroke, with higher prevalence rates than the general population. Depression co-morbid with these diseases is associated with increased risk of cardiovascular complications in CHD patients, diabetes related complications in diabetes patients, reduced functional recovery in stroke patients and increased mortality in all three conditions. There is some evidence to show that treatment with psychological and pharmacological interventions leads to short term improvement in depressive symptoms in patients with CHD, diabetes and stroke. However, there is no strong evidence of these interventions reducing cardiovascular complications, diabetes related complications, functional recovery and mortality. Various bio psychosocial and genetic theories have been hypothesized to explain the relationship between depression and cardiometabolic diseases and most likely multiple mechanisms are responsible with considerable overlap; the available evidence is inconclusive and there is lack of evidence about its potential clinical applicability.

In this thesis, the underlying hypothesis is that patients with cardiometabolic disease and co-morbid depression with abnormal values of certain cardiovascular biomarkers may be at higher risk of adverse cardiovascular outcomes as compared to those with normal cardiovascular biomarker values. This hypothesis is explored in the DepChron dataset, where all patients had existing cardiometabolic disease. In the next chapter, the allostatic load theory is discussed in detail and the two datasets and methods used for AI score calculation are described.

Chapter 4 Methods- Allostatic Load Theory, Allostatic Index Score and Description of Datasets

4.1 Chapter Summary

In this chapter, six issues are addressed: 1) the allostatic load (AL) model, which proposes that multi-system physiological dysregulation induced by stress can contribute to various disease trajectories; 2) the statistical methods proposed in the literature, as a means of calculating a composite allostatic index score, using multi-system markers of allostatic load; 3) findings from studies which examine the relationships between markers of allostatic load and depressive symptoms; 4) the methods of data collection, depression assessment and participant characteristics of the two cohorts used in my study, DepChron and Psobid; 5) the different biomarkers available for allostatic index score calculation in DepChron and Psobid; & 6) the different methods of AI score calculation used in the study are described. The potential strengths and weaknesses of each dataset for the purpose of this study are also discussed. The next section of this chapter provides a summary and appraisal of the first three issues in the list above and sets the context of the study and selection of datasets. The third section of this chapter describes the datasets, while the fourth section describes the AI score calculation in the two datasets. The chapter concludes with a summary section of all of the issues described above.

4.2 Allostatic Load Theory, Allostatic Index Score Calculation and Relationship between Allostatic Load and Depression

4.2.1 Allostatic Load Theory

4.2.1.1 Definitions

<u>Allostasis</u>: Allostasis refers to the process whereby an organism maintains physiological stability by changing parameters of its internal milieu and matching them appropriately to environmental demands (26).

<u>Allostatic Load</u>: AL represents the "wear and tear" the body experiences when repeated allostatic responses are activated during stressful situations (199).

The potential effect of stress on the human body has been a subject of medical research for many decades. In 1936, Hans Selye proposed that the physiological systems activated by stress may have a role, not only in protecting and restoring, but also in damaging the body (200). Selye suggested that the long term effects of stress can lead to enlargement of the adrenal gland, atrophy of the thymus, spleen and other lymphoid tissue, and gastric ulcerations (200). Sterling and Eyer discussed the multi-system physiological changes in response to arousal which are directed towards maintaining body stability and referred to them as "Allostasis". In 1993, McEwen and Stellar, proposed the formulation of AL to study the relationship between stress and processes leading to disease. The concept has evolved significantly with widely reported associations in population studies between AL and various diseases (28). In this section, there is a description of the physiological changes which constitute the AL.

4.2.1.2 Allostatic Load Conditions

Acute (major life events) and chronic (everyday minor) stress can have long-term physiological effects. There are two important factors which contribute to an individual's response to stressful stimuli-an individual's perception of stressful stimuli and an individual's state of physical health (see Figure 4-1), which in turn are influenced by multiple factors. An individual's perception of threat is instrumental in defining their behavioural response such as fighting or fleeing.

For example, the HPA (hypothalamic-pituatary-adrenal) axis measured by salivary cortisol was increased in most individuals when they were subjected to the stress of speaking or doing complex arithmetic in front of an audience (201). However, in some participants described as "low responders", salivary cortisol levels were unaltered after repeated stimuli; while others continued to have high salivary cortisol ("high responders") (201). Cardiovascular (CV) responses to stress obtained in a laboratory setting are, in real life, not only replicated but also often larger (202). Real life CV responses to stress can also be related to subjective ratings of stress and emotions (202). An individual's response to stress is influenced by genetic factors, lifestyle and behavioural choices. For instance, male adolescents with CYP17A1 gene loci were found to have increased blood pressure reactivity to mental stress(203).

Figure 4-1 Physiological Response to Stress and Allostatic Load (204)
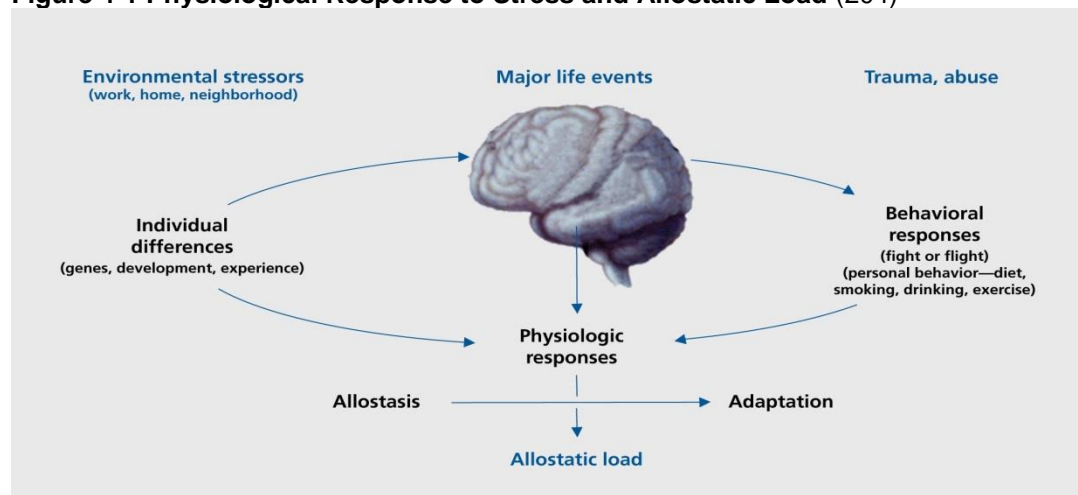
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McEwen proposed that the body's response to a challenging situation usually involves two steps (204). The first step is turning on an allostatic response, while the second is switching it off when the challenging situation has resolved. The allostatic response is usually mediated through the sympathetic nervous system and HPA axis, and involves increase in the levels of catecholamines and cortisol. When the challenging or stressful situation is resolved, these systems are deactivated and catecholamines and cortisol levels return to normal. McEwen proposed that there are three types of AL based on the variations of allostatic response to four different situations (see Figure 4-2). The first condition is due to repeated exposure to a stressful stimulus, with appropriate "allostatic"

response and similarly appropriate "switching off" and "normal adaptation" with reduced scale of responses. In the second condition, there is lack of adaptation to stressful stimuli and hence it leads to prolonged exposure to stress hormones as described earlier. In the third type, there is failure to shut off allostatic response after stressful stimuli, which again leads to prolonged exposure to stress hormones. Finally, in the fourth condition and the last type of AL variant, there is inadequate allostatic response to stressful stimuli. As a result of this, another physiological system such as the inflammatory response is activated.

Figure 4-2 Three types of Allostatic Load (AL) in four conditions (204)
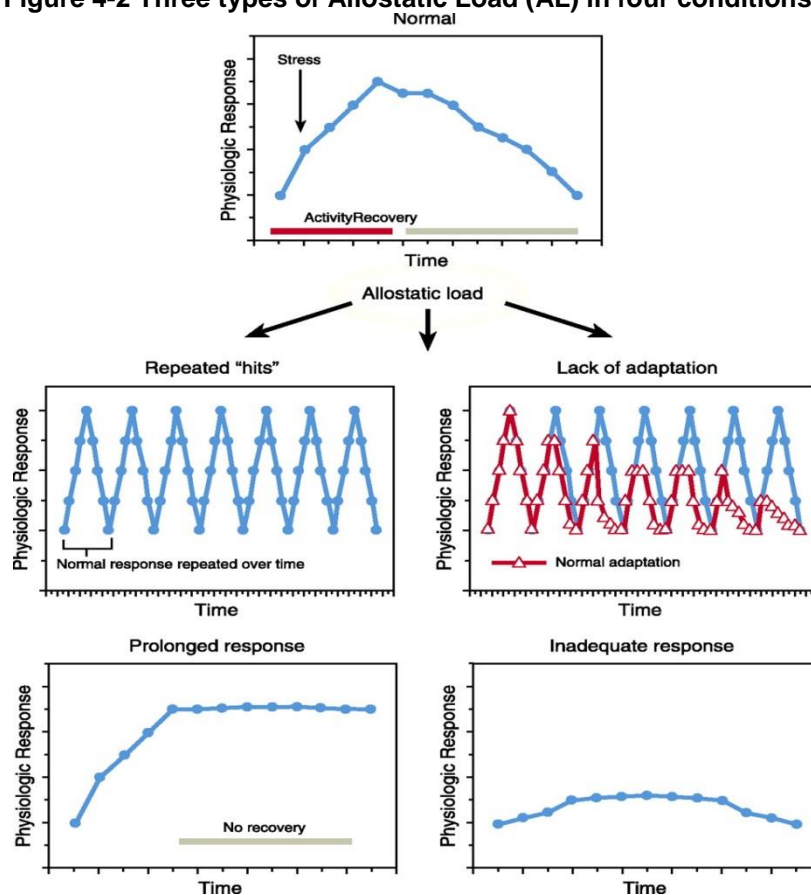
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4.2.1.3 Primary and Secondary Allostatic Load Markers

Chronic over activation of stress mediators and subsequent physiological response in allostatic load conditions can lead to damage to various organ systems in the body (204). Stress mediators in AL have been divided into primary and secondary mediators. The primary mediators of AL operate in a non-linear fashion, which means that each mediator has the ability to regulate the activity of the other mediators and reciprocally get regulated by other mediators as well

(205). At first, in response to stress, there is activation of the sympathetic-adrenal-medullary (SAM) activation, which leads to release of catecholamines such as epinephrine and norepinephrine. The other major group of "stress hormones" are glucocorticoids (antagonized by dehydroepiandrosterone-DHEA), which are released from the HPA axis. Catecholamines and glucocorticoids can influence the production of both pro and anti-inflammatory cytokines in a non-linear fashion (see Figure 4-3). The parasympathetic system also plays an important role in this network of allostatic mediators. It generally opposes the sympathetic system and it also has anti-inflammatory effect. Over time, compensatory mechanisms to over and under production of primary mediators such as catecholamines, glucocorticoids and cytokines, leads to increase in secondary mediators of AL or secondary outcomes.

Figure 4-3 Non-linear network of primary mediators of Allostatic Load in stress response (205)
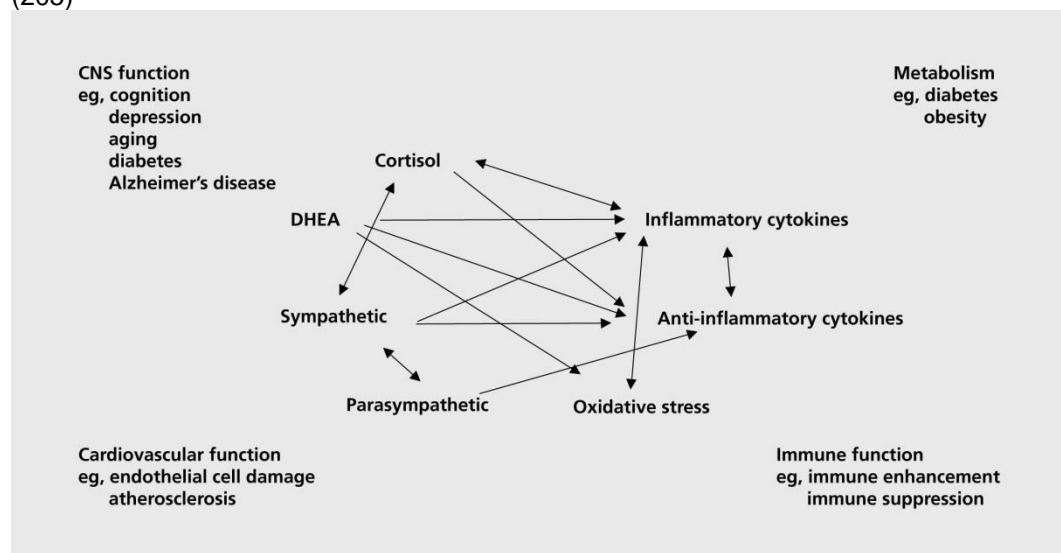
The activation of secondary mediators is often referred as the "prodromal" stage (28). The secondary mediators of AL are mainly divided into metabolic, cardiorespiratory and anthropometric systems. In this stage, the levels of secondary mediators or markers are at a sub-clinical stage; physicians routinely measure some of the secondary markers but clinical attention is only prompted when a marker reaches clinically significant levels. Examples of metabolic mediators are blood levels of: HDL cholesterol; low density lipoprotein-LDL cholesterol; triglycerides; creatinine; and glucose. Examples of cardiorespiratory markers are: systolic blood pressure; diastolic blood pressure; heart rate; and

peak expiratory flow. While examples of anthropometric markers are body mass index and waist hip ratio. The primary and secondary mediators of AL and their particular role are summarized in Table 4-1, adapted from a systematic review by Juster et al (28). The final stage of AL progression is the allostatic overload stage, where individuals experiencing chronic AL develop various health related diseases or tertiary outcomes. The AL models suggests that by measuring the levels of primary and secondary allostatic mediators, individuals at risk of developing tertiary outcomes can be identified earlier than they would by routine clinical measures (206). This has led to interest in developing methods for measuring AL, known as the allostatic index score.

Table 4-1 Primary and secondary mediators of allostatic load, overview of their function and measurement.

NEUROENDOCRINE (PRIMARY)	Cortisol	Glucocorticoid produced by the adrenal glands. Functions include role in fat metabolism, immunosuppression, regulating heart rate and blood pressure, regulation of limbic and prefrontal regions of brain.
	Dehydroepidrosterone-DHEA	Androgen produced by the adrenal glands. Functions include role in regulation of HPA-axis, inflammatory cytokines, lipid metabolism and reduction of oxidative stress.
	Epinephrine	Catecholamine produced by the adrenal glands and the brain, part of the "fight-or-flight" response, it increases heart rate and glucose levels.
	Norepinephrine	Catecholamine produced by the brain, part of the "fight-or-flight" response, it increases blood pressure and modulates brain activities.
	Dopamine	Catecholamine produced primarily in the brain and adrenal glands, it's a neurotransmitter involved in many neurological activities and also increases blood pressure and heart rate.
	Aldosterone	Minerocorticoid produced by the adrenal glands, functions include role in electrolyte and water balance and reduces blood pressure.
INFLAMMATORY (PRIMARY)	Interleukin-6	Cytokine produced by macrophages and T-cells, functions include major role in pro and anti-inflammatory responses.
	Tumour Necrosis Factor-Alpha	Cytokine produced by macrophages, functions include role in systemic inflammation by evoking mediators of acute phase reactions and role in tumour apoptosis.
	C-Reactive Protein	Protein synthesized in the liver, functions include role in acute phase reactions and promotes inflammation.
	Insulin Like Growth Factor-1	Polypeptide protein hormone produced primarily in the liver and pancreas, functions include cell growth stimulation and inhibition of cell apoptosis.
	Fibrinogen	Protein that synthesizes into fibrin in the liver, functions include role in blood clotting.
METABOLIC (SECONDARY)	High Density Lipoprotein Cholesterol	Lipoprotein synthesized in the liver, commonly referred to as "good cholesterol", as its' high protein/low cholesterol form is more easily removed by blood in the liver and excreted in bile.
	Low Density Lipoprotein Cholesterol	Lipoprotein synthesized in the liver, commonly referred to as "bad cholesterol", as its' low protein/high cholesterol form is more likely to be deposited in the walls of blood vessels and contribute to atherosclerosis.
	Triglycerides	Glyceride formed from glycerol and three chains of fatty acids, it is an important source of

		energy and a transporter of dietary fat.
	Glycosylated Haemoglobin	Haemoglobin used to index the average glucose concentration over weeks and months.
	Glucose	Monosaccharide synthesized in the liver and kidneys, functions as the main source of energy.
	Insulin	Protein hormone produced in the pancreas, functions include lowering glucose levels and promoting energy storage.
	Albumin	Protein produced by the liver, role in the maintenance of blood volume
	Creatinine	Nitrogenous waste product of muscle creatine phosphate that is filtered and excreted by the liver, it's a marker of glomerular filtration rate and renal function.
	Homocysteine	Amino acid biosynthesized from methionine, functions include role in remethylation and transsulfuration pathways that are in part dependent on nutritional intake of folic acid and vitamin B12.
CARDIOVASCULAR AND RESPIRATORY (SECONDARY)	Systolic Blood Pressure	Measured using a sphygmomanometer, represents the maximal force exerted by blood against the blood vessel walls when the left ventricle is contracting during systole.
	Diastolic Blood Pressure	Measured using a sphygmomanometer, represents the minimal force exerted by blood against the blood vessel walls when the left ventricle is relaxed during diastole.
	Peak Exploratory Flow	Measured using a peak flow meter, represents the maximum speed of expiration and the degree of obstruction of airflow through the bronchi.
	Heart Rate/Pulse	Measured at sites where arterial pulsation can be felt represents the number of heart beats within a period of time.
ANTHROPOMETRIC (SECONDARY)	Waist Hip Ratio	Measure of waist circumference and hip circumference using measuring tape values that are than calculated into a ratio by dividing waist by hip. Higher levels represent greater adipose fat distribution.
	Body Mass Index	Measure of weight and height that is then calculated into an index by dividing weight by height. Represents a proxy measure of an individual's relative body fat percentage.
Table has been adapted from Juster et al. systematic review (28)		

4.2.2 Allostatic Load Measurement, Allostatic Index Score Calculation and Health Implications

In this section, the methods of measuring AL, the Allostatic Index (AI) score and the relationship between AI score and different health consequences are summarized. The early work in AL measurement was done through the MacArthur Studies of Successful Ageing, which was a cohort of more than 1000 men and women, aged between 70 and 79 years recruited from communities in the North East of the United States of America (207). One of the first proposed methods of measuring AI score was based on a count based formulation consisting of 10 biomarkers-12 hour urinary cortisol, epinephrine and norepinephrine output, serum dehydroepiandrosterone-sulphate (DHEA-S), total cholesterol to HDL cholesterol ratio, HDL cholesterol, plasma glycosylated haemoglobin (HbA1c), systolic blood pressure, diastolic blood pressure and waist hip ratio (208). The biomarker values which fell into the high risk category of 75th percentile in relation to the sample's distribution in the cohort were given a score of "1" and the remaining values were given a score of "0". The two markers, DHEA-S and HDL cholesterol, were given a score of "1" if their value was in the lower 25th percentile. This was added up over the 10 biomarkers to give an AI score of 1-10. In the early work on this cohort, higher AI score was found to be a predictor of decline in cognitive and physical functioning and increase in cardiovascular risk over 3 years, independent of social and demographic factors (208). Subsequently, the association between higher AI score and these health related outcomes was also seen at 7 years of follow-up (27).

The health implications of AI score have been studied in other cohorts and in other countries apart from the USA. For example, the relationship between AI score and health outcomes was studied in Taiwan, using the Social Environment and Biomarkers of Ageing Study (SEBAS) cohort (209). The SEBAS study included more than 1000 men and women recruited form urban areas in Taiwan and aged 54-70 years. In this study, the AI score was calculated using 16 biomarkers and the cut-offs used were two tailed. A score of "1" was assigned if the biomarker value was in the higher than 90th percentile or lower than 10th percentile of the biomarker distribution in the cohort (209). In this group of participants, higher AI

score was associated with higher risk of 3 year mortality (209). The authors of this study did not explain the reasons for using cut-offs at both the low and high extremes or in other words measuring deviation in biomarker values in both directions of the sample mean. One of the possible explanations could be that the two tailed methods were designed to identify "inadequate physiological response", which is also a type of allostatic state (see Figure 4-2). If we measure deviation in biomarker values less than the sample mean, we are able to identify individuals who may have an "inadequate physiological response".

Seplaki and colleagues compared the count based formulation with a z-score technique of AI score calculation in the SEBAS cohort (210). The z-score technique involved calculating AI score as the sum of the standardized distances of a biomarker value from its respective mean value for the sample. The rationale of using the z-score technique was to use the whole distribution of a biomarker in calculating AI score and subsequently studying its relationship with adverse health outcomes. The study found a positive association between high AI score and health related outcomes such as physical mobility, self-rated health and cognitive function; but there was no difference in the observed relationship with AI score calculated with a count based formulation or a z-score technique (210). In Japanese earthquake survivors, researchers calculated AI score using a subset of AL biomarkers and three more (D-dimer, von Willebrand factor, and tissue-type plasminogen activator antigen), and found an association between high AI score and higher rates of myocardial infarction and stroke (211). The published evidence on relationship between AI score and health related tertiary outcomes has been summarized in two systematic reviews, which shows an association of AI score with a wide variety of health outcomes such as mortality, cognitive and functional decline, depressive symptoms and cardiovascular outcomes (28,212).

Various novel statistical techniques have been employed in AI score calculations, which are summarized in Table 4-2. In 2002, Karlamangla and colleagues used canonical correlations to show an association between higher AI score and 7 year functional and cognitive decline using the MacArthur Studies of Successful Ageing cohort (213). In this method, to assess the individual contribution of every single biomarker (allostatic load component) to the association between allostatic load and outcomes, the analysis was repeated on 200 bootstrap samples obtained

from the complete study sample. Allostatic load biomarkers and outcomes that made the least contribution were identified as the ones with the most variable canonical weights, and were dropped, one at a time, in a stepwise backward elimination process. Based on this method, the authors found certain biomarkers contributed more towards predicting physical decline (such as epinephrine, waist hip ratio and cortisol) while others contributed more to predicting cognitive decline (epinephrine, diastolic blood pressure and HbA1c) (213).

Gruenewald and colleagues used non-parametric regression techniques such as recursive partitioning to study the relationship between individual allostatic load biomarkers and gender in predicting 12 year mortality using the MacArthur Studies of Successful Ageing Cohort (214). They found that extreme values of neuroendocrine and immune biomarkers were associated with risk of death in males and systolic blood pressure was associated with risk of death in females (214). Using the same cohort, changes in AI score over 2.5 years was used to predict mortality at 4.5 years (215). Participants whose AI score increased over 2.5 years were found to have increased risk of mortality compared to those whose AI score reduced over follow-up (215). The different statistical techniques used for calculation of AI score have been reviewed by Juster and his colleagues and summarized in the table below, which is adapted from their systematic review (28).

Table 4-2 Different statistical techniques and algorithms for allostatic index score calculation.

Formulation	Description
Group allostatic load index	Count based formulation which represents the number of biomarkers falling within a high risk percentile (i.e., upper or lower 25th percentile or upper or lower 10th percentile) based on the sample's distribution of biomarker values. As each biomarker is dichotomized as 0 or 1 depending on cut-offs, each biomarker is allotted an equal weight in the index.
Norm allostatic load index (based on clinical cut-offs)	Count based formulation which represents the number of biomarkers falling within a high risk percentile (i.e., upper or lower 25th percentile) based on a population's distribution of normative biomarker values used in clinical practice.
z-Score allostatic load index	Summary measure representing the sum of an individual's obtained z-scores for each biomarker based on the sample's distribution of biomarker values. This standardized formulation allows the weight of each biomarker to be different depending on its deviation from the sample's mean.
Difference allostatic load score	Difference between two time-points for a single biomarker or an index measure of multiple biomarkers. For example, an index measure of pro-coagulation responses using several homeostatic biomarkers or two measures of cortisol before and after exposure to an acute stressor.
Dynamic allostatic load score	Repeated measures analysis or change scores between three or more time-points for single or multiple biomarkers.
Bootstrapping	Resampling technique used to make inferences about population parameters by generating multiple repetitive computations that estimate the shape of a statistic's sampling distribution.
Canonical correlation	Multiple correlational analyses that measure the association between two sets of latent variables representing an independent set and a dependent set. It has been used to determine the best linear combinations of weighted allostatic load biomarkers at baseline that are maximally correlated to tertiary outcomes like mortality at follow-up.
Recursive partitioning	Multivariate reduction technique that generates categories aimed at precisely classifying participants based on several dichotomous dependent variables. It has been used to classify participants into outcome risk categories by first identifying the biological markers and cut-points that best differentiate across participants. These have been used to define allostatic load categories (e.g., high, intermediate, low) and tertiary outcomes (e.g., mortality).
Table has been adapted from Juster et al. systematic review (28)	

4.2.3 Allostatic Load Markers and Depression

The relationship between allostatic load biomarkers and depressive symptoms has been the subject of research in a number of studies. In 2004, Seplaki and colleagues used the SEBAS cohort from Taiwan to show a significant cross sectional association between some allostatic load biomarkers such as IGF-1 (Insulin-like growth factor), total Cholesterol and triglycerides with depressive symptoms (216). Later on, Goldman and colleagues did a longitudinal analysis using the same cohort and found a significant association between composite AI score at baseline and depressive symptoms at 3 years of follow-up (209). Juster and his colleagues found a significant association between depressive symptoms and AI score in cross-sectional analysis but did not find an association between AI score at baseline and development of depressive symptoms at 3 and 6 years of follow-up (217). In another study, the same author and his colleagues found no cross-sectional association between AI score and depressive symptoms in 30 healthy workers in Canada (218). Finally in 125 elderly US residents, AI score was found to have a significant cross-sectional association with affective and somatic type of depressive symptoms, as well as with overall depressive symptoms (219). These study characteristics are summarized in Table 4-3.

In the next section, some of the criticisms faced by the allostatic load theory and its potential clinical application are discussed.

Table 4-3 Summary of studies investigating relationship between allostatic load biomarkers and depressive symptoms

Lead Author; Study Design	Method of Depression Assessment	Sample Size; Age; Country	Allostatic Load Biomarkers
Seplaki (216) Cross-sectional	CES-D (Centre for Epidemiological Studies- Depression Scale)	N=820; >54 years Taiwan	Cortisol, Dopamine, Epinephrine, Norepinephrine, DHEA-S (dehydroepiandrosterone sulfate), IL-6 (Interleukin), IGF-1 (Insulin-like Growth Factor, HbA1c (Glycosylated Hemoglobin), Glucose, TC (Total Cholesterol), HDL (High Density Lipoprotein) Cholesterol: TC Ratio, Triglycerides, SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), WHR (Waist Hip Ratio), BMI (Body Mass Index)
Goldman (209) Longitudinal	CES-D	N=820 >54 years Taiwan	Same as above
Juster (217) Cross-sectional and Longitudinal	GDS (Geriatric Depression Scale)	N=58 Age: 52-80 US	Cortisol, TC, HDL Cholesterol, Triglycerides, Glucose, SBP, DBP
Juster (218) Cross-sectional	BDI (Beck Depression Inventory)	N=30 Age:27-65 Canada	Diurnal Cortisol, CRP (C-Reactive Protein), Albumin, Insulin, Glucose, HDL Cholesterol, TC, SBP, DBP, HR (Heart Rate), WHR, fibrinogen, amylase, HbA1c
Kabrosly (219) Cross-sectional	CES-D	N=125 Age: 67-94 US	Diurnal Cortisol, IGF1, IL-6, WHR, HR, SBP, DBP

4.2.4 Critique of Allostatic Load Theory

Allostatic load theory has come under criticism from some reviewers involved in stress related research. Day suggested that the concepts of allostatic load and allostatic load mechanisms were simply "rebranding" of the homeostatic mechanisms concept, and not entirely useful (220). He also suggested that future research should focus on defining and differentiating between a "homeostatic response" (usually selective, specific and minor) and a "stress response" (usually non-selective and involving multi-system) (220). On the other hand, Chaney argued that the concept of allostatic load ignores the neurobiological responses involved with providing resilience to psychological stress in general and to specific forms of psychopathology (221) and that an understanding of psychobiological responses which maintain neural systems in the face of a stressful challenge may be better able to provide the understanding of why some individuals are able to cope with extreme stress with minimal consequences (221).

Most of the research to date on the association of allostatic load biomarkers and tertiary health outcomes has primarily been conducted using two major cohorts (28,212). These datasets are the MacArthur Studies of Successful Ageing (n=1189, Age Range=70-79, USA) and the SEBAS cohort (n=820, Age Range=54-90, Taiwan) (28). Hence, there are questions around the reproducibility of these findings, and their applicability in different age groups and different populations.

Another issue is that the allostatic load concepts suggests that measuring multi-system dysregulation by the means of biomarkers has clinical utility in predicting various tertiary health related outcomes. However, various studies discussed above have suggested that certain biomarkers may have better predictive utility than a composite score in predicting specific tertiary outcomes (213,214,216); for example predictive utility of systolic blood pressure in predicting all-cause mortality (214). This leads to the question: why use multiple markers if a single marker is equally good or better in predicting a specific clinical outcome?

So it would appear that further research is needed to establish the clinical implications of the findings so far from the allostatic load literature. If measuring a composite AI score using multiple biomarkers is found to have clinical usefulness in predicting health outcomes at a prodromal or asymptomatic stage, it has to be targeted at individuals who are at highest risk of having these outcomes. It is neither productive nor feasible to measure a battery of biomarkers on the general population and it would be a difficult task to integrate this into routine clinical practice.

4.2.5 Study hypothesis and selection of datasets

The existing knowledge of relationship between AL biomarkers and depressive symptoms, albeit with its various limitations, led to the development of my hypothesis that a combination of AL biomarkers will form a predictive algorithm in defining clinically meaningful outcomes in a population of patients with depressive symptoms. The objectives of this thesis are to investigate the cross-sectional association of AL biomarkers and depressive symptoms and association of AL biomarkers with clinical outcomes in those with depressive symptoms. Clinical outcomes pertaining to physical health are of special interest as the review findings (chapter 2) suggested a lack of research in this area. To test the hypothesis, two different datasets- DepChron and Psobid are used, which are described in detail in sections below. Any dataset has its own strengths and limitations and this was certainly true of DepChron and Psobid. Consequently, three other research data sources were considered and it was not feasible to use them for this study due to different reasons. The datasets considered were: UK Biobank (http://www.ukbiobank.ac.uk), Twenty-07 (http://2007study.sphsu.mrc.ac.uk/) and Generation Scotland (http://www.generationscotland.org). UK Biobank did not have any of the blood results available for research purposes when this study started. Twenty 07 and Generation Scotland did not have information on clinical outcomes available at the start of this study.

4.3 Description of Datasets-DepChron and Psobid

4.3.1 Data Collection in DepChron

DepChron is a dataset with 125,143 participants with at least one of the three cardiometabolic diseases-Coronary Heart Disease (CHD), Diabetes, and previous Stroke over the period of 2008-2010. The participants in the DepChron dataset come from the West of Scotland, with a population of circa 1.8 million served by two different health boards. The local health boards oversaw a programme of incentivised depression screening in chronic disease as part of a wider chronic disease management programme of LES. The Quality & Outcomes Framework (QOF) is part of the UK wide, pay for performance, General Medical Services contract for family practitioners (222). LES are contractual arrangements at a local health board level with general practices designed to augment the basic QOF specification by incentivising additional indicators that are deemed to be particularly important by a given area and there are no penalties for non-adherence. General practices were paid under the LES scheme to carry out a comprehensive annual health assessment for patients with three common chronic diseases, CHD, diabetes and stroke, including depression screening. Besides depression screening, the annual health assessment also included assessment and management of other health related behaviours such as smoking status, alcohol consumption, diet and activity levels. The annual health assessment was usually carried out by a practice nurse and lasted for approximately one hour. The remuneration offered varied according to disease area with £31 each for patients with diabetes, £26 each for patients with previous stroke and £23 each for patients with CHD. The remuneration was dependent on the level of coverage of indicators achieved in the health assessment, with full payment offered for >90% coverage, 3/4 payment for 75-90% coverage and half payment for 60-75% coverage.

The permission to access the data was given by the data guardian, NHS Greater Glasgow and Clyde Keep Well Enhanced Services Data Group. Approval was also obtained from the NHS Practice Advisory Committee (PAC) (reference PAC 85/12) and the NHS Research Ethics Committee (NRES) (reference 12/LO/1622)

for electronic data linkage to study the outcomes data for the DepChron patients (see Appendix 4).

In 2008-9, the period in which data collection took place, the comprehensive annual health assessment was offered to all patients on the practice register with one of the three aforementioned chronic diseases and was usually carried out by practice nurses. The results of the assessment were entered into the template with "Read codes" assigned to each data entry. Read codes are the coded thesaurus of clinical terms, by which clinicians in the UK record patient findings and procedures (223). The assessment included detailed history taking, various physical examinations and blood tests, and recording of certain drugs prescribed including antidepressants, anti-psychotics and cardiovascular drugs.

4.3.2 Assessment of Depression Status in DepChron

The health assessment included screening patients for depression using the depressive subscale of the Hospital Anxiety and Depression Score (HADS-D (152). Analysis was restricted to adults aged from 18 to 90 and health assessments recorded between 01/04/2008 to 31/03/2009. The rationale for the restriction in age range was to exclude young adolescents and extremely frail elderly patients who may have specific physical and mental health problems which may not be generalizable to all patients with chronic cardiometabolic disease. Patients who were noted to be under treatment for depression were exempt from depression screening and should not have been included in the dataset, but there was evidence that some had been. Based on the information available it was not possible to explicitly differentiate which of the patients in the dataset were being newly prescribed antidepressants from those who were under existing treatment for depression (and who should have been exempt from screening, but may nonetheless have been screened and included in the dataset). However based on the situational knowledge of primary care practice in Scotland, that the average prescription duration is not usually longer than 90 days, patients who were first prescribed antidepressants more than 90 days after the start of the observation period, they were labelled as 'likely' to be newly started on antidepressants without undergoing depression screening. Patients were labelled as 'under treatment' for depression and exempt from depression screening if: (a) they were noted to be on antidepressants based on their

prescription record with no record of depression screening during the 12 month observation period (01/04/2008 to 31/03/2009); or (b) the first prescription for antidepressants was issued in the first 3 months of the observation period.

The depressive subscale of HADS (HADS-D), which has a potential total score of 21, was used as the depression screening tool. The threshold of HADS ≥8 was used as a cut-off for a 'positive screening result' as there is evidence to suggest that this offers an optimal balance of sensitivity and specificity (224,225) and such an approach has been endorsed by national guidelines (148). All patients who underwent depression screening were checked for a new prescription of antidepressants in the six months after the date of assessment. No reliable information was available on the number of patients who were referred for psychological therapies following their depression screening. Information on history of previous episodes of depression or history of antidepressants prescribed in the past, prior to the observation period was not available.

4.3.3 Participant Characteristics in DepChron

Socioeconomic status was recorded in patient notes in the form of the Scottish Index of Multiple Deprivations (SIMD) score, which identifies small area concentration of multiple deprivations across all of Scotland in a consistent way. The SIMD score can be divided into quintiles with lower quintiles representing the most deprived areas. The socioeconomic status in DepChron was divided into SIMD quintile, quintiles from 1-5 representing the most deprived areas; quintiles 6-10 representing affluent population (29). A total of 125,143 patients were listed as having CHD, diabetes or stroke in the year 2008-09. Figure 4-4 shows the distribution of patients across the three diseases and their respective proportional combinations.

Figure 4-4 Distribution of patients across three diseases for the year 2008-09 in DepChron (226)
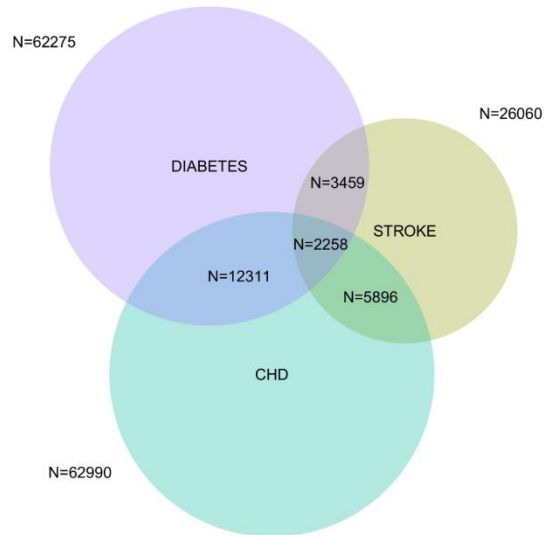
Of the total sample, 10,670 (8.5%) patients were under treatment for depression, having received their first antidepressant prescription of the within the first 3 months observation period, and were thus assumed exempt from screening. The remaining 114,473 (91.5% of total sample size) patients were eligible for depression screening.   However, depression screening was only undertaken in 35,537 (31.1% of those eligible) and 78,936 (68.9%) were not screened. Figure 4-5 shows the distribution of patients according to their depression status.

Table 4-4 compares the demographic characteristics of the subset of the population who underwent depression screening with the whole participant population. The continuous variables are compared using mean and standard deviation, while the categorical variables are presented using counts and percentages. The table shows that the distribution of variables between these populations was very similar.

Table 4-4 Comparison of demographic characteristics between total population (n=125143) with the subset which underwent depression screening (n=35537) in DepChron (227)

Mean (SD) are presented for continuous variables and count (%) for categorical.

		Total Population n=125143	Screened Population n=35537
Age (years)		67.6 (13.6)	69.0 (11.9)
	missing	42	11
Sex	Female	57566 (46.0%)	14861 (41.8%)
	Male	67507 (53.9%)	20658 (58.1%)
	missing	70	18
Socio-economic status	Deprived (SIMD 1-5)	82267 (67.4%)	22726 (65.3%)
	Affluent (SIMD 6-10)	39680 (33.5%)	12079 (34.7%)
	missing	3196	732
Comorbid Condition	One	101219 (80.8%)	27356 (76.9%)
	Two	21666 (17.3%)	7410 (20.8%)
	Three	2258 (1.8%)	771 (2.1%)
	missing	0	0

Figure 4-5 Flow chart showing the outcome of depression screening in DepChron (226)

Total number of patients with stroke, diabetes, CHD n=125,143

Patients under treatment for depression = 10,670 (8.5%) *Exempt from screening*

Patients eligible for depression screening = 114,473 (91.5%)

Depression screening results not recorded = 78,936 (68.9%)

Depression screening results recorded = 35,537 (31.1%)

'Likely' newly treated with antidepressants in unscreened population = 4989 (6.3%)

HADS <8 = 28,457 (80.1% of screened)

HADS 8–10 = 4,155 (11.7% of screened)

HADS ≥ 11 = 2,925 (8.2% of screened)

696 (2.4%) started on anti-depressants {388 (1.4%)}*

269 (6.5%) started on anti-depressants {193 (4.6%)}*

303 (10.4%) started on anti-depressants {258 (8.9%)}*

* Indicates results without amitryptyline

4.3.4 Data Collection in Psobid

The psychological, social, and biological determinants of ill health (Psobid) study is an existing research data source with 666 participants recruited in 2006-07 by the Glasgow Centre for Population Health (GCPH). The methods for recruitment and data collection have been described in detail in the literature (228-230). The recruitment of participants was based on SIMD (29) which has been described previously. The least and most deprived areas in the Greater Glasgow and Clyde health area were identified using the SIMD score. Five general practices each with the highest percentage of patients aged 35-64 years living in the most deprived and affluent areas as classed by SIMD were approached, and all agreed to participate in the recruitment process. A target population of 21,672 people from the lists of these 10 practices was identified. A sampling frame of 3600 participants from the least and most deprived areas was constructed from general practice lists and, therefore, included individuals regardless of whether they actually visited their general practitioner. The most deprived group had poor response rates and hence required oversampling. From the sampling frame of 3600 subjects a total of 2,712 invitations were issued to recruit a cohort of 700 (25.8%) participants. Out of the 2,712 invitations sent, 812 (29.9%) people declined to participate and 1,200 (44.3%) did not respond (see Figure 4-6). Data collection was completed in April 2007 and data quality was tested over the summer of 2007. Of the 700 subjects who participated in the study only 34 (4.9%) did not complete both visits, which led to a sample size of 666 participants.

The permission to access the Psobid participants' data was obtained from the GCPH. GCPH did have an existing ethical approval in place from the NHS Research Ethics Committee (reference number 05/S0705/40) (see appendix 5) which was applicable to this study. In addition, an approval was obtained from the NHS PAC for electronic data linkage and study of outcomes for Psobid participants (reference number PAC86/12) (see Appendix 5).

Figure 4-6 Patient recruitment in Psobid
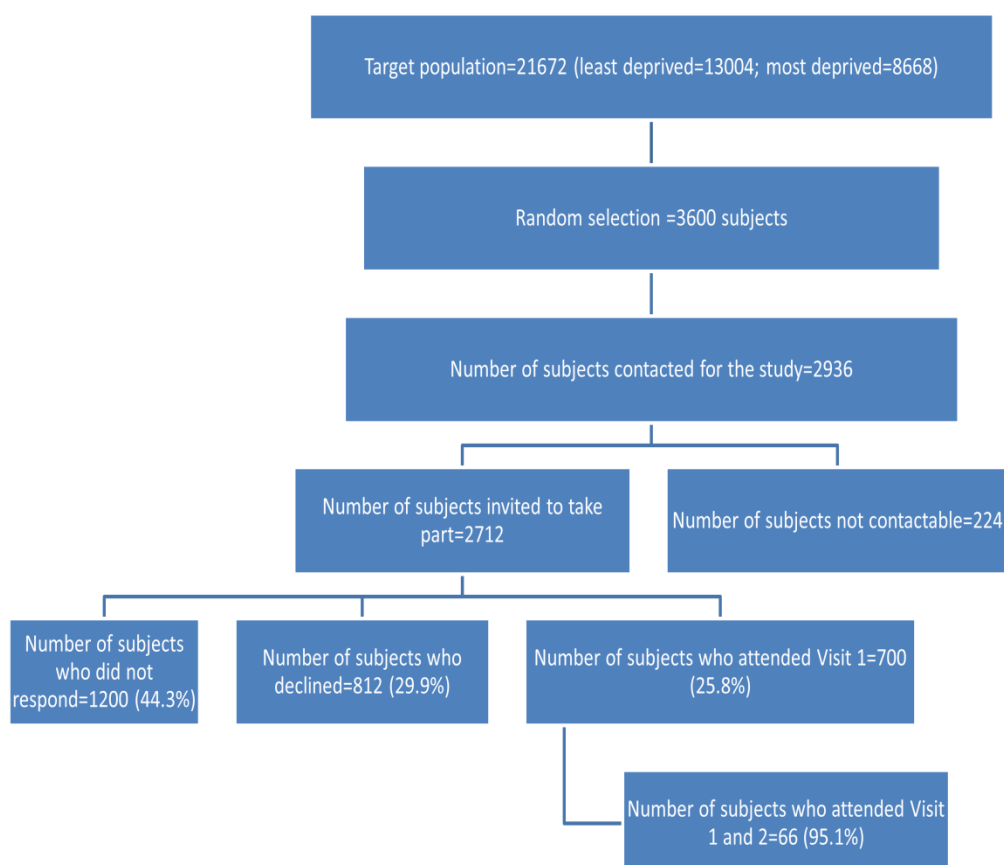
Figure adapted from Velupillai et al. (224)

4.3.5 Assessment of Depression Status in Psobid

Participants were screened for the presence of psychological symptoms using the General Health Questionnaire (GHQ-28) questionnaire score which has 4 sub-scales: somatic symptoms, anxiety and insomnia, social dysfunction and severe depression (231). A cut-off score of GHQ-28≥5 was used as this cut-off has been endorsed by national guidelines to be consistent with major depressive disorder (148).

4.3.6 Participant Characteristics in Psobid

The sampling procedure in Psobid was stratified to achieve an approximately equal distribution across age bands (35-44, 45-54 and 55-64 years), sex and

socio-economic status. The target population was identified from two different socioeconomic groups in the general population, affluent (least deprived) and most deprived. The least deprived group had the top 20% SIMD score while the most deprived group had the bottom 5% SIMD scores. Table 4-5 shows the demographic characteristics of participants in Psobid, divided into the most deprived and least deprived socio-economic groups. The occupation status was classified using the Registrar General Social Class Classification on the basis of current job, or if not currently working, on the basis of participants' last paid job. Only those participants who had never been in paid employment were classed as "unemployed". The classification categories were: I-professional occupations; II-managerial and technical occupations; II-manual and non-manual skilled occupations; IV-partly skilled occupations; V-unskilled occupations.

Table 4-5 Comparison of Demographics between the least deprived and most deprived participant groups in Psobid (232)

Mean (SD) are presented for continuous variables and count (%) for categorical.			
		Least Deprived Group (n=342)	Most Deprived Group (n=324)
Age (years)		51.8 (8.0)	51.5 (8.5)
Sex	Female	171 (50.0%)	168 (51.9%)
	Male	171 (50.0%)	156 (48.1%)
Household Income		£41,699 (£11,921)	£16,461 (£10,056)
Education (total years)		16.1 (3.6)	11.8 (2.5)
Residential Status	Owner	334 (97.7%)	97 (29.9%)
	Tenant	8 (2.3%)	227 (70.1%)
Occupation Status	I and II	251 (73.4%)	62 (19.1%)
	III	77 (22.5%)	139 (42.9%)
	IV and V	12 (3.5%)	105 (32.4%)
	Unemployed	1 (0.3%)	2 (0.6%)
	Undetermined	1 (0.3%)	16 (5.0%)

4.4 Allostatic Load Biomarkers and AI score calculation in DepChron and Psobid

In this section, the biomarkers used to calculate AI score in the two datasets- DepChron and Psobid are summarized. In addition, the different statistical methods used for AI score calculation for both datasets are also described. Finally, the distinct advantages and drawbacks of using these two data resources for the purpose of AI score calculation are also discussed.

4.4.1 AL Biomarkers in DepChron and Psobid

As previously discussed, there were 35,537 patients within the DepChron dataset with recorded HADS-D results (see Figure 4-5). The available AL biomarkers in DepChron and their respective AL biomarker category as suggested by the review by Juster et al (28) are listed below:

1. Systolic Blood Pressure (Cardiovascular)

2. Diastolic Blood Pressure (Cardiovascular)

3. Body Mass Index (Anthropometric)

4. Total Cholesterol (Metabolic)

5. Glycosylated Haemoglobin-HbA1c (Metabolic) (only in a subset of patients with diabetes)

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were recorded in mm Hg and body mass index (BMI) in kg/m2 determined from height and weight measurements. A blood sample was collected by the practice nurse at the time of assessment; the result for total cholesterol was reported in mmol/l and HbA1c was reported in Diabetes Control and Complications Trial (DCCT) units. HbA1c was available only in the subset of dataset for patients with Diabetes (n=18,453).

The Psobid dataset had a greater number of biomarkers available for AI score calculation for its participants. The AL biomarkers and their respective AL biomarker category are listed below:

1.   Systolic Blood Pressure (Cardiovascular)

2.   Diastolic Blood Pressure (Cardiovascular)

3.   Body Mass Index (Anthropometric)

4.   Waist Hip Ratio (Anthropometric)

5.   Total Cholesterol (Metabolic)

6.   HDL Cholesterol (Metabolic)

7.   Serum Triglycerides (Metabolic)

8.   Fasting blood glucose (Metabolic)

9.   Serum Creatinine (Metabolic)

10.   Fibrinogen (Inflammatory)

11.   Interleukin-6 (Inflammatory)

12.   Highly sensitive CRP (Inflammatory)

The biomarker measurement for participants took place over two visits conducted between December 2005 and May 2007, which has been described in detail elsewhere (228). In visit one, a research nurse measured systolic and diastolic blood pressure in mm Hg; body mass index in kg/m^2, and waist hip ratio. A fasting blood sample was collected during visit two by the research nurse. Serum triglycerides, fasting blood glucose, total cholesterol and HDL cholesterol were measured in mmol/l. CRP was measured in mg/dl, Interleukin-6 in pg/ml and Fibrinogen was measured in g/l. Creatinine was recorded in umol/l.

4.4.2 Comparison of AL Biomarkers in DepChron and Psobid

There were key differences between DepChron and Psobid with respect to availability of biomarkers and patient and participant characteristics. In DepChron, only four biomarkers were available for AI score calculation, while the subset of the population with Diabetes had an additional biomarker available. The available biomarkers in DepChron were mainly secondary markers of AL. In contrast, Psobid had 12 biomarkers available for AI score calculation. This facilitated a more comprehensive AI score calculation with both primary and secondary markers of AL available in the Psobid dataset.

DepChron had distinct advantages which included the large sample size, comprising mainly older patients, which resembled the age distribution of the majority of the cohorts previously used in the study of AI score and relationship with tertiary outcomes. On the other hand, Psobid had a much smaller sample with a mixture of middle aged and older participants. Moreover, DepChron patients were recruited from primary care with existing cardiometabolic disease, while Psobid participants were recruited from the general population, with only a subset of them suffering from any chronic disease. Thus, there were some important differences in the two datasets in relation to their method of data collection, age distribution and health status which contributed to the strengths and limitations of the study findings. The strengths and limitations of each dataset are discussed in more detail, along with the results in Chapters 5 and 6.

4.4.3 AI score Methods in DepChron and Psobid

Table 4-2 provides a summary of some of AI score calculation methods reported in the literature. These methods can be broadly divided into count based formulations or summary measures and those methods needing complex statistical calculations such as canonical correlations and recursive partitioning (see Table 4-2). A third type of method is those which require more than one measurement of AL biomarkers; this approach was not feasible for either of the two datasets available.

Four different methods for AI score calculation which included count based and summary formulations were used in this thesis. The four methods used were:

1. The clinical cut-off method- (Method 1- count based formulation)

2. The sample distribution cut-offs (25th and 75th percentile) method- (Method 2- count based formulation)

3. The sample distribution cut-offs (10th and 90th percentile) method – (Method 3- count based formulation)

4. The z-score method-(Method 4- summary measure)

There are various reasons for choosing these particular methods of AI score calculation. Firstly, count based formulation or z-score methods were the most widely used AI score calculation methods, based on the findings of three systematic reviews on allostatic load studies (28,233,234). Secondly, few studies which compared different methods of AI score calculation have generally found that the choice of method was less important than utilizing the whole distribution of AL biomarkers, as far as the value of AI score in predicting adverse health outcomes is concerned (210,213,214,216,233). Finally, these methods were relatively straightforward to compute and hence they had more potential to be implemented in routine clinical practice in future.

4.5 Conclusion

The AL framework has been put forward as the "price" an individual's body has to pay to maintain internal stability, in response to stress. The AL theory suggests that by measuring multi-system biomarker levels as a proxy of measuring multi-system physiological dysregulation, it is possible to identify individuals at risk of having adverse health outcomes at a prodromal or asymptomatic stage. Various statistical formulations have been proposed for calculating a comprehensive AI score using multiple biomarkers representing different physiological systems. The two datasets used in this thesis, with different age groups, sample sizes, health status of participants and sets of biomarkers, have been described. The AL biomarkers and methods used for AI score calculation in the respective datasets have also been outlined. The next five chapters present the results from analysis of these data sets. The next chapter, Chapter 5, describes the cross-sectional relationship between AL biomarkers and depressive symptoms in DepChron patients.

Chapter 5 Cross Sectional Analysis in DepChron Dataset

5.1 Chapter Summary

This chapter provides the results of investigations of the cross-sectional relationship between depressive symptoms and allostatic load biomarkers in patients with existing cardiometabolic disease (DepChron cohort). The objective of this analysis is to explore the aetiology of depressive symptoms in cardiometabolic disease, without attributing any causation due to the cross-sectional nature of the analysis. This addresses research questions 1 and 2 using the DepChron dataset. These research questions are:

Research Question 1 (RQ1):

What is the association, if any, between a composite Allostatic Index (AI) score calculated using available allostatic load biomarkers and depressive symptoms in patients with existing cardiometabolic disease (DepChron cohort)?

2.   Research Question 2 (RQ2):

What is the association, if any, between individual allostatic load biomarkers and depressive symptoms and how does it compare with the relationship between the composite AI score and depressive symptoms in patients with existing cardiometabolic disease (DepChron cohort)?

This chapter has four sections: the first addresses the statistical methods used for this analysis; the second provides findings concerning the cross-sectional relationship between a composite AI score and depressive symptoms (assessed by HADS-D) (152) in DepChron; the third examines the relationship between individual AL biomarkers in DepChron and depressive symptoms is and compares the relationship of a composite AI score versus individual constituent biomarkers with depressive symptoms; finally, the various strengths and limitations of the analyses, comparison of findings with the existing literature and the potential implications are discussed in the final section.

5.2 Methods

5.2.1 AI Score Calculation and Depression Screening in DepChron

All statistical analyses undertaken using R statistical software version 3.2.0 (235).

The following five biomarkers were used for AI score calculation:

1.   Systolic Blood Pressure- SBP (Cardiovascular)

2.   Diastolic Blood Pressure-DBP (Cardiovascular)

3.   Body Mass Index-BMI (Anthropometric)

4.   Total Cholesterol (Metabolic)

5.   Glycosylated Haemoglobin-HbA1c (Metabolic)-only available in subset of patients with diabetes

The values for individual biomarkers were restricted to a clinically plausible range based on both clinical judgement and the findings of general population studies. SBP measurements were restricted to a range between 90 to 240 mm Hg and DBP to a range between 50 to 130 mm Hg (236,237). Similarly, BMI was restricted to a range between 15 to 55 (238), total cholesterol to 2-10 (239) and HbA1C to 3-18% (240). All observations with missing values were excluded from analysis.

Four different count based formulations for AI score calculation were used in the analyses and the reasons for choosing these methods are discussed in Chapter 4, section 4.4. The methodology employed for the four AI score methods was consistent with those used by previous studies and reported in the three systematic reviews on allostatic load studies (28,233,234).

These methods were:

1. Clinical cut-off method- Method 1: Each biomarker was assigned a score of 0 or 1 based on their value being below or above the clinical cut-off value respectively. The AI score was computed by adding the score for each constituent biomarker. For SBP and DBP, a clinical cut-off of 140 and 90 mm Hg was used respectively (241–244). BP was classified into five different categories based on clinical judgement to improve interpretability of results. The cut-offs used for BMI and total cholesterol levels were 30 kg/m2 and 5 mmol/l respectively (244) (245). Finally, the clinical cut-off for HbA1c was 7 DCCT(246).

2. Sample distribution cut-offs (25th and 75th percentile)- Method 2: This method was also based on a similar count based formula; however it used cut-offs both below and above the mean instead of using single clinically relevant cut-off used in method 1. The cut-offs were defined on the basis of an observation value below the 25th percentile or above the 75th percentile of the sample distribution. Similarly to method 1, a score of 0 or 1 was assigned based on the distribution of the observation value in relation to the cut-offs. The AI score was derived by adding the score for each constituent biomarker.

3. Sample distribution cut-offs (10th and 90th percentile) - Method 3: This method was the same as method 2, except that the 10th and 90th percentiles were used as cut-offs for this method.

4. Z-score method- Method 4: This method was based on a continuous distribution of a biomarker rather than categorical. Each biomarker was assigned a score, which was based on the absolute amount of deviation of the observation value from their respective sample mean. The z-score calculations were standardized so that a score of 1 corresponded to a difference between the observation value and its respective mean of one standard deviation (SD). The z-score was a positive value regardless of whether the value was below or above the mean, thereby giving the same weight to a very low value as a very high one. The allostatic index score

was calculated by adding the absolute z-score values for each constituent biomarker.

The depressive subscale of HADS (HADS-D), which gives a total potential score of 21, was used as a screening tool within the DepChron dataset. The threshold of HADS ≥8 was used as a cut-off for a 'positive screening result' as there is evidence to suggest that this offers an optimal balance of sensitivity and specificity (224,225) and such an approach has been endorsed by national guidelines (148). All patients who underwent depression screening were checked for a new prescription of antidepressants in the six months after the date of initial assessment. No reliable information was available on the number of patients who were referred for psychological therapies following their depression screening. Information on history of previous episodes of depression or history of antidepressants prescribed in the past, prior to the observation period was not available.

5.2.2 Statistical Modelling - Logistic Regression

Several logistic regression models were used with HADS-D≥8 as the outcome variable (20,152) and AI score as a predictor variable. The utility of the AI score calculated using the four different methods outlined above was compared, both in univariable and multivariable analysis. Age (as a continuous variable), and sex (as a categorical variable) were added to the regression models as potential confounders. Socio-economic status, divided into affluent (Scottish Index of Multiple Deprivation (SIMD)(29) scores 1-5) and deprived (SIMD 6-10), and number of conditions 1-3 out of diabetes, stroke and coronary heart disease (CHD) were also added as potential confounders. The cross-sectional association of the four different AI scores with depressive symptoms (HADS-D≥8) was analysed, after adjusting for the various potential confounders mentioned above.

In the next part of the analysis, the cross-sectional relationships between individual biomarkers and raised HADS-D were analysed using logistic regression models, including the aforementioned potential confounders. The quadratic terms for each individual biomarker were entered into regression models to allow for a non-linear relationship. The turning point for each individual

biomarker with a significant non-linear relationship was calculated using the formula (nadir/min = −b/2a or peak/max=b/-2a) where "a" represents the coefficient of the quadratic term and "b" represents the coefficient of the linear term. The turning point will be a "nadir" in the event of a "U-shaped" non-linear relationship, while the turning point will be a "zenith" in the event of a "bell-shaped" non-linear relationship. The strengths of these relationships were evaluated in univariable and multivariable analyses adjusting for the same potentially confounding factors age, sex, socio-economic status and number of conditions.

The entire analysis was first performed for the whole sample (N=35,537) and then repeated for the subset of patients with diabetes (N=18,453). In patients with diabetes, HbA1c was utilized for AI score calculation and the association between raised HADS-D and HbA1c was analysed, in addition to the other four biomarkers.

The results of each regression model were presented as odds ratios (OR) with 95% confidence intervals (CI) and corresponding p-values, for AI scores and individual biomarkers. Different logistic regression models used were compared by using the Hosmer and Lemeshow's R^2 measure (247) and Akaike information criterion (AIC) (248). Hosmer and Lemeshow's R^2 is a measure of how well a given regression model fits the data; a better value usually implies a better fit of the model to the data(247). AIC is a measure of the relative quality of how well a given regression model fits the data; a lower value implies a better fit (248).

In the next step, model diagnostics was performed for a selected few models by checking for the presence of multicollinearity using the parameter variance inflation factor (VIF <10). Presence of multicollinearity implies that two or more predictor variables in a model are highly correlated to each other. A logistic regression model assumes that there is no multicollinearity among its predictor variables.

To visualise the results of regression models, predicted probability graphs were constructed for a raised HADS-D (≥8) against corresponding values for the individual biomarkers and the AI score that was shown to have the best fit and predictability out of the four methods in regression modelling. A new data set

was constructed using all possible combinations of the potential confounders: age (18 to 90 in increments of 10), gender, socio-economic status (deprived and affluent) and number of co-morbid conditions (1-3). In the new data set, a predicted value was calculated for the probability of a raised HADS-D based on a range of values of significant predictors and the values of the potential confounders using their respective logistic regression modelling results. Finally, six different predicted probability charts were created for the probability of raised HADS-D against the best AI score and each of the five individual biomarkers.

5.2.3 Sensitivity Analyses

For a sensitivity analysis, interactions of the six predictors (best AI score and five individual biomarkers) with age, gender, number of comorbid condition and deprivation status were tested for each of the corresponding logistic regression models to check for potential effect modification. If any predictor was found to have a significant interaction with a confounder, the results of the interaction were visualised using the predicted probability charts.

In a subsequent sensitivity analysis, multivariable linear regression models were constructed using the HADS-D as a continuous variable. As HADS-D was not normally distributed, it was transformed using the square root transformation. Similarly, the best AI score and the five individual biomarkers were used as predictor variables in six different regression models. Age, sex, socio-economic status and number of comorbid conditions were added to all models as potential confounding factors. The quadratic terms for AI score and individual biomarker were added to regression models to allow for a non-linear relationship. Similarly, the turning point was calculated for each of the nine predictors using the formula explained above, in the event of a significant non-linear relationship. The results of linear regression were presented using standardized regression coefficients for predictors with 95% CI and p-values, adjusted R^2 values, AIC values. For individual biomarkers, regression estimates for their respective quadratic terms with CI were also presented, in the event of a significant non-linear relationship.

5.2.4 Individual Biomarkers vs. Multiple Biomarkers

In this part of analysis, DepChron patients were divided into eight categories based on their individual biomarker values after excluding missing values and values outside a clinically plausible range as explained earlier. The reference category was the group of patients with all four individual biomarkers (only SBP, only DBP, only BMI or only total cholesterol) within 1 standard deviation (SD) of the sample mean. The next four categories were the group of patients with only one of the four biomarker values outside 1 SD of the sample mean (only SBP, only DBP, only BMI and Only total cholesterol). The next two categories were the group of patients with at least two and three biomarkers respectively outside 1 standard deviation of the sample mean. Finally, the last category was the group of patients with all four biomarker values outside their respective 1 standard deviation ranges. The absolute number and percentage of patients in each of the eight categories with raised HADS-D (≥ 8) were compared. The entire analysis as described above was repeated for two standard deviation biomarker values instead of one standard deviation. Results were visualised with the help of frequency tables and bar charts.

5.3 Results

5.3.1 Results of Depression Screening, Biomarker and AI score distribution in DepChron

DepChron is a routinely collected data set with a suboptimal completion rate of a range of variables. A total of 125,143 patients were listed as having CHD, diabetes or stroke in the year 2008-09. Of the total sample, 10,670 (8.5%) patients were considered under treatment for depression, having received their first antidepressant prescription within the first 3 months of the observation period, and were thus exempt from screening. HADS-D results were recorded for 35,537 (31.1%) of those who were eligible for screening. In the remainder of the patients (n=78,936, 68.9% of eligible) depression screening was not recorded. Figure 5-1 shows the results of depression screening using HADS-D in the study population (n=35,537).

Figure 5-1 Results of Depression Screening in DepChron(226)
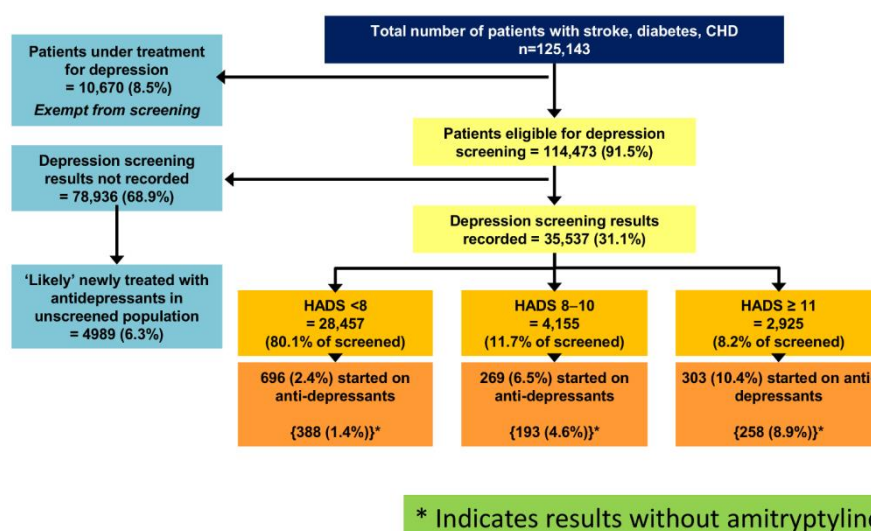
7,080 (19.9%) patients had a positive HADS-D result (HADS-D≥8) on depression screening in 2008-09, while nearly 4 in 5 who were screened (28,457) had

negative results. The majority of patients (4,155/7,080; 58.6%) with a positive result had a HADS-D score of 8-10 which is suggestive of mild depressive symptoms (152).

Table 5-1 describes the distribution of the five biomarkers measured in DepChron, their missing values, and the different cut-offs used for various AI score calculations. Most of the biomarkers had significant numbers of missing values. All missing values were excluded from the entire analysis. The clinical cut-off value was equal to the value of the 75th percentile for SBP, less than the value of the 75th percentile for BMI and more than the value of the 75th percentile for the rest of the three biomarkers. Except for DBP, the value of clinical cut-off was lower than the 90th percentile for all biomarkers.

Table 5-1 Distribution of Allostatic Biomarkers in DepChron (N=35,537).

Biomarker	Range	Missing Values	Method 1 AI score calculation (clinical cut-off)	Method 2 AI score calculation (25th/75th percentile)		Method 3 AI score calculation (10th/90th percentile)		Method 4 AI calculation (z-score method)
			Clinical cut-offs	25th percentile	75th percentile	10th percentile	90th percentile	Mean (Standard Deviation)
Systolic Blood Pressure in mm Hg	90 to 240	32139	140	120	140	110	155	133 (17.5)
Diastolic Blood Pressure in mm Hg	50 to 130	32139	90	69	80	60	88	74·5 (10.3)
Body Mass Index in kg/m^2	15 to 55	30139	30	24.92	34.03	22.43	36.51	28·9 (6.0)
Total Cholesterol in mg/dl	2 to 10	31311	5	3.6	4.8	3.1	5.6	4·3 (1.0)
HbA1c in DCCT N=18,453	3 to 18	15678	7	6.4	8.2	5.9	9.9	7·5 (1.6)

A small number of observations for each biomarker were excluded from the analysis as the observed values were outside the predetermined clinically plausible range: SBP 110 observations (SBP >240=0; SBP <90=110), DBP 167 observations (DBP >130=2, DBP < 50=165), BMI 97 observations (BMI>55=68; BMI<15=29) and total cholesterol 67 observations (total cholesterol >10=17; total cholesterol <2=50). Only 2 patients (HbA1c>18=0; HbA1c<3=2) had HbA1c observations outside the predetermined clinically plausible range.

Table 5-2 compares AI scores calculated using methods 1-4 described above, in the study population N=35,537 using four biomarkers (SBP, DBP, BMI and total cholesterol). The AI scores calculated by methods 1, 2 and 3 had big standard deviations relative to their mean value; the standard deviation calculated using method 3 AI score was larger than the mean. The range of AI score for the first three methods was 0 to 4, while the range was much larger for the method 4 AI score calculation. For method 1, the mean was less than 1 and the 75th percentile value was 1. This implies that most of the patients had on average only 1 individual biomarker value higher than the respective clinical cut-off value. The distribution of AI score with method 3 was very similar to method 1 with values of mean, median and 75th percentile not greater than 1.

Table 5-3 compares the AI score calculated with five biomarkers (above four plus HbA1c) in the sub-group of patients with diabetes (N=18,453) using the four different methods. Similar to the trend observed in the whole sample, patients with diabetes were found to have large standard deviations relative to the sample mean for AI scores calculated by methods 1 to 3. The range of AI score calculated using method 4 was much wider compared to methods 1 to 3, as observed in the whole sample.

Table 5-2 Distribution of AI score in the DepChron dataset (N=35,537) with four different statistical methods

N=35,537. Missing values and exclusions=10,162. Available N= 25,401				
Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Total Cholesterol				
	AI score- Method 1- clinical cut-off (Range 0-4)	AI score-Method 2- $25^{th}/75^{th}$ percentile (Range 0-4)	AI score-Method 3- $10^{th}/90^{th}$ percentile (Range 0-4)	AI score – Method 4- z score method (Range 0.2-12.4)
Mean	0.85	1.82	0.65	3.09
Standard Deviation	0.86	1.06	0.79	1.40
Median	1	2	0	2.86
25%- 75%	0-1	1-3	0-1	2.07-3.85

Table 5-3 Distribution of AI score in N=18,453 subset of DepChron patients with Diabetes using four different statistical methods

N=18,453 patients with Diabetes. Missing values and exclusions=7561. Available N=10,892				
Biomarkers used: Systolic Blood Pressure, Diastolic Blood Pressure, Total Cholesterol, Body Mass Index and HbA1c				
	AI score- Method 1 (Range 0-5)	AI score-Method 2 (Range 0-5)	AI score-Method 3 (Range 0-5)	AI score – Method 4 (Range 0.5-12.93)
Mean	1.54	2.34	0.87	3.85
Standard Deviation	1.07	1.18	0.91	1.60
Median	1	2	1	3.59
25%- 75%	1-2	2-3	0-1	2.70-4.69

5.3.2 Results of Logistic Regression Modelling

5.3.2.1 AI score as a predictor variable

AI scores calculated with all of the four methods described above were found to have a significant cross-sectional association with the probability of having a positive depression screening result (HADS-D≥8) in the univariable analysis. Table 5-4 compares the odds ratios with 95% confidence intervals from the logistic regressions of AI score on the odds of a positive depression screening result, along with Hosmer and Lemeshow's measure R^2 and AIC for the four different AI score methods. The AIC was the lowest and the Hosmer and Lemeshow's measure R^2 was the highest for method 4- this implies a better fit of model with method 4 AI score; however the difference between the four methods was not large.

The association between AI score and probability of positive HADS-D screening result remain unchanged after adjusting for various confounding factors such as age, sex, socio-economic status and number of co-morbid conditions (see Table 5-4). The effect sizes for AI scores for all four methods were reduced after adjusting for confounders but remained statistically significant predictors of raised HADS-D in all multivariable analysis models. The quadratic term for AI score was entered into multivariable regression models and it did not have a significant p-value; hence the association between AI score and raised HADS-D was linear in nature.

The odds ratio was biggest for the AI score calculated by method 2 (18% increase in odds, 95% CI 3 to 22%) with the provision that it was not comparable to method 4 (11%, 95% CI 9 to 13%). This implies that with each unit increase in AI score, the odds of raised HADS-D were increased by 18% if we use method 3 and 11% if we use method 4. The result of method 4 (z-score) is of greater clinical significance as the distribution of AI score is much wider with method 4 (range for AI score method 4= 0.2-12.4); while only a small number of patients (3515 patients) had AI score values >1 with method 3. So if we use method 3 AI score as compared to method 4 AI score, the significant relationship between AL biomarkers and depressive symptoms will be investigated for a much smaller proportion of DepChron patient.

Table 5-4 Logistic regression with AI score as a predictor and depressive symptoms (HADS-D≥8) as outcome variable.

	AI score- Method 1- clinical cut-off N=25,375 (Range 0-4)	AI score-Method 2- 25th/75th percentile N=25,375 (Range 0-4)	AI score-Method 3- 10th/90th percentile N=25,375 (Range 0-4)	AI score – Method 4- z score method N=25,375 (Range 0.2-12.4)
N=35,537. Missing values and exclusions=10,690. Available N= 25,401. HADS-D= Hospital Anxiety and Depression Scale-Depressive Subscale. AI=Allostatic Index. Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Total Cholesterol				
Univariable Analysis				
AI score-Odds Ratio	1.15	1.13	1.23	1.15
95% Confidence Intervals	1.11-1.19	1.09-1.16	1.18-1.27	1.12-1.17
p-value	p<0.001	p<0.001	p<0.001	p<0.001
Hosmer and Lemeshow's measure R^2	0.002	0.002	0.004	0.006
Akaike Information Criteria	25250	25249	25201	25149
Multivariable Analysis. Adjusted for confounder-age, sex, socio-economic status and number of co-morbid conditions				
AI score- Odds Ratio	1.07	1.10	1.18	1.11
95% Confidence Intervals	1.03-1.11	1.06-1.13	1.03-1.22	1.09-1.13
p-value	p<0.001	p<0.001	p<0.001	p<0.001
Hosmer and Lemeshow's measure R	0.032	0.033	0.035	0.036
Akaike Information Criteria	23948	23922	23891	23867

Moreover, the AIC was smallest for method 4 while the Hosmer and Lemeshow's measure R2 was largest for method 4 in multivariable analysis as well; although the difference between the four methods was not large. This implies that method 4 provided a better fit and representation of the data in both univariable and multivariable analyses for logistic regression models using the whole sample. Hence, method 4 AI score was used for predicted probability charts and sensitivity analyses. Model assumptions were checked for the multivariable model using method 4 AI score. The model assumption for logistic regression model- of multicollinearity using the parameter variance inflation factor (VIF <10) was held. This implies that the predictor variables used in the model were not highly correlated.

Key Findings (AI score as predictor variable):

- **AI score, calculated using SBP, DBP, BMI and total cholesterol and by applying four different statistical formulations, was found to have a statistically significant linear association with the presence of depressive symptoms as assessed by HADS-D≥8 (OR 1.15). The association persisted after adjusting for the effect of potential confounders.**

- **The model containing AI score calculated by method 4, the z-score method, had the best AIC and Hosmer and Lemeshow's measure R2 values which implied best fit to the available model.**

5.3.2.2 Individual Biomarkers as Predictor Variables

Each of the individual five biomarkers had statistically significant cross-sectional associations with the odds of having a positive HADS-D result. Each biomarker was found to have a non-linear association with the probability of HADS-D ≥8. Hence, a quadratic term for each biomarker was added to the respective logistic regression models. The results of univariable analyses with individual biomarkers are presented in Table 5-5.

The non-linear relationship between all individual biomarkers and HADS-D≥8 remained significant after adjusting for age, sex, socio-economic status and number of co-morbid conditions in multivariable analysis (see Table 5-5). The AIC was lowest and Hosmer and Lemeshow's measure R^2 was lowest for the model with BMI, which implies best fit of that model to the data. The observed nadirs (the value with lowest odds of having concurrent depressive symptoms) for all biomarkers (except SBP) for having a raised HADS-D were increased in value after adjusting for confounding factors. The nadirs for DBP and total cholesterol (74 and 3.6) were below their respective clinical cut-off values (90 and 5); BMI's nadir of 30.5 was very close to its clinical cut-off of 30 and the value of observed nadir of 148 was higher for SBP than its clinical cut-off of 140.

Table 5-5 Logistic Regression using individual biomarkers as predictors and depressive symptoms (HADS-D≥8) as outcome variable.

	Systolic Blood Pressure N=25,375 Range: 90 to 240	Diastolic Blood Pressure N=25,375 Range: 50 to 130	Total Cholesterol N=25,375 Range: 15 to 55	Body Mass Index N=25,375 Range: 2 to 10
N=35,537. Missing values and exclusions=10,690. Available N= 25,401. HADS-D= Hospital Anxiety and Depression Scale-Depressive Subscale.				
Univariable Analysis				
Odds Ratio (OR) with 95% Confidence Intervals (CI); p-value	0.95 (0.93-0.96); p<0.001	0.95 (0.92-0.98); p<0.001	0.79 (0.67-0.92); p=0.003	0.88 (0.85-0.91); p<0.001
OR for quadratic term with 95% CI; p-value	1.00016 (1.0001-1.0002); p<0.001	1.0003 (1.00016-1.00053); p<0.001	1.035 (1.018-1.051); p<0.001	1.0023 (1.0017-1.0029); p<0.001
Hosmer and Lemeshow's measure R^2	0.001	0.001	0.002	0.005
Akaike Information Criteria	25271	25294	25253	25169
Nadir	149	68	3.3	26.5
Multivariable Analysis. Adjusted for confounder-age, sex, socio-economic status and number of co-morbid conditions				
Odds Ratio (OR) with 95% Confidence Intervals (CI); p-value	0.95 (0.94-0.97); p<0.001	0.96 (0.93-0.99); p<0.001	0.80 (0.68-0.95); p=0.01	0.86 (0.83-0.89); p<0.001
Hosmer and Lemeshow's measure R^2	0.033	0.032	0.033	0.035
Akaike Information Criteria	23933	23957	23928	23897
Nadir	148	74	3.6	30.5

Overall, the model parameters AIC and Hosmer and Lemeshow's measure R^2 for BMI (the strongest individual biomarker predictor) and AI score calculated by method 4 were very similar in values (see Table 5-4 and Table 5-5). Model assumption of multicollinearity was checked for the multivariable model using the four individual biomarkers. The model assumption of multicollinearity using the parameter variance inflation factor (VIF <10) were satisfactory for all of the four regression models which implies that the predictor variables in these models were not highly correlated.

Key Findings (Individual biomarkers as predictor variables):

- **Individual biomarkers (SBP, DBP, BMI and total cholesterol) were found to have a "U-shaped" non-linear association with the probability of concurrent depressive symptoms (as assessed by HADS-D≥8), after adjusting for the effects of age, sex, socio-economic status and number of cardiometabolic conditions.**

- **The observed "nadir" or the values with the lowest probability of concurrent depressive symptoms were 148 for SBP, 74 for DBP, 3.6 for total cholesterol and 30.5 for BMI. The probability of having HADS-D positive was higher with biomarker values lower or higher than their respective nadir values.**

- **The strength of association between peripheral biomarkers and depressive symptoms was very similar to the observed associations between AI score and depressive symptoms.**

5.3.2.3 Results in Subset of Patients with Diabetes using Logistic Regression

In univariable analyses of the subset of patients with diabetes, AI score calculated by all four methods were found to have a statistically significant association with raised HADS-D ($\geq$8) (see Table 5-6).

In multivariable analysis after adjusting for various confounders, all AI scores were found to have a statistically significant relationship in predicting raised HADS-D. The effect sizes observed in multivariable analysis for the diabetes subset was very similar to the ones observed for the whole sample (see Table 5-4 and Table 5-6). For instance, with each unit increase in AI score calculated by method 4, the odds of raised HADS-D were raised by 11% (95% CI 8% to 15%) in the diabetes subset. This effect size was same as the one observed for method 4 AI score in the whole data. The z-score method (method 4) performed consistently better in all analyses judging by the model parameters (lower AIC and higher Hosmer and Lemeshow's measure R^2 value). The quadratic term for AI score was again found to have no significant effect on raised HADS-D in the subset of patients with diabetes.

Table 5-6 Logistic Regression in Diabetes subset using AI score as predictor variable and depressive symptoms (HADS-D≥8) as outcome variable.

N=18,453 patients with Diabetes. Missing values and exclusions=7561. Available N=10,892. Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Total Cholesterol and HbA1c. HADS-D= Hospital Anxiety and Depression Scale-Depressive Subscale				
Univariable Analysis				
	AI score- Method 1- clinical cut-off N=10,629 (Range 0-5)	AI score-Method 2- 25th/75th percentile N=10,629 (Range 0-5)	AI score-Method 3- 10th/90th percentile N=10,629 (Range 0-5)	AI score – Method 4- z score method N=10,629 (Range 0.5-12.93)
AI score – Odds Ratio with 95% Confidence Intervals; p-value	1.12 (1.07-1.17); p<0.001	1.16 (1.12-1.21); p<0.001	1.21 (1.15-1.28; p<0.001	1.14 (1.11-1.18); p<0.001
Hosmer and Lemeshow's measure R^2	0.002	0.005	0.005	0.008
Akaike Information Criteria	10794	10767	10763	10731
Multivariable Analysis. Adjusted for confounder-age, sex, socio-economic status and number of co-morbid conditions				
AI score – Odds Ratio (OR) with 95% Confidence Intervals (CI); p-value	1.09 (1.04-1.14); p<0.001	1.13 (1.08-1.18); p<0.001	1.17 (1.11-1.23); p<0.001	1.11 (1.08-1.15); p<0.001
Hosmer and Lemeshow's measure R2	0.036	0.038	0.038	0.040
Akaike Information Criteria	10172	10151	10151	10130

Table 5-7 Logistic Regression in Diabetes subset using HbA1c as predictor variable and depressive symptoms (HADS-D≥8) as outcome variable.

	HbA1c-univariable analysis N=10,892 (Range 3-18)	HbA1c-multivariable analysis N=10,629 Range (3-18) Confounders- age, sex, socio-economic status and number of co-morbid conditions.
OR with 95% CI; p-value	0.80 (0.67-0.97); p=0.02	0.81 (0.67-0.98); p=0.03
OR for quadratic term with 95% CI; p-value	1.016 (1.006-1.027); p=0.001	1.014 (1.004-1.025); p=0.006
Hosmer and Lemeshow's measure R^2	0.003	0.036
Akaike Information Criteria	10786	10169
Nadir	6.47	7.04

In the analysis using HbA1c alone as a predictor variable, HbA1c was found to have a non-linear association with the probability of positive depression screening result. Hence, a quadratic term for HbA1c was added to univariable and multivariable analyses and found to have a statistically significant association with the probability of raised HADS-D in logistic regression. The observed nadir for HbA1c in multivariable analysis was 7.04 DCCT which is very close to the clinical cut-off value of 7 (see Table 5-7).

Key Findings (Diabetes subset):

- **The observed trend in results from the whole sample was unchanged for the diabetes subset.**

- **AI score (OR 1.14) , calculated using four biomarkers in the main analysis + HbA1c and by applying four different statistical formulations, was found to have a statistically significant linear association with the presence of depressive symptoms as assessed by HADS-D≥8.**

- **HbA1c was found to have a non-linear "U shaped" association with the possibility of having concurrent depressive symptoms (HADS-D≥8), with the observed nadir value at 7.04 DCCT.**

5.3.2.4 Predicted Probability Charts

Figure 5-2 Predicted Probability of raised HADS-D (≥8) against AI score (z-score method)shows the predicted probability of raised HADS-D against AI score (method 4; z-score) with 95% CI, based on the logistic regression model reported above. The probability of raised HADS-D has a linear relationship with AI score and ranged from approximately 25% to 45%.

Figure 5-2 Predicted Probability of raised HADS-D (≥8) against AI score (z-score method)
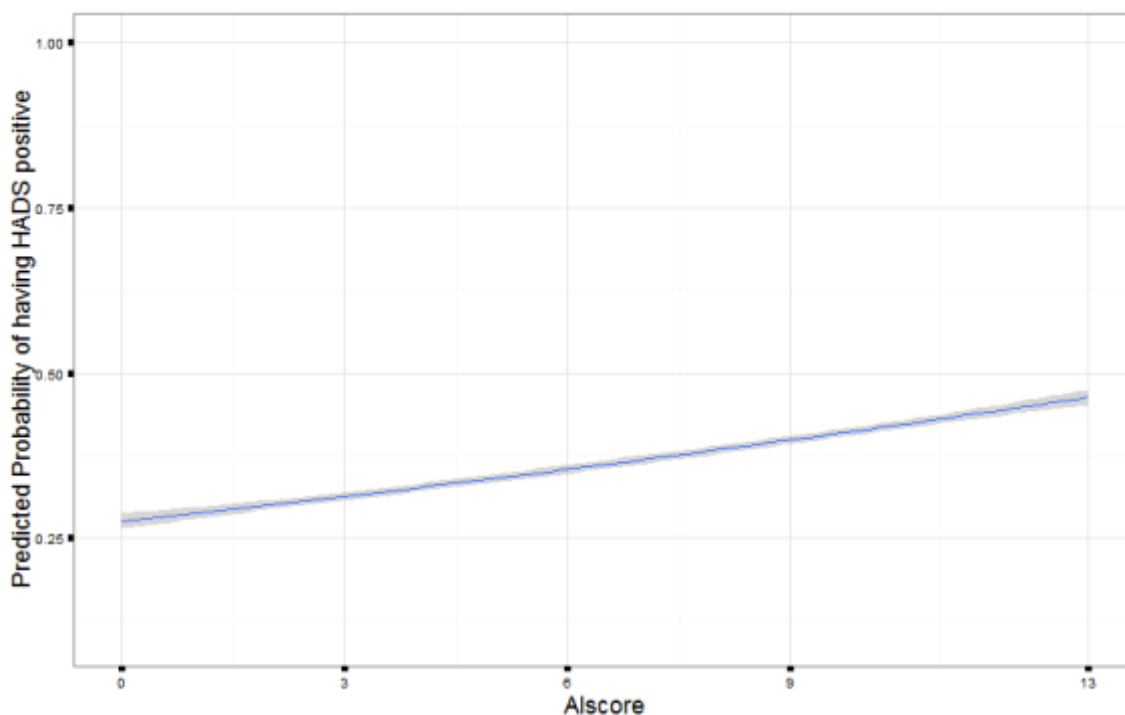
Figure 5-3 to Figure 5-6 are predicted probability charts (with 95% CI) for individual biomarkers- SBP, DBP, total cholesterol and BMI which were constructed using the logistic regression models for the whole sample. These charts demonstrate the non-linear relationship between each biomarker and probability of raised HADS-D. The chart for SBP is "U-shaped" while the charts for other biomarkers are "J-shaped".

Figure 5-3 Predicted Probability of raised HADS-D (≥8) against Systolic Blood Pressure
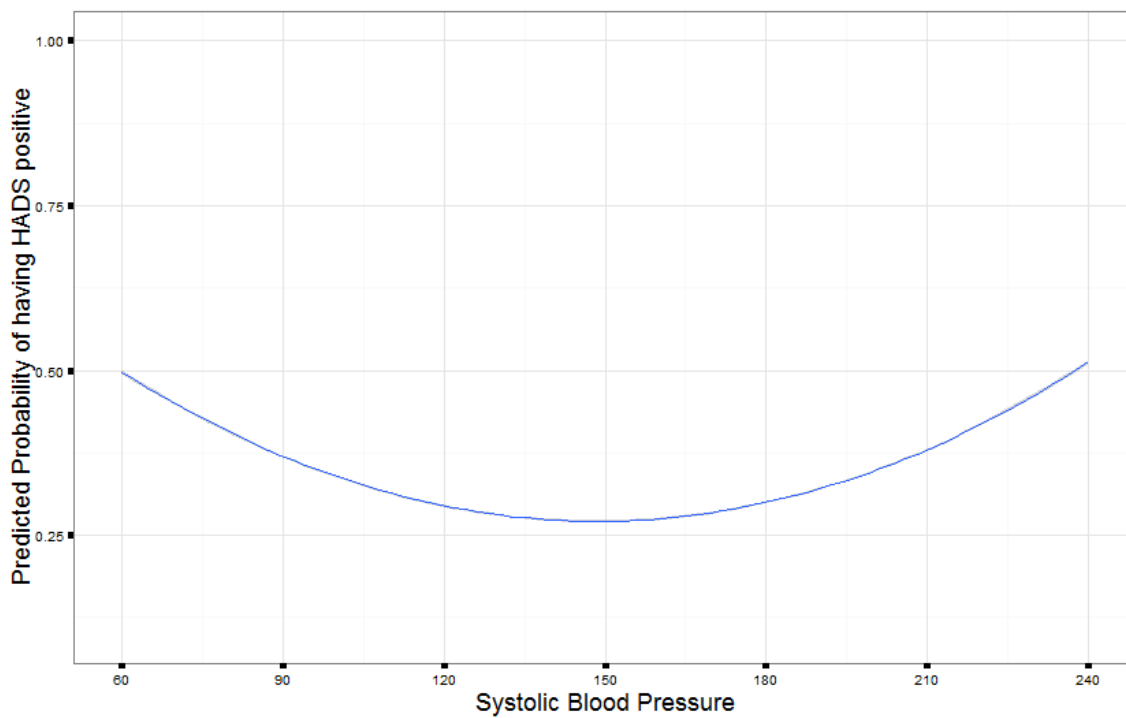
Figure 5-4 Predicted Probability of raised HADS-D (≥8) against Diastolic Blood Pressure
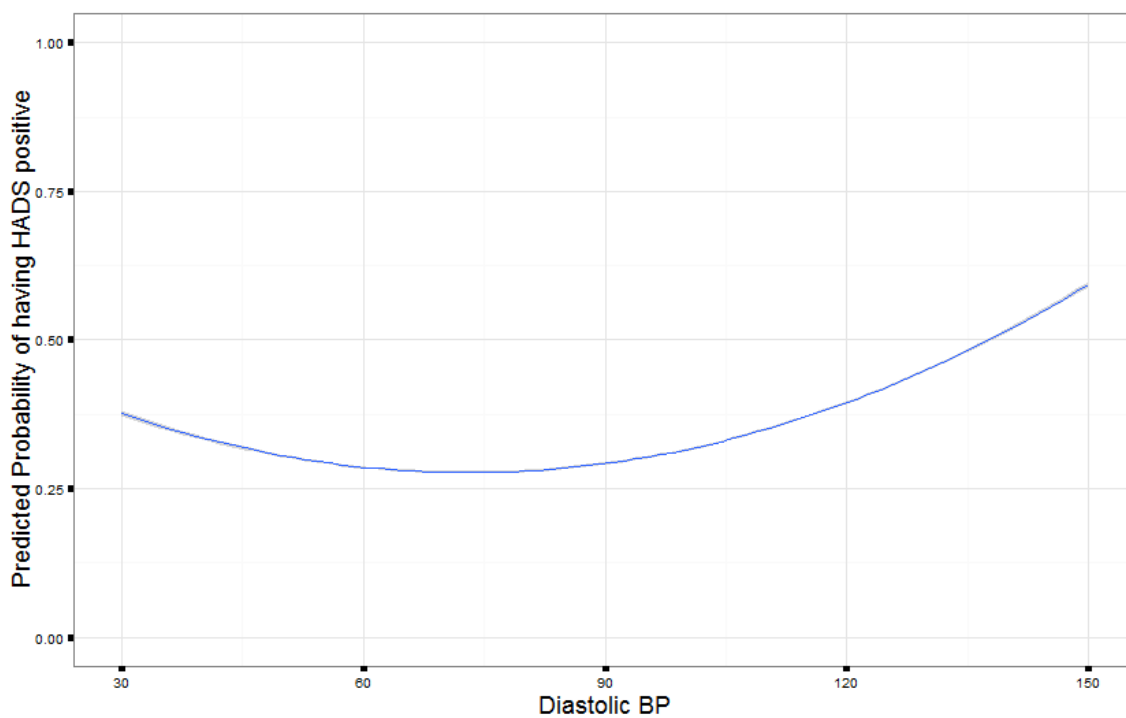
Figure 5-5 Predicted Probability of raised HADS-D (≥8) against Total Cholesterol.
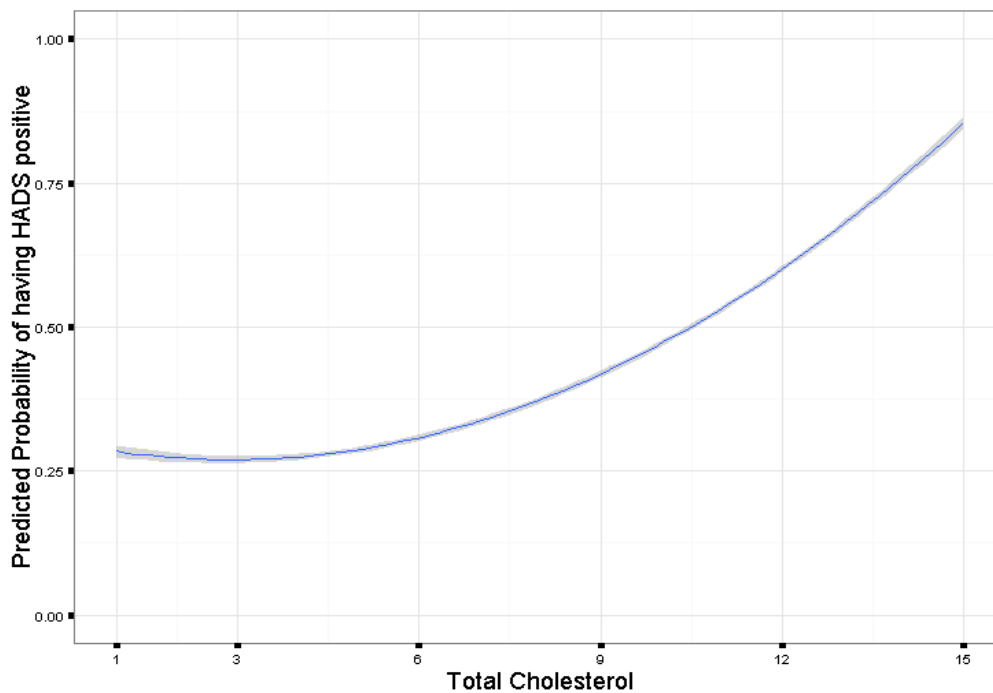
Figure 5-6 Predicted Probability of raised HADS-D (≥8) against Body Mass Index.
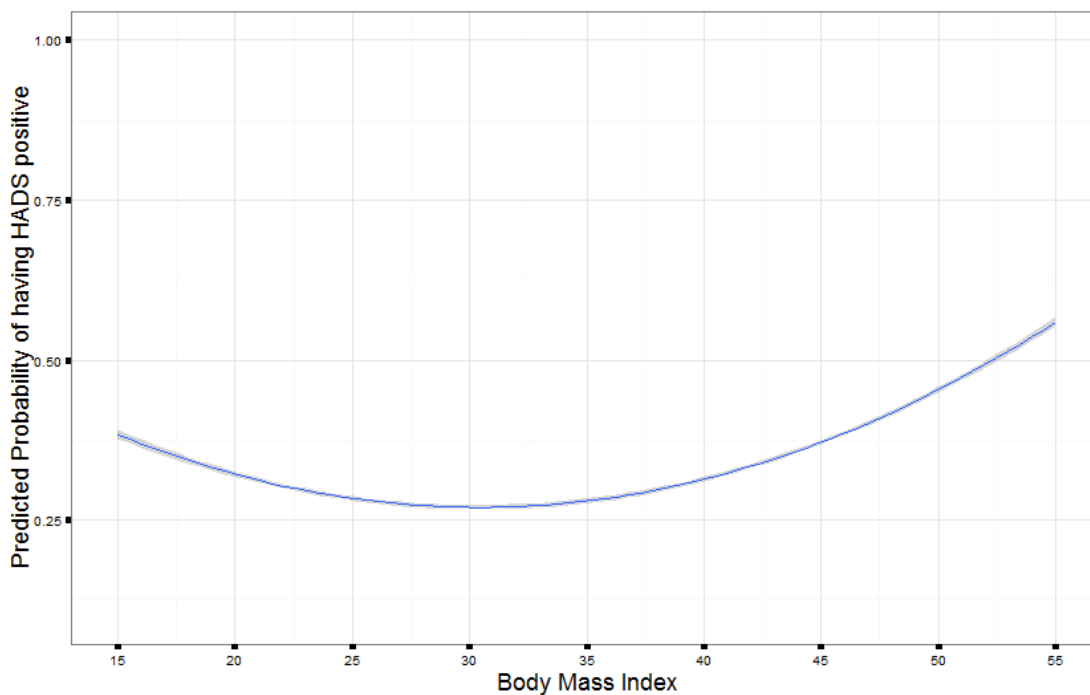
Using the subset of patients with diabetes, a predicted probability chart was constructed for raised HADS-D against HbA1c based on the results of

multivariable logistic regression model. Figure 5-7 demonstrates a "J-shaped" relationship between HbA1c and concurrent depressive symptoms.

Figure 5-7 Predicted Probability of raised HADS-D (≥8) against HbA1c for patients with Diabetes.
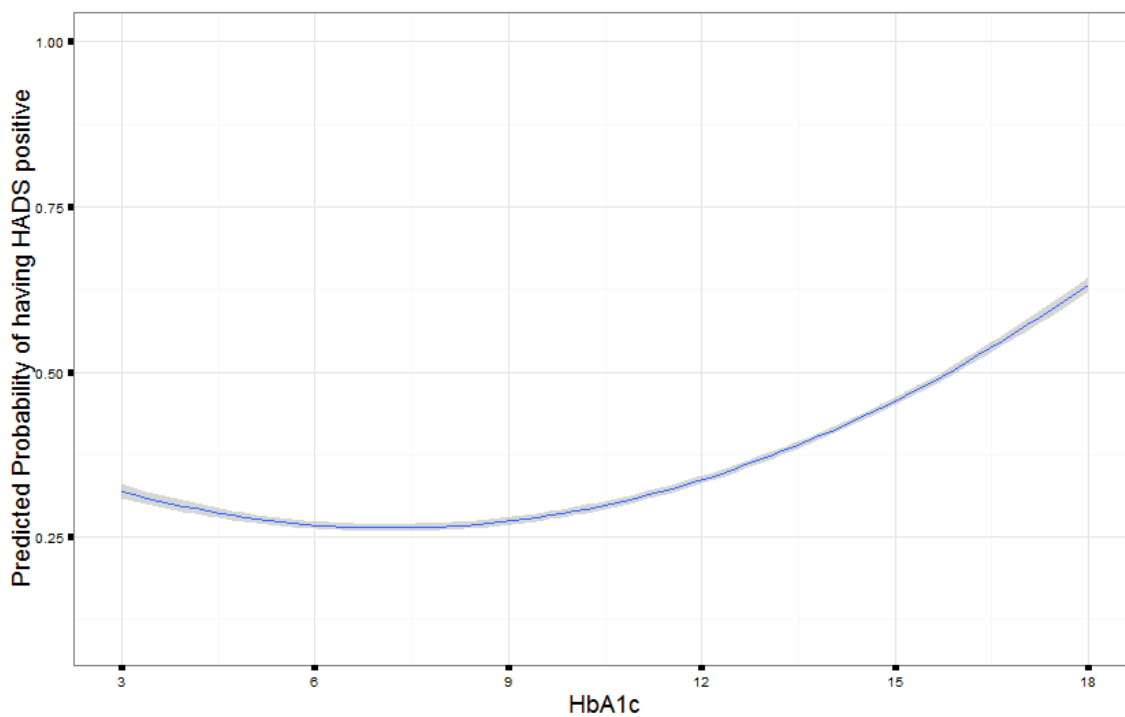
5.3.3 Sensitivity Analysis

5.3.3.1 Results of Interaction Analysis

In the multivariable analysis for the whole sample, AI score (method 4-z-score) was found to have a statistically significant interaction with age **(p=0.01)**, but no significant interactions with sex (p=0.61**)**, socio-economic status (p=0.051) and number of co-morbid conditions (p=0.65). Patients in the younger age group (18-64) had a larger increase in probability of a raised HADS-D with a rise in AI score as compared to those in the older age group (65-90). These results are visualised using the predicted probability chart in Figure 5-8.

Figure 5-8 Predicted Probability of raised HADS-D (≥8) against AI score (z-score method) for the two different age groups.
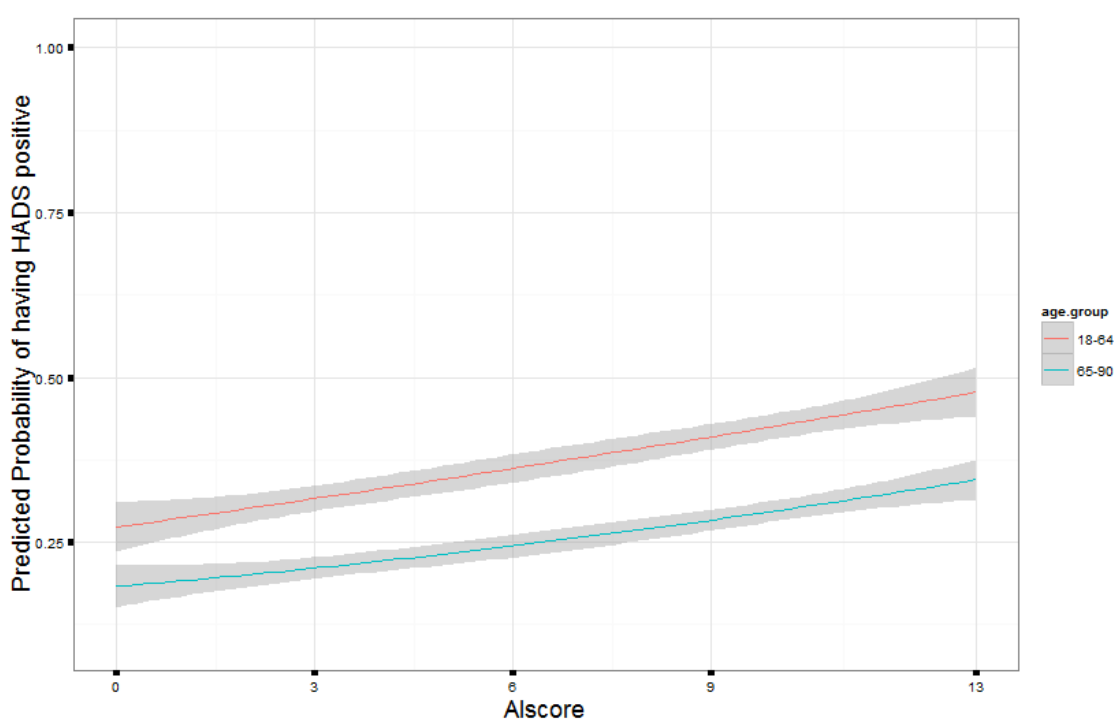
In the interaction analyses for individual biomarkers, none of the individual biomarkers were found to have a significant interaction with any of the confounding factors. In the multivariable analysis for the whole sample:

- SBP did not have a significant interaction with age (p=0.96), sex (p=0.18), socio-economic status (p=0.10) or number of co-morbid conditions (p=0.94).

- DBP did not have a significant interaction with age (p=0.87), sex (p=0.12), socio-economic status (p=0.88) or number of co-morbid conditions (p=0.98).

- Total cholesterol did not have a significant interaction with age (p=0.86), sex (p=0.84), socio-economic status (p=0.38) or number of co-morbid conditions (p=0.89).

- BMI did not have a significant interaction with age (p=0.87), sex (p=0.51), socio-economic status (p=0.41) or number of co-morbid conditions (p=0.27).

Finally, in the multivariable analysis for the subset of patients with diabetes, HbA1c did not have a significant interaction with age (p=0.14), sex (p=0.16), socio-economic status (p=0.29) or number of co-morbid conditions (p=0.32).

5.3.3.2 Results of Linear Regression Modelling

A similar trend in relationship was observed between AI score and individual biomarkers and HADS-D in multivariable linear regression models using HADS-D as a continuous outcome variable. AI score (method 4; z-score) was found to have a positive linear relationship with HADS-D; standardized regression coefficient was 0.051 for AI score. All individual biomarkers were found to have a non-linear relationship. The strengths of relationship and the model parameters were very similar between composite AI score and individual biomarkers (see Table 5-8).

The values of nadirs for the four biomarkers (SBP, DBP, total cholesterol and HbA1c) observed in linear relationship were similar to the ones observed in logistic regression models (compare Table 5-5 &Table 5-7 with Table 5-8). However, for BMI the observed nadir was approximately 20% higher with linear regression at 36.2 as compared to the nadir value of 30.3 observed with logistic regression.

Key Findings (Sensitivity Analysis):

- **AI score (calculated by z-score method in the whole sample and by using SBP, DBP, BMI and total cholesterol) was found to have a statistically significant interaction with age categories in predicting probability of concurrent depressive symptoms (HADS-D≥8).**

- **The increase in probability of having HADS-D≥8 with increase in AI score was significantly higher in the younger and the middle age group (18-64) as compared to the older age group (65-90).**

- **The relationship of AI score and individual biomarkers with concurrent depressive symptoms was unchanged using HADS-D as a continuous outcome variable in linear regression models.**

Table 5-8 Multivariable Linear Regression Models with HADS-D as continuous outcome variable.

N=35,537. Missing values and exclusions=10,690. Available N= 25,401. Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Total Cholesterol. HADS-D as outcome variable after square root transformation. Confounders- age, sex, socio-economic status and number of co-morbid conditions. * For HbA1c subset of patients with Diabetes was used (N=18,457, missing values and exclusions=7824).

	AI score – Method 4- z score method N=24,847	Systolic Blood Pressure N=24,847	Diastolic Blood Pressure N=24,847	Body Mass Index N=24,847	Total Cholesterol N=24,847	HbA1c* N=10,629
Standardized regression coefficient with 95% Confidence Intervals (CI); p-value	0.051 (0.043-0.059; p<0.001	-1.72 (-2.36 to -1.08); p<0.001	-1.98 (-3.12 to -0.008); p<0.001	-0.072 (-0.087 to -0.05); p<0.001	-0.09 (-0.15 to -0.027); p=0.005	-0.08 (-0.15 to -0.007); p=0.03
Standardized regression coefficient for quadratic term with 95% CI; p-value	Not applicable	5.99 (3.68 to 8.30); p<0.001	1.32 (0.0002 to 5.83); p<0.001	0.0012 (0.0010 to 0.0014); p<0.001	0.012 (0.006 to 0.189); p<0.001	1.006 (1.001-1.010); p=0.004
Adjusted R^2	0.040	0.037	0.036	0.041	0.038	0.040
Akaike Information Criteria	66417	66540	66557	66443	66528	28773
Nadir	Not applicable	143	75	36.2	3.59	6.65

5.3.4 Individual Biomarkers vs. Multiple Biomarkers

5.3.4.1 Impact of Biomarker Values outside 1 Standard Deviation (Individual versus Multiple Biomarkers)

The range for mean +/- 1 SD was 116 to 150 for SBP, 64 to 84 for DBP, 23.2 to 34.4 for BMI and 3.3 to 5.3 for total cholesterol. Table 5-9 shows a steady incremental rise in percentage of patients with raised HADS-D across the eight categories. These categories were: patients with no biomarker values outside 1 SD, four categories of having only one biomarker value outside 1 SD, patients with two, three and four biomarkers values outside 1 SD. Interestingly, the rise in percentage was proportionately smaller for patients with 2 biomarkers outside 1 SD as compared to those with only 1 biomarker value outside 1 SD of mean. On the other hand, the rise in percentage was much higher for patients with all four biomarkers outside 1 SD as compared to those with 3 biomarkers values outside 1 SD of sample mean.

The absolute count of patients across the eight categories of patients can be compared in Figure 5-9. The figure shows that the highest number (30.9%) of patients was those with all four biomarkers within 1 SD of sample mean and lowest number (1.1%) of patients had all four biomarkers outside 1 SD. There were proportionately a large number of patients (22.5%) with two biomarkers outside 1 SD of sample mean. Overall, patients with all four biomarker values outside 1 SD had much higher percentage of patients with raised HADS-D.

Table 5-9 Percentage of raised HADS-D (≥8) for DepChron patients with biomarker values outside 1 standard deviation of sample mean.

Patient Categories based on 4 biomarker values (Systolic Blood Pressure-SBP, Diastolic Blood Pressure-DBP, Body Mass Index-BMI and Total Cholesterol) N=25,375 (after exclusions and missing values)							
	No biomarkers n=7850	Only SBP n=1905	Only DBP n=2260	Only BMI n=2858	Only Total Cholesterol n=2528	2 Biomarkers n=5710	3 Biomarkers n=1959	4 Biomarkers n=303
Percentage with raised HADS-D	16.4%	18.6%	17.6%	22.2%	20.0%	22.6%	24.1%	30.6%

Figure 5-9 Bar Chart for DepChron patients with biomarker values outside 1 standard deviation and frequency of raised HADS-D.
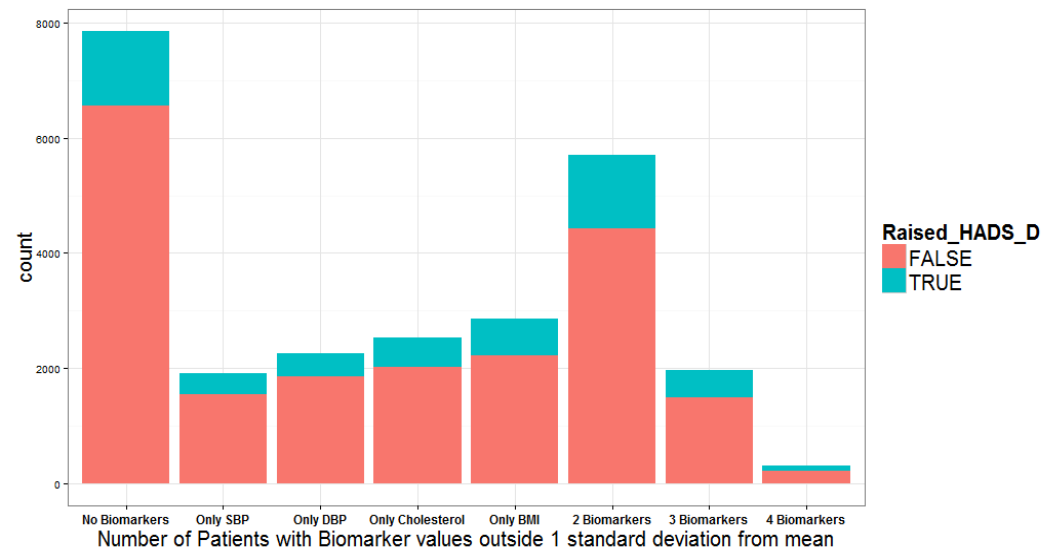
5.3.4.2 Impact of Biomarker Values outside 2 Standard Deviations (Individual versus Multiple Biomarkers)

The range for mean +/- 2 SD was 99 to 167 for SBP, 54 to 94 for DBP, 17.6 to 40.0 for BMI and 2.3 to 6.3 for total cholesterol. The results for analysis with 2 SD biomarker values were very different to that of 1 SD. Table 5-10 shows that the variation in percentage of patients with raised HADS-D was very high and not incremental.

There were very few patients with two or more biomarker values outside 2 SD of the sample mean. Hence, it is difficult to compare the frequency of raised HADS-D across different patient categories in this analysis (see Figure 5-10). The most relevant part of this analysis was that the majority of patients had biomarker values within 2 SD of the sample mean.

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Key Findings (Individual biomarkers vs. multiple biomarkers):

- **Patients observed to have multiple biomarker values outside the mean +/- 1 SD range were incrementally more likely to have a raised HADS-D as compared to patients with no or only one biomarker value within the specified range.**

- **There were relatively fewer patients with biomarker values outside the mean +/- 2 SD range and hence it was not possible to make the comparison between individual and multiple biomarkers effectively.**

</div>

Table 5-10 Percentage of raised HADS-D (≥8) for DepChron patients with biomarker values outside 2 standard deviations of sample mean.

Patient Categories based on 4 biomarker values (Systolic Blood Pressure-SBP, Diastolic Blood Pressure-DBP, Body Mass Index-BMI and Total Cholesterol) N=25,375 (after exclusions and missing values)								
	No biomarkers n=21218	Only SBP n=825	Only DBP n=774	Only BMI n=1006	Only Total Cholesterol n=946	2 Biomarkers n=527	3 Biomarkers n=75	4 Biomarkers n=4
Percentage with raised HADS-D	18.6%	22.9%	21.5%	29.3%	26.8%	28.8%	24%	100%

Figure 5-10 Bar Chart for DepChron patients with biomarker values outside 2 standard deviations and frequency of raised HADS-D.
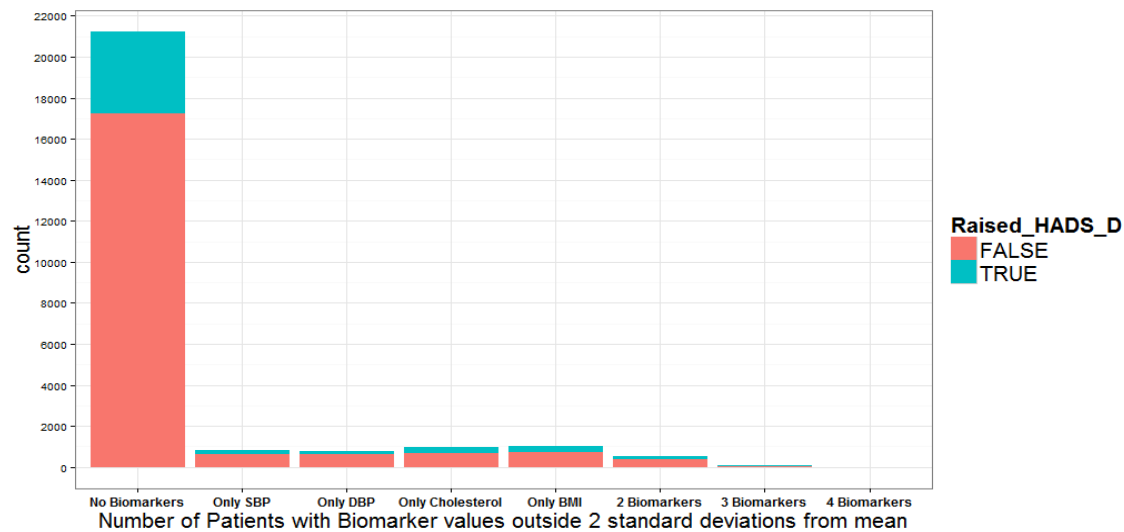
5.4 Discussion

5.4.1 Summary of Findings

AI score had a statistically significant association with concurrent depressive symptoms measured by raised HADS-D (≥8) in patients with existing cardiometabolic disease. This relationship was linear in nature. The regression model using AI score calculated by method 4 (z-score method) had better model parameters as compared to the models using the other three AI score calculation methods, but the differences between the regression models using different AI score methods were small. All individual biomarkers used in AI score calculation, SBP, DBP, total cholesterol, BMI and HbA1c (in diabetes subset), were found to have a non-linear relationship with concurrent depressive symptoms. The non-linear relationships were "J-shaped" and "U-shaped". Method 1 of AI score calculation (clinical cut-off method) was unable to utilize the strengths of relationships as method 1 was one tailed. These relationships remained significant after adjusting for potential confounders such as age, sex, socio-economic status and number of co-morbid conditions. Although there were non-linear relationships, in general the risk of concurrent depressive symptoms was higher with very high biomarker values than very low biomarker values with SBP being a notable exception.

The strength of association between AI score and depressive symptoms was very similar to the observed associations between individual constituent biomarkers and depressive symptoms. Thus, AI score as a construct did not offer any additional benefits over using individual biomarkers for this population and these biomarkers. An important caveat of this finding is that the biomarkers used for AI score in this analysis were secondary biomarkers (only those which were clinically available) and there were no inflammatory or neuroendocrine biomarkers available. AI score had a significant interaction with age; patients in the younger age group (18-64) have more quickly increasing risk of concurrent depressive symptoms with increasing AI score as compared to the older age group (65-90).

The trends in results were unchanged after repeating the analyses with linear regression models using HADS-D as a continuous outcome variable. The group with multiple biomarker values outside 1 SD of the sample mean had a greater number of patients with raised HADS-D as compared to those with either none or a single biomarker value outside 1 SD; with an incremental rise in percentage of raised HADS-D seen with each increase in the number of biomarkers outside 1 SD range.

5.4.2 Strengths

There are certain key strengths of these analyses. They are:

1. The DepChron dataset has a large sample size with a good distribution of demographic factors.

2. The data was collected in routine clinical practice and not in a simulated environment.

3. Depression screening was conducted in individuals with cardiometabolic disease using a standardized and validated screening tool in HADS-D (152).

4. These findings have clinical applicability as the biomarkers used for AI score calculation are those which are used in routine clinical practice.

5.4.3 Limitations

There are several limitations of these analyses. They are discussed in detail below along with their possible implications for the interpretation of the observed results where applicable.

1. A majority of patients with cardiometabolic disease in DepChron were not screened for depression. There are several potential explanations for this. The observed uptake for depression screening was much lower when compared with the uptake for depression screening in the Quality and Outcomes Framework (QOF) programme in Scotland (90.5%) for the year 2008-09 (249). However, the QOF programme had target driven incentivisation where practices were only paid if they achieved 90%

coverage in depression screening. In comparison, the incentivisation for the LES programme used in this study depended on achieving a proportion of clinical indicators across the health assessment protocol so there were no direct monetary benefits of performing depression screening. Secondly, the screening tool used for the QOF programme consisted of two stem questions which are much quicker to administer when compared to the HADS-D questionnaire which consists of 7 items (149).

In addition the other influencing factors for the observed poor uptake of depression screening could be previously reported barriers to discussing depression (or mental health) in patients with chronic disease in primary care, such as stigma associated around the 'label' and physicians' preconception of normalizing depression in patients with chronic disease (250,251).

2. The observed association between allostatic load biomarkers (composite score and individual biomarkers) and depressive symptoms could be due to reverse causality. The extreme values of individual biomarkers could be a sign of the severity of the underlying cardiometabolic disease which may influence the prevalence of co-morbid depressive symptoms.

3. Moreover, the study only included those patients who were able to attend their GP practice for annual health assessment. Hence, housebound patients were excluded who may have more severe form of illness.

4. The results may not be truly due to depression screening. GPs or practice nurses may have reviewed a patient whom they consider to have depression and thus targeted depression screening in some way.

5. Since only a minority of the patients were actually screened, depression status was unknown for a large number of patients, which remains an important limitation. There may be important differences between patients who underwent depression screening and those who did not, which are not clearly evident from their baseline demographic data. The

demographic characteristics between patients who were screened for
depression and those who were not are compared in chapter 4.

6. We did not have complete information on biobehavioural factors such as
   smoking status, alcohol intake and levels of physical activity which are
   likely to influence the values of cardiovascular risk factors considered and
   also the prevalence of depressive symptoms (252–255).

7. We also did not have information on history of previous episodes of
   depression for patients in our study which may influence the prevalence
   levels for depressive symptoms.

8. Finally, the overall accuracy of depression screening in our study was
   reliant on HADS-D which is a self-reported measure and it is reported to
   have moderate sensitivity and specificity in assessing depressive
   symptoms in patients with cardiometabolic disease in a primary care
   setting (114,256,257).

5.4.4 Comparison of Findings with Existing Literature

There have been five previous studies analysing the cross-sectional relationship
between AI score and depressive symptoms (209,216–219) (Please see Table 4-3
in Chapter 4). There are several important differences between the findings in
DepChron and the existing literature. Firstly, AI score calculation in DepChron
only used five secondary biomarkers of AL construct while other studies in this
area have used more biomarkers (range: 7-17 biomarkers) and have included
both primary and secondary AL biomarkers for AI score calculation. Secondly,
participants in existing studies have been recruited from the general population
while DepChron patients were recruited from primary care and all patients had
an existing diagnosis of cardiometabolic disease. On the other hand, DepChron
has a larger sample size. Out of the five previously published studies, two
studies (209,216) used the Social Environment and Biomarkers of Ageing Study
(SEBAS) cohort with a sample size of 820 participants; while the other 3 studies
had a sample size of fewer than 150 participants (217–219). DepChron is also
different from previous studies of AI score and depressive symptoms in the
method of depression assessment with three studies using CES-D (Centre for

Epidemiological Studies- Depression Scale)(209,216,219); while GDS (Geriatric Depression Scale)(217) and BDI (Beck Depression Inventory)(218) were used by one study each. These scales are also self-reported depression symptom questionnaires and similar in sensitivity and specificity to HADS-D (257).

Only four out of five studies analysed the cross-sectional association between depressive symptoms and AI score; while 1 study only looked at AI score at baseline and depressive symptoms at three years of follow-up (209). Selpaki et al did not report any regression estimates for cross-sectional association between a composite AI score and depressive symptoms (216); while the study by Juster et al reported no significant association between AI score and depressive symptoms assessed by BDI(218). A standardized regression coefficient of 0.44 was reported in multivariable linear regression for cross-sectional association between AI score and GDS(217); while a regression estimate of 1.44 (standardized regression coefficient was not reported) was observed in multivariable linear regression for cross-sectional association between AI score and CES-D(219). The regression estimate for multivariable linear regression for AI score and HADS-D was 0.05 and standardized beta co-efficient was 0.07 in DepChron. None of the five studies used logistic regression models to study the association between AI score and depressive symptoms.

The relationship between individual AI biomarkers used in DepChron and depressive symptoms has been previously studied, mainly in the general population. Barrett-Connor et al reported a non-linear relationship between DBP and depression with an observed nadir of 75 mm Hg DBP for concurrent depressive symptoms in a general population sample (258). In various cross-sectional studies involving mainly elderly populations, depression has been observed to have a non-linear association with SBP (259–262) and DBP (258,263). Similarly, increased prevalence of depressive symptoms has been observed with extreme values of total cholesterol (264,265) and HbA1c in general population samples (266), in a non-linear trend. There is as yet no published literature to the best of my knowledge that examines the relationship between these biomarkers and depressive symptoms in those with cardiometabolic disease. A number of these population studies have found a significant cross-sectional association between cardiovascular biomarkers and depressive symptoms, even after adjusting for lifestyle factors (such as smoking, alcohol and physical

activity) (262,265,266). Non-linear relationships between extreme values of SBP, DBP, BMI and HbA1c and adverse clinical outcomes such as increased incidence of vascular events and deaths in patients with cardiometabolic conditions have been reported extensively (267–271).

A previous meta-analysis has studied the nature of cross-sectional association between depressive symptoms and having abnormal levels of multiple biomarkers (central obesity, hyperglycaemia, elevated blood pressure, hypertriglyceridemia, and decreased HDL cholesterol) (272). However, direct comparison between the findings of this study and the meta-analysis is not feasible mainly because the cluster of biomarkers used in the meta-analysis are different from those used in DepChron (272). Secondly, the meta-analysis only investigated the relationship between depressive symptoms and cardiovascular biomarker values higher than clinical cut-offs and did not examine the possibility of non-linear relationship (272). This meta-analysis reported a cross-sectional association between a cluster of cardiovascular biomarkers and depressive symptoms based on findings of 27 cross-sectional studies, with most of the primary studies adjusting for lifestyle factors such as smoking, alcohol and physical activity levels (272).

5.4.5 Implications of Findings

There are three potential implications of the findings. Firstly, for most of these cardiovascular biomarkers it is still better to have very low than very high values, as far as concurrent association of depressive symptoms is concerned.

Secondly, if the association between extreme values of these biomarkers or cardiometabolic risk factors with depressive symptoms in those with cardiometabolic disease is supported by prospective studies, then this relationship could be used to identify those at "high risk" of depression. This would then offer a mechanism for targeting of depression screening in those with cardiometabolic disease. These results need to be replicated using other datasets and also prospectively to further explain the nature and direction of the observed association between depressive symptoms and cardiometabolic biomarkers. Such further investigation is necessary in order to determine whether the lower values of these biomarkers is due to other disease processes

(for example, low total cholesterol levels associated with malnutrition, liver diseases and haematological diseases) (273-275) that may make patients more vulnerable to experiencing depressive symptoms.

Finally, AI score did not offer any added clinical value in predicting concurrent depressive symptoms, over and above the use of individual constituent biomarkers on their own. This may be due to the selection of patients with existing cardiometabolic disease and use of only secondary cardiovascular biomarkers for AI score calculation.

5.4.6 Conclusion

In a large community dwelling sample of patients with existing cardiometabolic disease, dysregulation of allostatic biomarkers (mainly cardiometabolic) was found to have a non-linear association with increased probability of co-morbid depressive symptoms. A composite AI score constructed using four or five biomarkers did not lead to any improvement in the observed strength of the association. Further investigation of the relationship between cardiometabolic biomarkers and depressive symptoms is needed, in order to determine whether they have potentially important implications for clinical practice in relation to risk stratification for secondary prevention in individuals with cardiometabolic disease and concurrent depressive symptoms.

In the next chapter, the findings from the cross-sectional analysis in Psobid dataset are presented.

Chapter 6 Cross-sectional Analysis in Psobid Dataset

6.1 Chapter Summary

In this chapter, the cross-sectional relationship between allostatic load biomarkers and depressive symptoms in the general population (using the psychological, social, and biological determinants of ill health (Psobid) cohort) is described. The objective of this analysis is to explore the eitiology of depressive symptoms in general population, without implying any causality. This addresses research questions 1 and 2 in Psobid dataset. These research questions are:

Research Question 1 (RQ1):

What is the association, if any, between a composite Allostatic Index (AI) score calculated using available allostatic load biomarkers and depressive symptoms in general population (Psobid cohort)?

2.   Research Question 2 (RQ2):

What is the association, if any, between individual allostatic load biomarkers and depressive symptoms and how does it compare with the relationship between composite AI score and depressive symptoms in general population (Psobid cohort)?

This chapter is divided into three sections: the first explains the statistical methods used; the second provides the results of the analysis of the cross-sectional relationship between a composite allostatic index (AI) score and depressive symptoms as assessed by the 28 items General Health Questionnaire (GHQ-28)(231) and a comparison of the association of individual allostatic biomarkers versus a composite score AI score with depressive symptoms; while the final section discusses the strengths and limitations of the analyses, how these findings fit with the existing literature and their potential implications.

6.2 Methods

6.2.1 AI Score Calculation and Depression Screening in Psobid

The 12 biomarkers used for AI score calculation are listed below with their respective allostatic load category. The four biomarkers with a * mark are those which were also used in the DepChron dataset for AI score calculation.

1.   Systolic Blood Pressure (SBP) (Cardiovascular) *

2.   Diastolic Blood Pressure (DBP) (Cardiovascular) *

3.   Body Mass Index (BMI) (Anthropometric) *

4.   Waist Hip Ratio (WHR) (Anthropometric)

5.   Total Cholesterol (Metabolic) *

6.   High Density Lipoprotein (HDL) Cholesterol (Metabolic)

7.   Triglycerides (Metabolic)

8.   Fasting glucose (Metabolic)

9.   Creatinine (Metabolic)

10.   Fibrinogen- (Inflammatory)

11.   Interleukin-6 (IL-6) (Inflammatory)

12.   Highly sensitive C - reactive protein (CRP) (Inflammatory)

Psobid was a research study led by the Glasgow Centre for Population Health (GCPH) with 666 participants recruited in 2006-07. The methods for recruitment and data collection for Psobid have been described in detail in the published literature (228–230) and a summary has been presented in Chapter 4-section 4.3.

Four different methods for AI score calculation were used in Psobid, similar to the analysis with the DepChron dataset. A brief description of these four methods and reasons for choosing these methods for AI score calculation have been discussed in Chapter 4 while these methods are described in detail in Chapter 5.

5. The clinical cut-off method- (Method 1)

6. The sample distribution cut-offs (25th and 75th percentile) method- (Method 2)

7. The sample distribution cut-offs (10th and 90th percentile) method – (Method 3)

8. The z-score method-(Method 4)

The inflammatory biomarkers in the Psobid study, highly sensitive CRP, IL-6 and fibrinogen, are not currently used in routine clinical practice and hence their clinical cut-offs are not very well defined. However, highly sensitive CRP has been recognised as an emerging cardiovascular risk factor and its use in cardiovascular risk assessment has been recommended in a recent guideline published by the American College of Cardiology (ACC) and American Heart Association (AHA) (276). Based on the recommendation of this guideline, a clinical cut-off score of highly sensitive CRP $\geq$ 2 was selected for use in this study (276). The approach of using the 75th percentile of a biomarker distribution as a cut-off for AI score calculation has been used by various other studies in the published allostatic load literature (28), when the biomarker in question does not have a widely accepted clinical cut-off. For IL-6 and fibrinogen, the 75th percentile value of the sample distribution was used as a cut off and values higher than the 75th percentile were labelled as abnormal.

Participants were screened in the Psobid study for the presence of psychological symptoms using the General Health Questionnaire (GHQ-28) score. The GHQ-28 consists of 4 sub-scales with 7 questions each: somatic symptoms, anxiety and insomnia, social dysfunction and severe depression(231). Each question is given a score of 0 or 1, giving a total score out of 28. A cut-off score of GHQ-28$\geq$5 was

used, as recommended by the national guidelines for case identification of depressive symptoms in a community setting (20).

6.2.2 Statistical Modelling-Logistic Regression

A raised GHQ-28 (≥5) was used as the outcome variable for logistic regression models. For raised GHQ-28, the strength of its cross-sectional association with AI score (calculated by the 4 different methods) in univariable and multivariable analysis was assessed. For multivariable analysis, adjustments were made for age (as a continuous variable), sex, socio-economic status (affluent and deprived), number of existing cardiometabolic conditions (coronary heart disease/stroke, diabetes, and hypertension), with a range from 0-3. These potential confounders were the same as those used in the DepChron analysis apart from the number of existing cardiometabolic conditions since there was no control group in DepChron, i.e. all patients in DepChron had at least one of the three cardiometabolic conditions. In addition, stroke and CHD were categorized as one problem in the Psobid dataset at the time of data collection, so it was not possible to differentiate between those who had stroke and those who had CHD among Psobid participants. Participant's smoking status (current vs. former smoker vs. non-smoker) was also used as a confounding factor. This information on smoking status was not available in the DepChron dataset.

In the next part of the analysis, the cross-sectional relationships between individual biomarkers and raised GHQ-28 was analysed using univariable logistic regression models. If individual biomarkers were found to have a significant association with raised GHQ-28, their respective quadratic terms were entered into the regression models to allow for a non-linear relationship. The turning point for each individual biomarker with a significant non-linear relationship was calculated using the formula (nadir/min = $-b/2a$ or zenith/max=$b/-2a$) where "a" represents the coefficient of the quadratic term and "b" represents the coefficient of the linear term. The turning point will be a "nadir" in the event of a "U-shaped" non-linear relationship, while the turning point will be a "zenith" in the event of a "bell-shaped" non-linear relationship. The strengths of these relationships were evaluated in multivariable analyses adjusting for the potentially confounding factors- age, sex, socio-economic status, number of cardiometabolic conditions and smoking status as described above. If a

confounder was found to be a non-significant predictor, it was removed from the final multivariable models.

The results of each regression model were presented as an odds ratio (OR) with 95% confidence intervals (CI) and corresponding p-values, for AI scores and individual biomarkers. Different logistic regression models were compared by using the Hosmer and Lemeshow's R^2 measure (247) and Akaike information criterion (AIC) (248). The model assumption for multicollinearity for a multivariable logistic regression model was checked for a selected few models- by checking for multicollinearity using the parameter variance inflation factor (VIF <10). Presence of multicollinearity implies that two or more predictor variables in a model are highly correlated to each other. A logistic regression model assumes that there is no multicollinearity among its predictor variables.

To visualise the results of regression models, predicted probability charts were constructed for only those predictors - AI score or individual biomarkers which were found to have a statistically significant association with raised GHQ-28 in logistic regression. Predicted probability graphs were constructed for a raised GHQ-28 against corresponding values for significant predictors with the best fit in regression modelling. A new data set was constructed using all possible combinations of the following confounders (only included if a confounder had statistically significant association): age (35 to 65 in increments of 10), gender, socio-economic status (deprived and affluent), number of cardiometabolic conditions (0-3) and smoking status (current/former smoker and non-smoker). In the new data set, a predicted value was calculated for the probability of a raised GHQ-28 based on a range of values of significant predictors and the values of the potential confounders using their respective logistic regression modelling results. Finally, different predicted probability charts were created for the probability of raised GHQ-28 against each significant predictor using their respective new data set.

6.2.3 Sensitivity Analysis

For a sensitivity analysis, the interactions of significant predictors (AI score or individual biomarkers) with confounders-age, gender, number of cardiometabolic conditions, socio-economic status and smoking status were tested to check for a

potential effect modification. If any predictor was found to have a significant interaction with a confounder, the results of the interaction were visualised using the predicted probability charts.

In a subsequent sensitivity analysis, multivariable linear regression models were constructed using GHQ-28 as a continuous variable. As GHQ-28 was not normally distributed, it was transformed using the square root transformation. The best AI score and the twelve individual biomarkers were used as predictor variables in 13 different regression models. Age, sex, socio-economic status, number of cardiometabolic conditions and smoking status were added to all models as potential confounding factors. Only significant confounders were added to the final multivariable model. The quadratic terms for individual biomarker were added to regression models to allow for a non-linear relationship. The turning point was calculated for each of the predictors using the formula explained above in the event of a significant non-linear relationship. The results of linear regression were presented using standardized regression coefficients for predictors with 95% CI and p-values, adjusted R^2 values and AIC values. For individual biomarkers, standardized regression coefficients for their respective quadratic terms with CI were also presented only in the event of a significant non-linear relationship.

6.3 Results

6.3.1 Results of Depression Screening, Biomarker and AI score distribution in Psobid

The results for GHQ-28 were recorded for 639/666 (95.9%) participants in the Psobid dataset. 183 participants (27.4%) had a raised GHQ-28 (≥5) which is consistent with having significant depressive symptoms.

Table 6-1 describes the distribution of the 12 biomarkers, their range and missing values, and different cut-offs used for various AI score calculations. Blood glucose and the 3 inflammatory markers had a higher number of missing values than the other markers. All missing values were excluded from the entire analysis. IL-6, highly sensitive CRP and triglycerides had high standard deviation values relative to their respective mean values. The value of the clinical cut-off was lower than the 75th percentile of sample distribution for SBP, WHR (for females), BMI, total cholesterol, HDL cholesterol and highly sensitive CRP. With the exception of fasting blood glucose and creatinine, the value of clinical cut-off was lower than the 90th percentile of sample distribution for all biomarkers. The observed range for SBP, DBP, total cholesterol and BMI in the Psobid dataset was within the limits of the clinically plausible range defined for these biomarkers in the DepChron data. These ranges were SBP: 90 to 240 mm Hg, DBP: 50 to 130 mm Hg, BMI: 15 to 55, and total cholesterol: 2-10.

Table 6-1 Distribution of Allostatic Biomarkers in Psobid (N=666).

Biomarker	Range	Missing Values	Method 1 AI Calculation Clinical cut-offs	Method 2 AI Calculation		Method 3 AI Calculation		Method 4 AI Calculation (Z-score method)
				25th percentile	75th percentile	10th percentile	90th percentile	Mean (Standard Deviation)
Systolic Blood Pressure in mm Hg	90 to 202	4	140	123.3	147	112	159	135.7 (18.8)
Diastolic Blood Pressure in mm Hg	52 to 119	4	90	74	88	68	95	81.2 (10.9)
Waist Hip Ratio	0.64 to 1.31	7	0.9 F 0.95 M	0.84	0.94	0.78	0.99	0.89 (0.08)
Body Mass Index in kg/m2	16.3 to 51.1	4	30	24.1	30.4	21.4	35.1	27.7 (5.5)
Total Cholesterol in mmol/l	2.4 to 8.6	21	5.0	4.4	5.8	3.8	6.4	5.1 (1.0)
High Density Lipoprotein Cholesterol in mmol/l	0.4 to 3.0	21	1.2	1.1	1.6	0.9	1.8	1.3 (0.3)
Triglycerides in mmol/l	0.3 to 10.8	21	2.0	0.9	1.7	0.3	2.6	1.5 (1.0)
Fasting Blood Glucose in mmol/l	3.2 to 19.8	54	6.0	4.8	5.4	4.5	5.9	5.2 (1.4)
Creatinine in umol/l	55.2 to 159.6	28	110 F 120 M	74.2	89.6	68.2	97.3	82.4 (12.1)
Highly sensitive C-reactive protein in mg/l	0.6 to 18.9	30	1	0.6	3.3	0.3	6.8	2.7 (3.4)
Interleukin 6 in pg/ml	0.2 to 11.8	37	NA	1.0	2.75	0.6	4.0	2.1 (1.5)
Fibrinogen in g/l	1.1 to 6.4	33	NA	2.8	3.7	2.5	4.2	2.8 (0.7)
M=Male; F=Female; NA=Not applicable.								

Table 6-2 compares the AI scores calculated with methods 1-4 in the Psobid study population N=666 using 12 biomarkers (SBP, DBP, BMI, WHR, total cholesterol, HDL cholesterol, triglycerides, creatinine, fasting Glucose, highly sensitive CRP, IL-6 and fibrinogen). The AI scores calculated by methods 1 and 4 had large standard deviations values relative to their respective mean value. The range of AI score for method 1 was from 0 to 10. This implies that there were some participants who had all of the 12 biomarker values less than the respective clinical cut-off values, which is appropriate as participants were recruited from the general population (as opposed to a setting) and likely to be healthier. The range of AI score was the widest for the method 4 AI score. In addition, the values of mean and median were very different for the method 4 AI score. The AI score calculated by method 3 had the narrowest total and interquartile range. Observations with missing values for AI scores were excluded from regression analysis.

Table 6-2 Distribution of AI score in the Psobid dataset (N=666) with four different statistical methods.

N=666. Missing values and exclusions=96.Available N=570. Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Waist Hip Ratio, Total Cholesterol, High Density Lipoprotein Cholesterol, Triglycerides, Fasting Glucose, Creatinine, Highly sensitive C-reactive Protein, Interluekin-6 and Fibrinogen.				
	AI score-Method 1- clinical cut-off method Range=0-10	AI score-Method 2- 25th/75th percentile method Range=1-12	AI score-Method 3- 10th/90th percentile Range=1-9	AI score – Method 4- z score method Range=3.7-22.3
Mean	3.3	5.7	3.0	8.5
Standard Deviation	2.1	2.0	1.5	8.0
Median	3.0	6.0	3.0	2.7
25%- 75%	2.0-5.0	4.0-7.0	2.0 – 4.0	6.6-9.9

6.3.2 AI score as a predictor variable

6.3.2.1 Univariable Analysis

In the univariable analysis in the Psobid dataset, AI score did not have a statistically significant association with depressive symptoms (defined by raised GHQ-28≥5). In logistic models with raised GHQ-28 (≥5) as the outcome variable, all four AI scores calculated with the four different statistical methods were found to have a statistically non-significant p-value (see Table 6-3). The ranges and distribution of the four AI scores were different and hence their odds ratios were not directly comparable. However, the values of odds ratios for the 4 AI scores were close to 1 which suggests that effect sizes were very small. The Hosmer and Lemeshow's measure R^2 value was small for all of the 4 models which imply that these models were a poor fit to the data.

6.3.2.2 Multivariable Analysis

The association between the AI scores and raised GHQ-28 remained statistically insignificant after adding various confounding factors (see Table 6-3). The odds ratios for the respective AI scores were close to 1 which suggests that the respective effect sizes were unchanged after adding confounders to these models. The Hosmer and Lemeshow's measure R^2 values improved after adding confounders. Among the confounders, sex (OR for male 0.62, 95% CI 0.43-0.90) socio-economic status (OR for deprived group 1.58, 95% CI 1.06-2.36) and smoking status (OR for smoker 1.51, 95% CI 1.02-2.25) were found to have a significant association with raised GHQ-28. Age and cardiometabolic co-morbidity were again not significant predictors of depressive symptoms and hence they were removed from the final model. The model assumptions were checked for the multivariable model using the method 4 AI score. The model assumptions for logistic regression model- multicollinearity using the parameter variance inflation factor (VIF <10) was satisfactory for the multivariable logistic regression model using the method 4 AI score as the predictor variable. This implies that the predictor variables used in the model were not highly correlated.

Key Findings (AI score as Predictor Variable):

- **AI score (calculated by 4 different methods) did not have a significant cross-sectional association with the presence of depressive symptoms (as assessed by raised GHQ-28) in univariable or multivariable logistic regression analyses.**

Table 6-3 Logistic Regression with AI score as a predictor and depressive symptoms (GHQ-28≥5) as outcome variable.

N=666. Missing values and exclusions=97. Available N=570. Biomarkers used for AI score calculation: Systolic Blood Pressure, Diastolic Blood Pressure, Body Mass Index, Waist Hip Ratio, Total Cholesterol, High Density Lipoprotein Cholesterol, Triglycerides, Fasting Glucose, Creatinine, Highly sensitive C-reactive Protein, Interluekin-6 and Fibrinogen.				
Univariable Analysis				
	AI score- Method 1-clinical cut-off method N=570 Range=0-10	AI score-Method 2- $25^{th}/75^{th}$ percentile method N=570 Range=1-12	AI score-Method 3- $10^{th}/90^{th}$ percentile N=570 Range=1-9	AI score – Method 4- z score method N=570 Range=3.7-22.3
AI score-Odds Ratio	1.06	1.04	0.98	1.01
Odds Ratio-95% Confidence Intervals; p-value	0.97-1.16; p=0.15	0.95-1.14; p=0.36	0.87-1.11; p=0.85	0.95-1.08; p=0.61
Hosmer and Lemeshow's measure R^2	0.003	0.001	<0.001	<0.001
Akaike Information Criteria	650	651	652	652
Multivariable Analysis. Adjusted for confounders: age, sex, socio-economic status, number of cardiometabolic conditions, smoking status.				
AI score- Odds Ratio	1.00	1.00	0.92	0.97
Odds Ratio-95% Confidence Intervals; p-value	0.91-1.10; p=0.91	0.90-1.10; p=0.99	0.80-1.05; p=0.25	0.90-1.05; p=0.56
Hosmer and Lemeshow's measure R^2	0.043	0.043	0.045	0.043
Akaike Information Criteria	634	634	633	634
Only sex, socio-economic status and smoking status included in the final analysis as the other confounders were not statistically significant.				

6.3.3 Individual Biomarkers as Predictor Variables

6.3.3.1 Univariable Analysis

The univariable modelling results for GHQ-28≥5 as the outcome variable and each of the 12 biomarkers as predictors are presented in Table 6-4. Six individual biomarkers were found to have a statistically significant association with raised GHQ-28 in the Psobid dataset. These biomarkers were SBP, BMI, triglycerides, creatinine, highly sensitive CRP and fibrinogen. The odds ratio for SBP and creatinine were less than 1 in value which suggests that these two biomarkers had an inverse association with the probability of having a raised GHQ-28.

A quadratic term was added for these six biomarkers to allow for a non-linear relationship. The quadratic term was found to have a significant association with raised GHQ-28 for only highly sensitive CRP. The other five biomarkers with significant association were found to have a linear relationship. The odds ratios with univariable analysis for the linear and the quadratic terms for highly sensitive CRP were 1.27 (95% CI 1.10-1.47) and 0.98 (95% CI 0.97-0.99) respectively. The odds ratio of quadratic term was less than 1 in value which implies that highly sensitive CRP had a "bell shaped" non-linear association with the odds of raised GHQ-28. The zenith of the "bell" for highly sensitive CRP was calculated with the formula –b/2a and found to be at 9.4 mg/l. The possibility of having raised GHQ-28 concurrently was highest for CRP value of 9.4 and the possibility of raised GHQ-28 was lower with CRP values above and below 9.4 mg/l.

Table 6-4 Univariable Logistic Regression using individual biomarkers as predictors and depressive symptoms (GHQ-28≥5) as outcome variable.

N=666. Available N=639 GHQ-28=General Health Questionnaire							
Biomarker	Missing Values	Range	Odds Ratio	95% Confidence Intervals	p-value	Akaike Information Criteria	Hosmer and Lemeshow's measure R^2
Systolic Blood Pressure in mm Hg	4	90 to 202	0.99	0.98-0.99	*0.04*	760	0.005
Diastolic Blood Pressure in mm Hg	4	52 to 119	0.09	0.98-1.01	0.73	764	<0.001
Waist Hip Ratio	7	0.64 to 1.31	1.44	0.77-11.62	0.72	757	0.001
Body Mass Index in kg/m2	4	16.3 to 51.1	1.04	1.01-1.07	*<0.01*	757	0.009
Total Cholesterol in mmol/l	21	2.4 to 8.6	0.86	0.73-1.02	0.09	737	0.003
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	0.79	0.50-1.26	0.34	739	0.001
Triglycerides in mmol/l	21	0.3 to 10.8	1.18	1.00-1.39	*0.03*	732	0.005
Fasting Blood Glucose in mmol/l	54	3.2 to 19.8	0.97	0.83-1.10	0.69	707	<0.001
Creatinine in umol/l	28	55.2 to 159.6	0.98	0.96-0.99	*0.02*	729	0.007
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	1.06	1.01-1.12	*0.01*	720	0.008
Interleukin 6 in pg/ml	37	0.2 to 11.8	1.09	0.98-1.22	0.09	718	0.003
Fibrinogen in g/l	33	1.1 to 6.4	1.32	1.03-1.70	*0.02*	722	0.006

6.3.3.2 Multivariable Analysis

The association between the six (SBP, BMI, triglyceride, creatinine, highly sensitive CRP and fibrinogen) individual biomarkers which were significant in univariable analysis and raised GHQ-28 were checked in six different multivariable models after adding various confounding factors (see Table 6-5). Among the confounders, sex, socio-economic status and smoking status only were added to the multivariable model. The other confounders, age and cardiometabolic co-morbidity were not significant predictors of depressive symptoms and hence they were removed from the final model. Only BMI was found to have a significant association with raised GHQ-28 in multivariable analysis. All the other five biomarkers had statistically non-significant associations. BMI was found to have a significant linear relationship with raised GHQ-28 in multivariable analysis. The effect size for BMI suggested that with each unit increase in BMI, there was a 4% (95% CI: 1% to 7%) increase in the probability of raised GHQ-28; this was consistent with the effect size observed in the univariable model for BMI. Model assumptions of multicollinearity were checked for the multivariable model using the parameter variance inflation factor (VIF <10) and it was satisfactory. This implies that the predictor variables used in the model were not highly correlated.

Table 6-5 Multivariable Logistic Regression using individual biomarkers as predictors and depressive symptoms (GHQ-28≥5) as outcome variable

N=666. Available N=639. GHQ-28=General Health Questionnaire. Confounders: sex, socio-economic status and smoking status							
Biomarker	Missing Values	Range	Odds Ratio	95% Confidence Intervals	p-value	Akaike Information Criteria	Hosmer and Lemeshow's measure R^2
Systolic Blood Pressure in mm Hg	4	90 to 202	0.99	0.98-1.00	0.16	702	0.037
Body Mass Index in kg/m2	4	16.3 to 51.1	1.04	1.01-1.07	**_<0.01_**	696	0.044
Triglycerides in mmol/l	21	0.3 to 10.8	1.16	0.97-1.38	<0.08	683	0.038
Creatinine in umol/l	28	55.2 to 159.6	0.99	0.98-1.01	0.89	681	0.032
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	1.03	0.98-1.09	0.14	669	0.039
Fibrinogen in g/l	33	1.1 to 6.4	1.22	0.94-1.58	0.12	672	0.035

Key Findings (Individual Biomarkers as Predictor Variables):

- **Six individual biomarkers (SBP, BMI, triglycerides, creatinine, highly sensitive CRP and fibrinogen were found to have a significant cross-sectional association with the presence of depressive symptoms (as assessed by raised GHQ-28) in univariable logistic regression analyses.**

- **BMI (OR 1.04) was the only peripheral biomarker found to have a significant cross-sectional association with the presence of depressive symptoms in multivariable logistic regression analysis, after adjusting for the effect of statistically significant confounders.**

6.3.3.3 Predicted Probability Charts

A predicted probability chart was created for BMI against raised GHQ-28. This was based on the multivariable model for raised GHQ-28, with BMI as predictor, and sex, smoking status and socio-economic status as confounders. The probability of raised GHQ-28 ranged from 18% to 52% for different BMI values (see Figure 6-1).

Figure 6-1 Predicted Probability Chart of raised GHQ-28(≥5) against BMI in Psobid dataset.
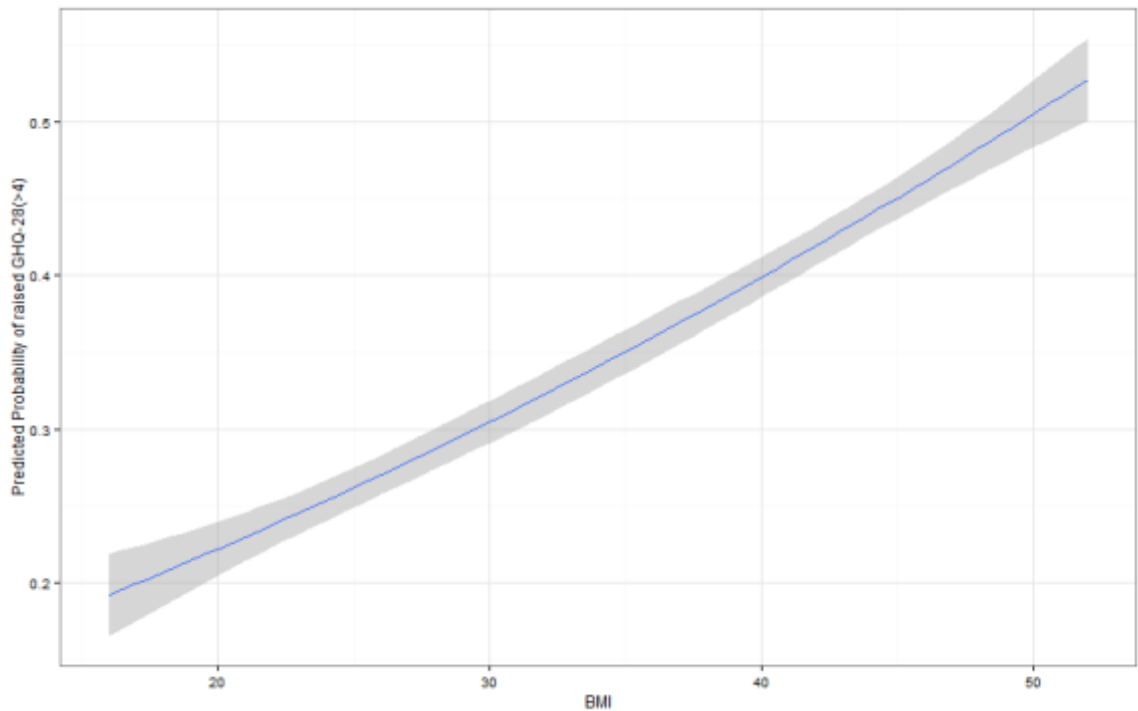
Legend: Adjusted for sex, smoking status and socio-economic status.

6.3.4 Sensitivity Analysis

6.3.4.1 Interaction Analysis between depressive symptoms, BMI and confounders

In the multivariable models showing a significant association between depressive symptoms (as assessed by raised GHQ-28) and BMI, the interactions between individual biomarkers and respective significant confounders were checked. For the multivariable model with raised GHQ-28 as the outcome, there was no interaction of BMI with sex (p=0.34), smoking status (p=0.07) and socio-economic status (p=0.07).

6.3.4.2 Linear Regression with GHQ-28 as continuous measure

Multiple multivariable linear regression models were performed with continuous GHQ-28 as the outcome variable. The GHQ-28 was square root transformed as the original score was not normally distributed. Among the confounders, sex, socio-economic status and smoking status were found to have a significant association with a continuous measure of GHQ-28. Age and number of cardiovascular co-morbidities were not significant predictors and hence removed from the models. Table 6-6 shows the results of separate multivariable linear regression models with AI score and 12 individual biomarkers as predictors respectively. AI score was not found to have a statistically significant association which is consistent with the findings from the logistic regression modelling. In the linear multivariable regression modelling, four individual biomarkers were found to have a significant association with GHQ-28: BMI, triglycerides, HDL cholesterol and highly sensitive CRP. The standardized regression coefficient was less than 0 in value for HDL cholesterol implying that lower HDL cholesterol values were associated with higher GHQ-28 scores.

Table 6-6 Multivariable Linear Regression with continuous GHQ-28 (square root transformed) as outcome variable and AI score and the 12 individual biomarkers as predictor variables.

N=666, Available N=639. Confounders- sex, socio-economic status and smoking status.							
Biomarker	Missing Values	Range	Standardized Regression Coefficients	95% Confidence Intervals	p-value	Akaike Information Criteria	Hosmer and Lemeshow's measure Adjusted R^2
AI score (z-score method)	96	3.7 to 22.3	0.005	-0.03 to 0.04	0.99	1809	0.069
Systolic Blood Pressure in mm Hg	4	90 to 202	-0.06	-0.01 to 0.001	0.15	2087	0.072
Diastolic Blood Pressure in mm Hg	4	52 to 119	0.006	-0.008 to 0.01	0.68	2089	0.069
Waist Hip Ratio	7	0.64 to 1.31	0.024	-1.13 to 1.99	0.51	2074	0.071
Body Mass Index in kg/m2	4	16.3 to 51.1	0.09	0.004 to 0.044	***0.01***	2080	0.081
Total Cholesterol in mmol/l	21	2.4 to 8.6	-0.03	-0.15 to 0.05	0.31	2046	0.069
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	-0.084	-0.61 to -0.011	***<0.01***	2040	0.078
Triglycerides in mmol/l	21	0.3 to 10.8	0.08	0.001 to 0.21	***0.04***	2043	0.074
Fasting Blood Glucose in mmol/l	54	3.2 to 19.8	-0.04	-0.11 to 0.03	0.53	1949	0.073
Creatinine in umol/l	28	55.2 to 159.6	-0.02	-0.01 to 0.007	0.81	2025	0.065
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	0.099	0.008 to 0.074	***0.01***	2012	0.079
Interleukin 6 in pg/ml	37	0.2 to 11.8	0.05	-0.02 to 0.12	0.15	1993	0.068
Fibrinogen in g/l	33	1.1 to 6.4	0.05	-0.04 to 0.26	0.16	2003	0.069

The possibility of a non-linear relationship for the 4 biomarkers that were found to have a significant association in multivariable linear regression models- BMI, triglycerides, HDL cholesterol and highly sensitive CRP was checked by adding their respective quadratic terms to each of the individual models. The p-values for quadratic terms were not significant for BMI, triglycerides and HDL cholesterol and highly sensitive CRP.

<u>**Key Findings (Sensitivity Analysis):**</u>

- **No significant statistical interaction observed between BMI and any of the significant confounders in multivariable logistic regression with raised GHQ-28 as the outcome variable.**

- **In the multivariable linear regression using the continuous measure of GHQ-28 as the outcome variable, AI score was not found to have a significant cross-sectional association.**

- **Four peripheral biomarkers were found to have a significant cross-sectional association with GHQ-28 in multivariable linear regression. They were: BMI, triglycerides, HDL cholesterol and highly sensitive CRP, with HDL cholesterol observed to have an inverse linear relationship with continuous GHQ-28.**

6.4 Discussion

6.4.1 Summary of Findings

The prevalence estimate for depressive symptoms in participants recruited from the general population in Psobid data was 27.4% (based on raised GHQ-28≥5). AI score did not have a significant cross-sectional association with depressive symptoms as assessed by GHQ-28 in either logistic regression (using raised GHQ-28) or in linear regression (continuous GHQ-28).

Six individual biomarkers were found to have a significant cross-sectional association with raised GHQ-28 in univariable analysis: SBP, BMI, triglycerides, creatinine, highly sensitive CRP and fibrinogen. Highly sensitive CRP was found to have a non-linear bell shaped relationship with a peak at the value of 9.4 mg/l for the highest probability of raised GHQ-28. In the multivariable analysis with raised GHQ-28 as the outcome variable, only BMI was found to have a significant linear relationship after adjusting for statistically significant confounders (sex, smoking status and socio-economic status). The effect size of the association implied that with increase in BMI by 5 units, there was 20% higher chances of having associated raised GHQ-28.

Four individual biomarkers (BMI, triglycerides, HDL cholesterol and highly sensitive CRP) had a significant cross-sectional association with the continuous measure of GHQ-28 in multivariable linear regression models. HDL-cholesterol had an inverse linear relationship with continuous GHQ-28; while BMI, triglycerides and highly sensitive CRP were found to have a direct linear relationship. The results were adjusted for the effects of the statistically significant confounders- sex, smoking status and socio-economic status. Individual biomarkers did not have a statistically significant interaction with any confounding factors in predicting co-existing depressive symptoms.

Interestingly, BMI was the only peripheral biomarker which had a significant cross-sectional association with depressive symptoms (assessed with both versions of GHQ-28- clinical cut-off and continuous scale), after adjusting for confounders.

6.4.2 Strengths

1. The rate of data completion was very good (>90% participants) for GHQ-28.

2. The study participants were well characterized with detailed information available on demographics and availability of both primary and secondary allostatic load biomarkers.

6.4.3 Limitations

1. The participants were chosen from the most deprived and the least deprived areas to highlight the impact of socio-economic status. However, socio-economic status is a continuum and hence the results of this study cannot be generalized to the whole population.

2. Secondly, the response rate for study participation was 25%. Hence there is a possibility of response bias.

3. In this analysis, GHQ-28 was used for estimating prevalence of depressive symptoms. GHQ-28 was originally designed to screen for minor psychological distress (not restricted to depressive symptoms) (231). However, it has been validated for screening of depressive symptoms in the general population (reported sensitivity 79.2% and specificity 79.6% against a composite international diagnostic instrument for DSM-IV criteria for major depressive disorder) (277) and for assessing depressive symptoms in patients with chronic physical health problems in primary care with good diagnostic accuracy (reported sensitivity 90%, reported specificity 80%) (257).

6.4.4 Comparison of findings with the existing literature and with DepChron

The prevalence estimate for depressive symptoms was 27.4% (based on GHQ-28≥5) in Psobid participants, which is higher than those reported in the general

population based on recent global estimates of <6%, even after taking dysthymia into account (8). This prevalence levels observed in Psobid is even higher than the prevalence levels observed in DepChron (19.9%). DepChron had patients with existing cardiometabolic disease who would be expected to be more likely to have co-existing depressive symptoms than the general population (106,107,278). There was no significant cross-sectional association between depressive symptoms and AI score in Psobid participants, either with logistic or linear regression modelling. As discussed previously in Chapters 4 and 5, 3 out of 4 studies have reported a significant cross-sectional association between AI score and depressive symptoms (216,217,219). None of the previously published studies have used GHQ-28 for estimating prevalence of depressive symptoms, however as previously suggested GHQ-28 has been validated for assessing depressive symptoms in the general population and primary care. The findings from Psobid are in contrast to those of DepChron, which showed a significant relationship between AI score and depressive symptoms; the important caveat being that the AI score constituents were very different for the two datasets.

In Psobid participants, a cross-sectional association was observed between individual biomarkers such as SBP, BMI triglycerides, HDL cholesterol, creatinine and the two inflammatory markers: highly sensitive CRP and fibrinogen) and depressive symptoms. However, the majority of these relationships, apart from the one between BMI and depressive symptoms, were no longer significant after adjusting for potential confounders. In DepChron, depressive symptoms had a significant non-linear relationship with SBP, DBP, BMI and total cholesterol, after adjusting for confounders. BMI has also been found to have a non-linear cross-sectional association (U-shaped) with depressive symptoms in the general population, in a large study published previously (279). In contrast, BMI had a positive linear relationship (with raised GHQ-28) in Psobid participants. Similarly, in various cross-sectional studies involving mainly elderly populations, depression has been observed to have a non-linear association with SBP (259–262), which was contradictory to the non-significant relationship observed in the Psobid participants. Also, DBP and total cholesterol did not have a significant association with depressive symptoms in Psobid, which is different to the findings of DepChron.

Finally, based on the results of a meta-analysis of 51 cross-sectional studies, raised highly sensitive CRP and IL-6 have been found to have a positive linear association with depressive symptoms (280). In Psobid, there was a significant cross-sectional association between depressive symptoms and highly sensitive CRP, but not with IL-6.

6.4.5 Implications of Findings

There are two potential implications of the observed relationships between AI score and peripheral biomarkers with depressive symptoms in Psobid participants. Previous evidence supporting a significant cross-sectional association between a composite AI score and depressive symptoms has mainly come from studies involving older populations (>65 years). The association may not be statistically significant in middle aged populations such as the Psobid participants. Further research should focus on investigating relationship between AI score and depressive symptoms in different age groups.

A significant cross-sectional association was observed between certain individual cardiometabolic (BMI) and inflammatory (highly sensitive CRP) biomarkers and concurrent depressive symptoms in Psobid participants, which resonates with the existing literature (281,282)(283). These relationships are cross-sectional in nature and hence it is not possible to infer causality based on these relationships. Previously reported meta-analyses have found a bi-directional relationship of depressive symptoms with raised inflammatory markers (281,282) and with obesity (283). In other words, studies have shown that obesity and raised inflammatory markers at baseline could be predictors of onset of depressive symptoms in future in someone who has not got depressive symptoms (281,282)(283). But the added risk of adverse clinical outcomes to the people who are obese or who have raised inflammatory markers and also concurrent depressive symptoms remains unclear. The hypothesis of this study is that these people with depressive symptoms and abnormal biomarkers are at higher risk of suffering from adverse physical outcomes which have been associated with depressive symptoms, such as death and cardiovascular events. This research question will be answered through longitudinal analysis of outcomes in Psobid participants in Chapter 8.

6.4.6 Conclusion

In a community dwelling sample of participants selected from the most affluent and deprived socio-economic sections of the general population, certain individual cardiometabolic and inflammatory biomarkers (such as BMI and CRP) were found to have a significant cross-sectional association with depressive symptoms. A composite AI score constructed using 12 biomarkers did not show a significant association with depressive symptoms. Future research should be directed towards longitudinal studies that examine clinical outcomes and the role of peripheral biomarkers in predicting clinical outcomes in patients with depressive symptoms.

In the next two chapters- Chapters 7 and 8, the findings from longitudinal analysis of the DepChron and Psobid datasets are presented respectively.

Chapter 7: Longitudinal Analysis in DepChron Dataset

7.1 Chapter Summary

In this chapter, the relationship between depressive symptoms, peripheral biomarkers and general and cardiovascular clinical outcomes is examined in patients with existing cardiometabolic disease, using the DepChron data. The objective of this analysis is to study the prognosis of depressive symptoms co-morbid with cardiometabolic disease. This addresses research questions 3 and 4 in the DepChron dataset. These research questions are:

Research Question 3 (RQ3):

What is the association, if any, between a composite AI score at baseline and the risk of future adverse health outcomes such as vascular events, hospitalisation and mortality in patients with depressive symptoms co-morbid with cardiometabolic disease (DepChron cohort)?

Research Question 4 (RQ4):

What is the association, if any, between individual allostatic load biomarkers at baseline and the risk of future adverse health outcomes, such as vascular events, hospitalisation and mortality in patients with depressive symptoms co-morbid with cardiometabolic disease and how does it compare with the relationship between composite AI score at baseline and risk of adverse outcomes?

The three sections in this chapter address: a)the methods for data collection of clinical outcomes and statistical analysis; b) the association of AL biomarkers (composite AI score and individual biomarkers) and depressive symptoms with the risk of adverse clinical outcomes (general and cardiovascular) at 4 years as well as the statistical analyses of the interaction of depressive symptoms with allostatic load biomarkers (composite AI score and individual biomarkers) in risk prediction of these outcomes; and c) a discussion of how the findings compare with the existing literature and strengths, limitations and possible implications.

7.2 Methods

7.2.1 Data Collection- Adverse Clinical Outcomes

Clinical outcomes were studied via electronic data linkage performed by the Information Services Division (ISD), Scotland data linkage services. The follow-up period was 4 years between April 2009 and March 2013. The process of electronic data linkage was completely anonymised and there was no access to any patient identifiable information at any point. Firstly, the list of the patients on the DepChron dataset was transferred to ISD with their dummy study numbers. The Keep Well Enhanced Services Data Group (data guardian for the DepChron dataset) transferred the list of original community health index (CHI) numbers along with their dummy study numbers to ISD. The ISD used patient CHI numbers to link the DepChron dataset with the general hospitalization and death registries. After extracting the information on clinical outcomes for DepChron patients, the CHI numbers were removed from the data by ISD and the data was forwarded to Glasgow University for analysis. This method of electronic data linkage has been shown to provide robust data on health care outcomes and has been used for a wide range of high profile epidemiological studies and large scale randomised controlled trials such as the West of Scotland Coronary Prevention Study and Heart Failure and Optimal Outcomes from Pharmacy Trial(284-286). The general and cardiovascular health outcomes, defined using the International System of Disease Classification- 10th Edition (ICD-10), were those studied (287). Only the first cause of death and the first cause of hospitalization were taken into account to improve accuracy, in line with the approach adopted by previously published studies using data linkage (284-286).

The general Health Outcomes studied were:

1. All-cause Death

2. All-cause Hospitalization

The cardiovascular health outcomes with respective ICD-20 codes are as follows:

1. Admission due to myocardial Infarction (MI)- I21

2. Admission due to stroke – I61-I64

3. Admission due to heart failure (HF): I50

4. Death due to cardiovascular causes: I00- I99.

Major adverse cardiovascular outcome (MACE) was used as the composite outcome variable, which included cardiovascular mortality or admission due to MI/stroke/HF. Patients were censored if they died due to reasons other than cardiovascular causes.

7.2.2 Clinical Variables

Depressive symptoms assessed by HADS-D were entered as a binary variable (present/absent) with HADS-D≥8 used as the threshold for presence of depressive symptoms, as per previous analyses.

The values for individual biomarkers were restricted to clinically plausible ranges based on both clinical judgement and the findings of general population studies as before. SBP measurements were restricted to a range between 90 to 240 mm Hg and DBP to a range between 50 to 130 mm Hg (236,237). Similarly, BMI was restricted to a range between 15 to 55 (238), total cholesterol to 2-10 (239) and HbA1C to 3-18% (240).

The AI score was calculated using the four different methods which have been described in detail in Chapter 5. These methods were: 1. Method 1- Clinical cut-off 2. Method 2- Sample distribution cut-offs (25th and 75th percentile) 3. Method 3- Sample distribution cut-offs (10th and 75th percentile). 4. Method 4- z-score.

The 5 individual biomarkers (HbA1c was only available in the subset of patients with diabetes) were used both as continuous and categorical measures. BMI was classified into 4 categories: healthy 18.5-25, underweight 15-18.5, overweight 25-30, obese 30-55 (245) and total cholesterol levels into two categories (not raised vs. raised: >5 mmol/l (244)), based on published clinical guidelines.

There is no consensus among various guidelines published internationally for optimal BP targets in patients with existing cardiometabolic disease (241–244).

Therefore, BP was classified into five different categories based on clinical judgement to improve the interpretability of results. SBP was classified into five categories: very high (160-240), high (140-159), reference (130-139), tightly controlled (120-129), and low (80-119). Diastolic blood pressure (DBP) was also classified in to: very high (100-130), high (90-99), reference (85-89), tightly controlled (80-84) and low (40-79).

7.2.3 Statistical Analysis-AI score

Time to event analysis was used to study the association of the two predictors: a) AI score (4 different methods) and b) presence of depressive symptoms; with the risk of the two general health outcomes and the composite major adverse cardiovascular outcome in DepChron patients. A quadratic term of the AI score was added to check for a non-linear association between AI score and the 3 clinical outcomes.

If AI score or depressive symptoms were found to have a statistically significant relationship with a clinical outcome in multivariable analysis, a Kaplan-Meier plot was used to visualise the results of this relationship.

Multivariable analysis was performed adjusting for the following confounders: age (continuous), sex (male and female), socio-economic status (deprived: SIMD deciles 1-5 vs. affluent: SIMD deciles 6-10), initiation of antidepressants within 6 months of depression screening (yes/no), number of conditions (range 1-3, representing a combination of one or more of the three cardiometabolic diseases under investigation: CHD, stroke or diabetes. The results are presented as hazard ratios (HR) with 95% confidence intervals (CI) and Harrell's Concordance statistics (with standard errors) using the "survival" package from R (288). The final adjusted Cox proportional hazard models were fitted for each of the 3 adverse clinical outcomes with both AI score and depressive symptoms added together as predictors in all models, and in addition to the five potential confounders in the adjusted models.

To understand the relationship between AI score and depressive symptoms in risk prediction of clinical outcomes, if any, an analysis of variance (ANOVA) test was undertaken to check for interaction between the AI score and presence of

depressive symptoms. The results were presented as p-values with a p-value less than 0.05 regarded as statistically significant. In the event of a significant interaction, a sub-group analysis was also conducted to further study the nature of the interaction.

7.2.4 Statistical Analysis-Individual Biomarkers

Time to event analysis was used to study the association between the 4 individual biomarkers (SBP, DBP, BMI and total cholesterol, both as continuous and categorical measures based on categories described above) and the risk of general and cardiovascular adverse outcomes in DepChron patients. Cox proportional hazards regression analysis was performed and the results presented with hazard ratios and 95% confidence intervals.

If a peripheral biomarker was found to have a statistically significant relationship with any of the clinical outcome, a Kaplan-Meier plot was constructed to visualise the results of this relationship.

Three multivariable cox proportional models were constructed, with the 4 individual peripheral biomarkers and depressive symptoms as predictors, adjusting for the same confounders: age (continuous), sex (male and female), socio-economic status (deprived: SIMD deciles 1-5 vs. affluent: SIMD deciles 6-10), initiation of antidepressants (yes/no) within six months of depression screening, number of conditions (range 1-3, representing a combination of one or more of the three cardiometabolic disease under investigations: CHD, stroke or diabetes. The results of these models were presented with HR with 95% CI and C-statistics (with standard errors).

To understand the relationship between individual biomarkers and depressive symptoms in risk prediction of clinical outcomes, if any, an ANOVA test was undertaken to check for interaction between the individual biomarkers and the presence of depressive symptoms. The results were presented as p-values with a p-value less than 0.05 regarded as statistically significant. In the event of a significant interaction, sub-group analysis was performed to further study the nature of the interaction. In the sub-group analysis, the study sample was divided on the basis of individual biomarker categories. In each sub-group, a Cox

proportional hazards regression analysis was performed to study the risk of clinical outcomes with the presence of depressive symptoms at baseline, adjusting for potential confounders. The results of the interaction were visualised with the help of a forest plot for HRs with 95% CI.

7.2.5 Sensitivity Analysis in Diabetes Subset

For sensitivity analysis, the analyses for risk prediction of general and cardiovascular health outcomes were repeated in patients with diabetes. In these patients, Cox's regression modelling was performed as described above, firstly for AI score using HbA1c as one of the biomarkers for AI calculation. Final multivariable models were then constructed for the three clinical outcomes under study using AI score, depressive symptoms and the five potential confounders described previously for the diabetic subgroup. Testing for a potential interaction between AI score and depressive symptoms in the risk prediction of these outcomes was also performed.

In the next part, the analyses were repeated with individual biomarkers. The HbA1c was categorized, based on national guidelines (246), into reference: 6.5-7.4 DCCT, low 3-6.4 DCCT, and high 7.5-18 DCCT. Similarly, final multivariable models were constructed for the three clinical outcomes using depressive symptoms, five biomarkers (as categorical variables based on national guidelines) and five confounders in subset of patients with diabetes. Finally, a potential interaction between HbA1c and depressive symptoms were explored using the ANOVA test. In the event of a significant interaction, the results were further analysed using forest plots and sub-group analysis as described previously.

7.3 Results

7.3.1 Clinical Outcomes

Electronic data linkage between primary care disease registers and hospital discharge and mortality records was successful for 99.4% (124414/125143) of patients. Among the patients who had recorded results of depression screening, 19.9% (7080/35537) were identified as screen positives based on HADS-D ≥8. New antidepressants were initiated for 2.4% (696/28457) of patients with HADS-D negative and 8.1% (572/7080) of patients with HADS-D positive within six months of depression screening, while the overall rate of new antidepressant prescribing was 3.5% (1268/35537) of screened patients. 6.3% (4989/78936) of the unscreened population were started on new antidepressants during the observation period (with no clear explanation recorded) (see Figure 7-1).

Figure 7-1 Results of Depression Screening and Antidepressant Initiation in DepChron(226)
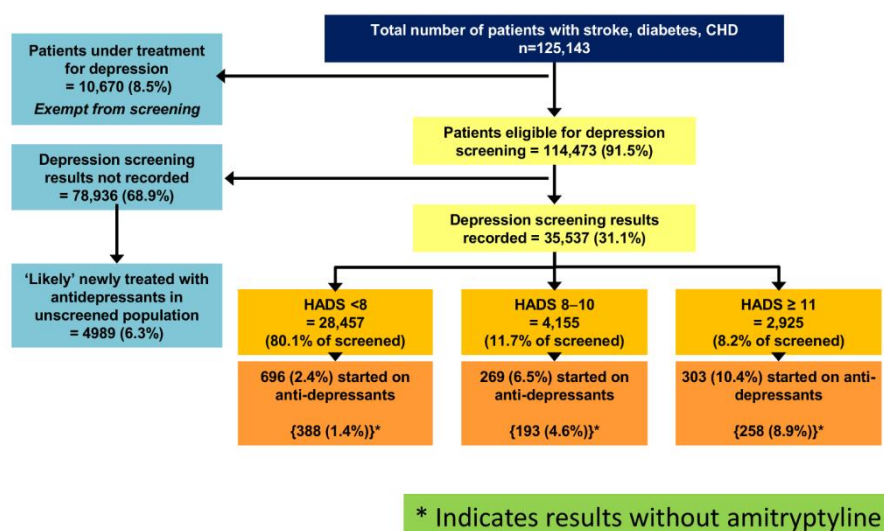
Table 7-1 shows that patients who were screened were more likely to be female, deprived, younger and less likely to have cardiometabolic multi-morbidity. The recording of biomarker values in the unscreened population was very poor across the 4 biomarkers with, for example, 59.5% missing values for BMI recordings.

Table 7-1 Comparison between screened and unscreened patients in DepChron

	Categories	Depression Screened N= 35537	Unscreened N=78936	p-value
Age(years) - mean (SD)		69.0 (11.9)	67.0 (14.3)	<0.001
White ethnicity (%)		30693 (92.4)	53343 (90.3)	<0.001
Male sex (%)		20658 (58.2)	42727 (54.2)	<0.001
Deprived socio-economic status SIMD deciles<=5 (%)		22726 (65.3)	51686 (67.4)	<0.001
Number of cardiometabolic conditions- (%)	One	27356 (77.0)	65417 (82.9)	
	Two	7410 (20.9)	12265 (15.5)	
	Three	771 (2.2)	1254 (1.6)	<0.001
Antidepressant initiation (%)		1268 (3.5)	4989 (6.3)	<0.001
Systolic Blood Pressure-(%)	130-139 mm Hg Reference	8389 (23.6)	13315 (16.8)	
	120-129 mm Hg	6864 (19.3)	10818 (13.7)	
	80-119 mm Hg	5711 (16.0)	9258 (11.7)	
	140-159 mm Hg	8624 (24.2)	15969 (20.2)	<0.001
	160-240 mm Hg	2514 (7.0)	5778 (7.3)	
	Not Available (NA)	3435 (9.6)	23802 (30.1)	
Diastolic Blood Pressure- (%)	85-89 mm Hg Reference	1909 (5.3)	4219 (5.3)	
	80-84 mm Hg	7070 (19.8)	13088 (16.5)	
	40-79 mm Hg	20585 (57.9)	31724 (40.1)	
	90-99 mm Hg	1981 (5.5)	4450 (5.6)	<0.001
	100-130 mm Hg	562 (1.5)	1649 (2.0)	
	NA	3430 (9.6)	23806 (30.1)	
Body Mass Index (%)	18.5-25 kg/m2 Reference	7349 (20.6)	7904 (10.1)	
	15-18.5 kg/m2	340 (0.9)	627 (0.8)	
	25-30 kg/m2	11379 (32.0)	11505 (14.5)	<0.001
	30-55 kg/m2	10974 (30.8)	11882 (15.0)	
	NA	5495 (15.4)	47018 (59.5)	
Total Cholesterol in mmol/l (%)	2-5 mmol/l Reference	25134 (70.7)	34027 (43.1)	
	5-10 mmol/l	6093 (17.1)	12264 (15.5)	<0.001
	NA	4310 (12.1)	32645 (41.3)	
Composite major adverse cardiac outcome (MACE) (%)		3939 (11)	10990 (13.9)	<0.001
All-cause mortality (%)		5021 (14.1)	13569 (17.2)	<0.001
All-cause hospital admissions (%)		23717 (66.7)	52089 (66.0)	0.01
SIMD=Scottish Index of Multiple Deprivation. SD= Standard Deviation. MI=Myocardial Infarction. MACE=Cardiovascular death or admission due to MI/stroke/heart failure. NA=Not available				

The unscreened group of patients were more likely to die of any cause and more likely to have experienced the composite adverse cardiovascular outcome. Although the rate of all-cause first hospital admission was statistically different, clinically the difference in rate was negligible between the screened and unscreened groups of patients. As shown in Table 7-1, the number of patients within the screened group who suffered from adverse clinical outcomes during 4 year follow-up were as follows: 5021 (14.1%) all-cause mortality, 23717 (66.7%) all-cause general hospital admission, and 3939 (11%) composite major adverse cardiovascular outcome-(cardiovascular death/ admission due to MI/stroke/heart failure).

7.3.2 Statistical Analysis- AI Score

7.3.2.1 Univariable Analysis with AI score and depressive symptoms

Table 7-2 shows that all 4 AI scores and the presence of depressive symptoms had a significant association with the 3 clinical outcomes in univariable analysis. The estimated elevated risk of all-cause death was 36% higher, all-cause hospital admission was 29% higher and MACE was 23% higher at 4 years with the presence of depressive symptoms (HADS-D≥8), when compared to patients with no depressive symptoms at baseline.

The AI score calculated from 4 different statistical methods were observed to have different types of associations with the 3 clinical outcomes, in their respective univariable models. The method 1 AI scores (clinical cut-off method) had an inverse linear association with the 3 clinical outcomes, while the other 3 AI scores had a direct linear association with the clinical outcomes. DepChron patients had a 19% lower risk of death at 4 years with each unit increase in the AI score calculated from method 1- clinical cut off method (mean 0.85, median 1.0) at baseline. Similarly, the risk of all-cause hospital admission and composite major cardiovascular outcome was lower with increasing AI score (method 1) at baseline. On the contrary, the risk of the 3 clinical outcomes was associated with increases in AI scores calculated from the other 3 methods (methods 2-4) at baseline. The effect sizes were largest for method 3 (10th/90th percentile cut-off method) with 18% higher risk of death, 3% higher risk of all-cause hospital admission and 12% higher risk of composite major adverse cardiovascular outcome with each unit increase in AI score. The confidence intervals were the narrowest for method 4 AI score- the z-score method. The effect sizes in the first 3 methods were not directly comparable to method 4 as the distribution of AI score was different for method 4; the range for methods 2 and 3 AI scores was 0 to 4, while the range of method 4 AI was 0.2 to 12.4. In general, the effects sizes observed for AI scores were lower than that of depressive symptoms (see Table 7-2).

Finally, comparing the concordance statistics (C-statistics) for the 4 AI scores, the z-score method had the best C-statistic values. However, the differences between the C-statistic values of the 4 AI score methods were very small and

unlikely to be statistically significant based on the values of corresponding standard errors.

Next, the possibility of a non-linear association between AI score and the 3 clinical outcomes was checked by entering a quadratic term for AI score in the respective regression models. There was no significant non-linear relationship between the 4 AI scores and the 3 clinical outcomes in their respective Cox regression models. The results in detail were as follows: The quadratic term for AI score (method 1) did not have a significant association with the risk of all-cause death (p-value = 0.26), all-cause general hospital admission (p-value = 0.67) or composite major adverse cardiovascular outcome (p-value = 0.31). The quadratic term for AI score (method 2) did not have a significant association with the risk of all-cause death (p-value = 0.99), all-cause general hospital admission (p-value = 0.35) or composite major adverse cardiovascular outcome (p-value = 0.54). The quadratic term for AI score (method 3) did not have a significant association with the risk of all-cause death (p-value = 0.99), all-cause general hospital admission (p-value = 0.79) or composite major adverse cardiovascular outcome (p-value = 0.23). The quadratic term for AI score (method 4) did not have a significant association with the risk of all-cause death (p-value = 0.21), all-cause general hospital admission (p-value = 0.19) or composite major adverse cardiovascular outcome (p-value = 0.98).

Key Findings (AI score-Univariable Analysis):

- **Method 1 AI score had an inverse linear association with the risk of 3 clinical outcomes.**

- **Methods 2-4 AI score had direct linear association with the risk of 3 clinical outcomes (HR for method 4 AI score=1.08).**

- **Presence of depressive symptoms was associated with a higher risk of 3 adverse clinical outcomes.**

Table 7-2 Univariable Cox's proportional hazards for the three clinical outcomes with AI score and presence of depressive symptoms as predictors.

Clinical Outcomes		AI score-Method 1- clinical cut-off (Range 0-4) Available N=25375	AI score-Method 2- 25th/75th percentile (Range 0-4) Available N=25375	AI score-Method 3- 10th/90th percentile (Range 0-4) Available N=25375	AI score – Method 4- z score method (Range 0.2-12.4) Available N=25375	Presence of depressive symptoms (HADS-D≥8) (Yes against No)
All-cause death	HR	0.81	1.12	1.18	1.08	1.36
	95% CI	0.78 to 0.85	1.09 to 1.16	1.13 to 1.22	1.06 to 1.11	1.27 to 1.45
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001
	C statistics- (SE)	0.543 (0.004)	0.536 (0.005)	0.537 (0.004)	0.543 (0.005)	0.527 (0.003)
All-cause hospital admission	HR	0.95	1.02	1.03	1.02	1.29
	95% CI	0.93 to 0.96	1.01 to 1.04	1.01 to 1.05	1.01 to 1.03	1.25 to 1.33
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001
	C-statistics (SE)	0.512 (0.002)	0.509 (0.002)	0.510 (0.002)	0.513 (0.002)	0.523 (0.002)
Composite major adverse cardiovascular outcome (MACE)	HR	0.95	1.08	1.12	1.07	1.23
	95% CI	0.91 to 0.99	1.05 to 1.12	1.07 to 1.17	1.04 to 1.10	1.14 to 1.32
	p-value	0.01	<0.001	<0.001	<0.001	<0.001
	C-statistics (SE)	0.511 (0.005)	0.526 (0.005)	0.527 (0.005)	0.534 (0.005)	0.518 (0.004)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.						

7.3.2.2 Kaplan-Meier plots for AI score, depressive symptoms and clinical outcomes

The absolute event rate for all-cause mortality (p-value <0.001) and major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) (p-value <0.001) was significantly higher for participants with raised AI score- the z-score method (defined as >3.85; 75th percentile value) at baseline as compared to participants without raised AI score (p-value < 0.001). Figure 7-2 shows that nearly 17% of patients with raised AI score at baseline were dead and 14% had experienced at least one major adverse cardiovascular event at 4 years as compared to approximately 13% and 12% event rates observed for patients without a raised AI score at baseline respectively.

Figure 7-2 Kaplan-Meier plot comparing clinical outcomes for different AI scores.
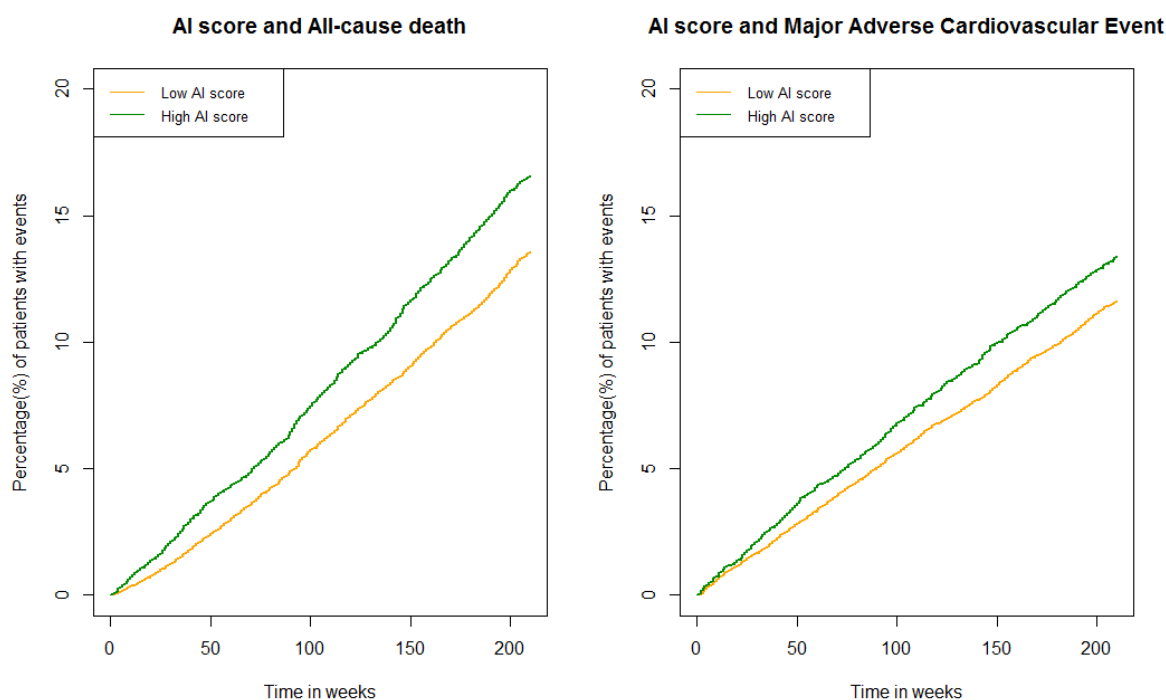
Legend: Raised AI score= defined as >3.85 (75th percentile value). AI score calculated by the z-score method.

Figure 7-3 shows a similar relationship between the presence of depressive symptoms and clinical outcomes, with a higher rate of adverse clinical outcomes observed for patients with the presence of depressive symptoms at baseline (p-value <0.001 for both outcomes).

Figure 7-3 Kaplan-Meier plot comparing clinical outcomes for presence and absence of depressive symptoms.
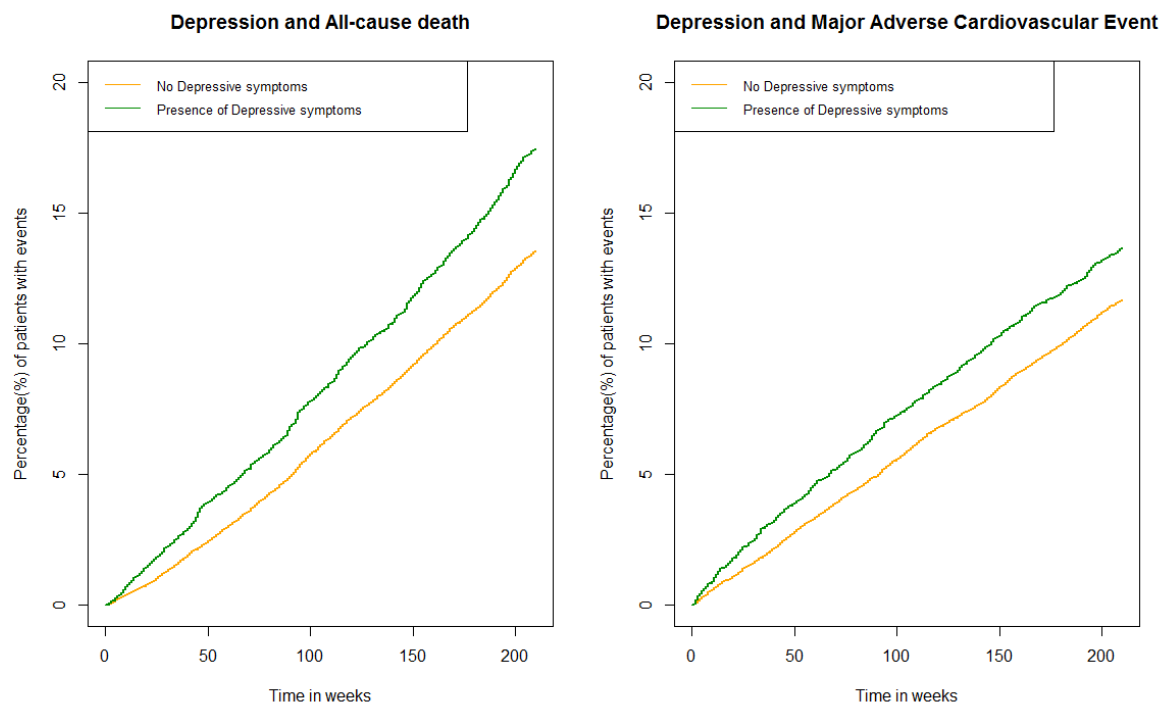
Legend: Depressive symptoms defined as HADS-D≥8

7.3.2.3 The final Model for three clinical outcomes using AI score and depressive symptoms

The z-score method AI score was chosen for the final models of risk prediction for the three clinical outcomes based on its marginally better C-statistics values and narrower confidence intervals. The final model consisted of both AI score and presence of depressive symptoms as predictors, along with all the other potential confounding factors added together (see Table 7-3). Patients with depressive symptoms at baseline were 46% more likely to die at the end of 4 years, while one unit increase in AI score (equals to 1 SD higher or lower biomarker value than the sample mean) was associated with 12% higher risk of all-cause death. Increase in age by one year was associated with 8% higher risk of death. The risk of death was higher for patients with multiple cardiovascular co-morbidities and lower for female sex, affluent socio-economic class and those who were initiated on antidepressants within 6 months of depression screening. In the risk prediction of all-cause hospital admission, the effect sizes of AI score and depressive symptoms were comparatively smaller. Sex was no longer a

significant predictor for hospital admission and patients who were initiated on antidepressants were more likely to get admitted during four years follow-up, which was contrary to the results observed for all-cause death.

Finally, in the risk prediction of major adverse cardiovascular outcomes, the presence of depressive symptoms was associated with a 21% higher risk while one unit in AI score was associated with a 10% higher risk of the event at 4 years. Notably, the presence of two cardiovascular co-morbidities was associated with 89% higher risk while presence of all three cardiovascular co-morbidities was associated with approximately three times higher risk of an event, compared to having only one of the three cardiometabolic diseases at baseline. Initiation of antidepressants within six months of depression screening did not have a significant impact on the event rate of major adverse cardiovascular outcome at four years. The C-statistic was highest in value for all-cause death which suggests the best predictability of the model out of the three clinical outcomes under study.

Key Findings (AI score-Multivariable Analysis):

- **Method 4 AI score had a direct linear association with the risk of 3 adverse clinical outcomes.**

- **Presence of depressive symptoms was associated with higher risk of the 3 adverse clinical outcomes.**

- **Results were adjusted for the effect of 5 potential confounders and the trends were unchanged.**

Table 7-3 Final multivariable Cox's proportional hazards for the three clinical outcomes with AI score and presence of depressive symptoms as predictors. Available N=25375.

	All-cause Death HR with 95% CI	All-cause Hospital Admissions HR with 95% CI	Composite major adverse cardiovascular outcome (MACE) HR with 95% CI
AI score (z-score method)	1.12 (1.10 to 1.15)	1.03 (1.02 to 1.04)	1.10 (1.07 to 1.13)
Presence of depressive symptoms (HADS-D≥8)	1.46 (1.35 to 1.58)	1.28 (1.23 to 1.32)	1.21 (1.11 to 1.33)
Age	1.08 (1.076 to 1.085)	1.025 (1.023 to 1.026)	1.054 (1.050 to 1.058)
Sex-female	0.71 (0.67 to 0.77)	1.00 (0.97 to 1.03)	0.83 (0.77 to 0.89)
Socio-economic status-affluent group	0.73 (0.68 to 0.78)	0.90 (0.87 to 0.93)	0.80 (0.74 to 0.87)
Number of cardiovascular co-morbidities			
2 vs. 1	1.32 (1.23 to 1.42)	1.25 (1.20 to 1.29)	1.89 (1.75 to 2.04)
3 vs. 1	1.95 (1.68 to 2.26)	1.56 (1.43 to 1.70)	3.06 (2.63 to 3.57)
Initiation of anti-depressants-treated vs. non-treated	0.76 (0.62 to 0.93)	1.35 (1.25 to 1.46)	0.84 (0.68 to 1.04)
Concordance (SE)	0.720 (0.005)	0.595 (0.002)	0.692 (0.005)
Confounders: Age, Sex, Socio-economic status, Number of cardiovascular co-morbidity, Initiation of anti-depressants within 6 months of depression screening.			
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.			

7.3.2.4 Interaction analysis- AI score and depressive symptoms

Table 7-4 shows that there was no statistically significant interaction between the AI scores and depressive symptoms (HADS-D≥8) in risk prediction of the 3 clinical outcomes under study. The results are presented as p-values for interaction analysis based on ANOVA and none of the p-values were statistically significant.

Table 7-4 Interaction Analysis for three clinical outcomes with AI score and presence of depressive symptoms as predictors in DepChron.

	AI score- Method 1-clinical cut-off (Range 0-4)	AI score-Method 2- 25th/75th percentile (Range 0-4)	AI score-Method 3- 10th/90th percentile (Range 0-4)	AI score – Method 4- z score method (Range 0.2-12.4)
All-cause death	p-value = 0.11	p-value = 0.89	p-value = 0.86	p-value = 0.21
All-cause hospital admission	p-value = 0.24	p-value = 0.07	p-value = 0.23	p-value = 0.26
Composite major adverse cardiovascular outcome	p-value = 0.18	p-value = 0.93	p-value = 0.76	p-value = 0.82
Confounders: Age, Sex, Socio-economic status, Number of cardiovascular co-morbidity, Initiation of anti-depressants within 6 months of depression screening.				

Key Findings (AI score-Interaction Analysis):

- **No significant statistical interaction between AI score (all 4 methods) and depressive symptoms in risk prediction of the 3 adverse clinical outcomes.**

7.3.3 Statistical Analysis – Individual Biomarkers

7.3.3.1 Individual Biomarkers as continuous measures (univariable analysis)

Table 7-5 shows the results of univariable survival analyses with the individual biomarkers as predictors for the 3 adverse clinical outcomes. DBP, BMI and total cholesterol had a significant association with all of the three clinical outcomes with the respective HR values of less than 1.0 suggesting that lower values of these biomarkers was associated with higher risk of adverse clinical outcomes. In other words, these biomarkers had an inverse linear association with the risk of adverse clinical outcomes. The exception was the association between SBP and MACE. The HR for MACE was higher than 1.0 for SBP suggesting lower risk of MACE with lower SBP values. The continuous measure of SBP did not have a significant association with all-cause death and all-cause hospital admissions.

7.3.3.2 Individual Biomarkers as categorical measures (univariable analysis)

The association between the clinical outcomes and individual biomarkers was further analysed using clinical categories, as described in the methods (see Table 7-6). DBP, BMI and total cholesterol were found to have an inverse linear association with the risk of 3 adverse clinical outcomes. For DBP and BMI, the highest risk for the 3 clinical outcomes at 4 years was observed with patients with the lowest values of DBP and BMI at baseline. Similarly, the risk of the 3 adverse clinical outcomes was significantly lower in patients with high total cholesterol (5-10) values at baseline. SBP had a significant non-linear association with all 3 adverse clinical outcomes with a higher risk in patients with the two extremes of SBP at baseline- 80-119 and 160-240. Thus, the nature of the observed relationship between the individual peripheral biomarkers and adverse clinical outcomes was different.

Key Findings (Individual Biomarkers-Univariable Analysis):

- **DBP, BMI and total cholesterol had an inverse linear relationship with the risk of the 3 adverse clinical outcomes.**

- **SBP had a non-linear relationship with the risk of the 3 adverse clinical outcomes, with the risk higher in the two extremes (very high and low) of SBP categories.**

Table 7-5 Univariable Cox's proportional hazards for the three clinical outcomes with the four individual biomarkers (continuous) as predictors.

Clinical Outcomes		SBP Range: 90 to 240	DBP Range: 50 to 130	Total Cholesterol Range: 15 to 55	BMI Range: 2 to 10
All-cause death Number of events = 3628	HR	1.001	0.975	0.939	0.860
	95% CI	0.999 to 1.003	0.972 to 0.979	0.932 to 0.945	0.831 to 0.890
	p-value	0.29	<0.001	<0.001	<0.001
	C statistics-(SE)	0.503 (0.005)	0.565 (0.005)	0.594 (0.005)	0.546 (0.005)
All-cause hospital admission Number of events = 17261	HR	1.00	0.990	0.985	0.971
	95% CI	0.99 to 1.01	0.988 to 0.991	0.983 to 0.988	0.956 to 0.985
	p-value	0.66	<0.001	<0.001	<0.001
	C-statistics (SE)	0.497 (0.002)	0.529 (0.002)	0.525 (0.002)	0.512 (0.002)
Composite major adverse cardiovascular outcome Number of events = 2939	HR	1.004	0.984	0.971	0.960
	95% CI	1.002 to 1.006	0.981 to 0.988	0.964 to 0.977	0.926 to 0.995
	p-value	<0.001	<0.001	<0.001	0.027
	C-statistics (SE)	0.515 (0.005)	0.543 (0.005)	0.545 (0.005)	0.518 (0.005)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.					

Table 7-6 Univariable Cox's proportional hazards for the three clinical outcomes with the four individual biomarkers (categorical) as predictors.

		All-cause Death Number of events = 3628 HR with 95% CI	All-cause Hospital Admissions Number of events = 17261 HR with 95% CI	Composite major adverse cardiovascular outcome Number of events = 2939 HR with 95% CI
Systolic Blood Pressure in mm Hg	80-119	*1.21 (1.11 to 1.32)*	*1.10 (1.06 to 1.15)*	*1.15 (1.04 to 1.27)*
	120-129	1.01 (0.92 to 1.10)	*1.04 (1.00 to 1.08)*	0.99 (0.90 to 1.09)
	130-139	1	1	1
	140-159	1.09 (1.00 to 1.18)	*1.04 (1.01 to 1.08)*	1.10 (1.00 to 1.20)
	160-240	*1.30 (1.16 to 1.46)*	*1.12 (1.06 to 1.18)*	*1.43 (1.26 to 1.61)*
Diastolic Blood Pressure in mm Hg	40-79	*1.45 (1.26 to 1.67)*	*1.22 (1.15 to 1.30)*	*1.24 (1.07 to 1.44)*
	80-84	1.10 (0.94 to 1.27)	*1.08 (1.02 to 1.16)*	1.05 (0.89 to 1.23)
	85-89	1	1	1
	90-99	*0.80 (0.66 to 0.98)*	0.95 (0.87 to 1.03)	0.83 (0.67 to 1.02)
	100-130	0.86 (0.64 to 1.16)	1.01 (0.89 to 1.14)	1.03 (0.77 to 1.39)
BMI in kg/m2	15-18.5	*2.13 (1.78 to 2.55)*	*1.30 (1.15 to 1.47)*	*1.37 (1.05 to 1.79)*
	18.5-25	1	1	1
	25-30	*0.63 (0.58 to 0.67)*	*0.87 (0.84 to 0.90)*	*0.78 (0.72 to 0.85)*
	30-55	*0.48 (0.44 to 0.52)*	*0.81 (0.78 to 0.83)*	*0.68 (0.62 to 0.74)*
Total Cholesterol in mmol/l	2-5	1	1	1
	5-10	*0.79 (0.73 to 0.86)*	*0.96 (0.93 to 0.99)*	0.98 (0.90 to 1.06)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.				
Significant results in bold.				

7.3.3.3 Kaplan Meier Plots for peripheral biomarkers and clinical outcomes

Kaplan-Meier plots were constructed for SBP, BMI and total cholesterol categories for two clinical outcomes- all-cause mortality and MACE (cardiovascular death or admission due to MI/stroke/HF).

On comparing the event rate for the five SBP categories, patients with a raised SBP (160-240) at baseline had the highest event rate for both adverse clinical outcomes (p-value <0.001). The elevated event rate observed for patients with high SBP was more pronounced for major adverse cardiovascular event than for all-cause death (see Figure 7-4).

Figure 7-4 Kaplan-Meier plot comparing clinical outcomes based on SBP values at baseline.
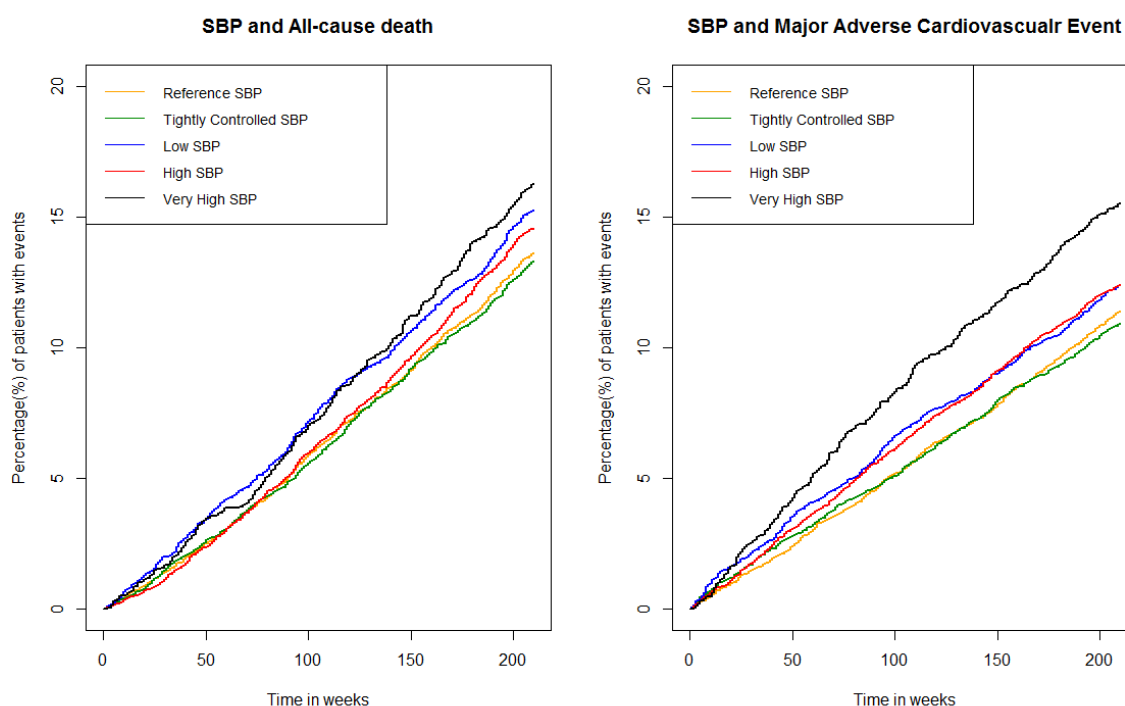
Figure 7-5 shows a significant inverse linear relationship between BMI values at baseline and risk of all-cause death and major adverse cardiovascular event at 4 years in DepChron patients (p-value <0.001). Notably, patients with low BMI (15 to 18.5) had a substantially higher rate of mortality (approximately 40%).

Figure 7-5 Kaplan-Meier plot comparing clinical outcomes based on BMI values at baseline.
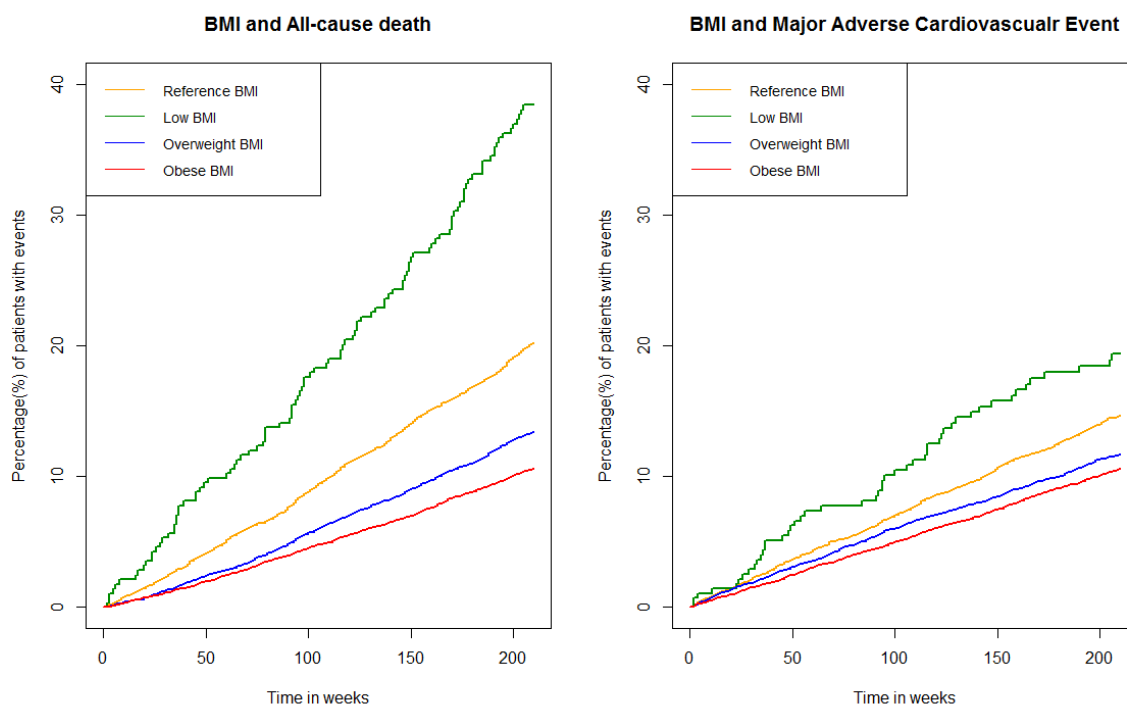
Finally, Figure 7-6 shows the effects of raised total cholesterol at baseline on the two clinical outcomes. DepChron patients with low total cholesterol (< 5.0 mmol/l) were more likely to die at the end of 4 years (p-value < 0.001). However, there was no significant difference in the two cholesterol categories as for major adverse cardiovascular event rate (p-value = 0.83).

Figure 7-6 Kaplan-Meier plot comparing clinical outcomes based on total cholesterol at baseline.
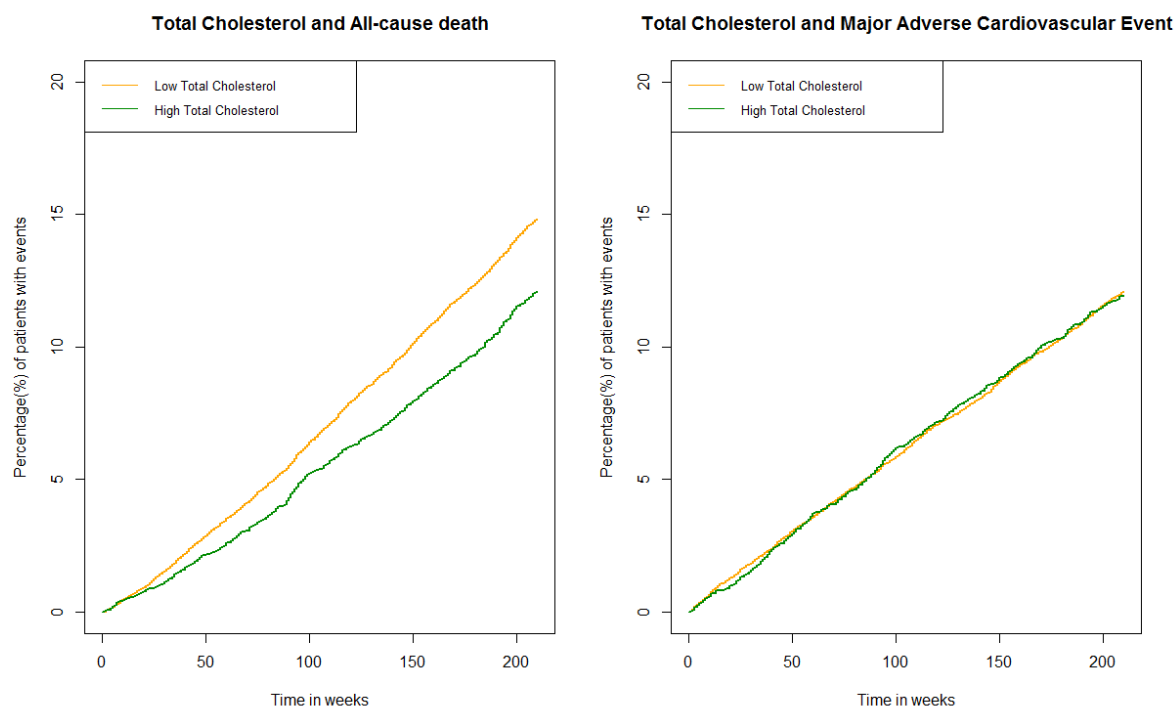
7.3.3.4 Final model of risk prediction for three clinical outcomes using individual biomarkers and depressive symptoms

Table 7-7 shows the results from multivariable analyses after adding four biomarkers, depressive symptoms and five potential confounders. The five confounders were: age, sex, socio-economic status, number of cardiovascular co-morbidities and initiation of antidepressants-within 6 months of depression screening. The non-linear association of SBP categories with MACE and with all-cause hospital admissions was significant, after adjusting for the confounders. The association of the DBP categories with the 3 adverse clinical outcomes was no longer statistically significant, after adjusting for confounders. The BMI categories were observed to have an inverse linear association with the 3 adverse clinical outcomes, in multivariable analyses.

On the contrary, the association between total cholesterol at baseline and the three adverse clinical outcomes at four years was completely changed in multivariable analysis. High cholesterol was associated with significantly higher

risk of major adverse cardiovascular outcome and all-cause hospital admission in multivariable analysis. This was changed from the lower risk of all-cause hospital admission and non-significant association with major adverse cardiovascular outcome observed in univariable analysis.

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<u>Key Findings (Individual Biomarkers- Multivariable Analysis):</u>

- **SBP categories had a linear association with the risk of MACE (HR 1.28) and all-cause hospital admissions (HR 1.07), while the risk of all-cause death was significantly lower in the low SBP category.**

- **DBP categories did not have a significant association with any of the 3 adverse clinical outcomes.**

- **BMI had an inverse linear association with the 3 adverse clinical outcomes, with higher observed risk in low BMI categories.**

- **Total cholesterol was found to have a higher risk of MACE and all-cause hospital admissions in the high category as compare to low total cholesterol.**

- **These results were adjusted for 5 confounders and for the presence of depressive symptoms and the trends were unchanged.**

</div>

Table 7-7 Final multivariable Cox's proportional hazards for the three clinical outcomes with four peripheral biomarkers as predictors. Available N=25375

		All-cause Death 3594 events HR with 95% CI	All-cause Hospital Admissions 17041 events HR with 95% CI	Composite major adverse cardiovascular outcome (MACE) 2920 events HR with 95% CI
Systolic Blood Pressure in mm Hg	80-119	*1.18 (1.06 to 1.30)*	*1.11 (1.06 to 1.16)*	*1.15 (1.03 to 1.29)*
	120-129	1.00 (0.90 to 1.10)	*1.05 (1.01 to 1.10)*	1.00 (0.90 to 1.12)
	130-139	1	1	1
	140-159	1.05 (0.96 to 1.15)	1.04 (0.99 to 1.08)	1.07 (0.96 to 1.18)
	160-240	1.12 (0.97 to 1.28)	*1.07 (1.00 to 1.14)*	*1.28 (1.11 to 1.49)*
Diastolic Blood Pressure in mm Hg	40-79	0.95 (0.80 to 1.12)	1.00 (0.93 to 1.07)	0.94 (0.78-1.12)
	80-84	0.93 (0.78 to 1.10)	0.99 (0.92 to 1.06)	0.93 (0.77-1.12)
	85-89	1	1	1
	90-99	0.83 (0.66 to 1.05)	0.93 (0.84 to 1.02)	0.90 (0.71-1.14)
	100-130	1.21 (0.86 to 1.68)	1.01 (0.87 to 1.16)	1.15 (0.82-1.62)
BMI in kg/m2	15-18.5	*1.99 (1.63 to 2.42)*	*1.21 (1.06 to 1.39)*	*1.36 (1.02 to 1.82)*
	18.5-25	1	1	1
	25-30	*0.71 (0.65 to 0.76)*	*0.92 (0.88 to 0.96)*	*0.83 (0.76 to 0.91)*
	30-55	*0.69 (0.63 to 0.75)*	*0.92 (0.88 to 0.96)*	*0.83 (0.75 to 0.92)*
Total Cholesterol in mmol/l	2-5	1	1	1
	5-10	1.01 (0.92 to 1.11)	*1.05 (1.01 to 1.09)*	*1.21 (1.10 to 1.33)*
Presence of depressive symptoms (HADS-D≥8)		*1.45 (1.34 to 1.57)*	*1.28 (1.23 to 1.32)*	*1.22 (1.11 to 1.33)*
Age		*1.07 (1.07 to 1.07)*	*1.02 (1.02 to 1.02)*	*1.05 (1.04 to 1.05)*
Sex-female		*0.72 (0.67 to 0.77)*	1.00 (0.97 to 1.03)	*0.81 (0.75 to 0.88)*
Socio-economic status-affluent group		*0.71 (0.66 to 0.76)*	*0.90 (0.87 to 0.93)*	*0.79 (0.73 to 0.86)*
Number of cardiovascular co-morbidities (vs. 1)	2	*1.38 (1.29 to 1.49)*	*1.26 (1.22 to 1.31)*	*1.95 (1.81 to 2.11)*
	3	*2.13 (1.83 to 2.48)*	*1.60 (1.47 to 1.75)*	*3.22 (2.76 to 3.76)*
Initiation of anti-depressants- treated vs. non-treated		*0.75 (0.61 to 0.92)*	*1.35 (1.25 to 1.45)*	0.84 (0.68 to 1.04)
Concordance (SE)		0.724 (0.005)	0.596 (0.002)	0.692 (0.005)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure. Significant results in bold.				

7.3.3.5 Interaction analysis- individual biomarkers and depressive symptoms

In the final phase of analysis of individual biomarkers, the presence of a statistical interaction between depressive symptoms (HADS-D≥8) and 3 biomarkers found to have a significant association with clinical outcomes in multivariable analysis (SBP, BMI and total cholesterol) was checked. The results are presented in Table 7-8 as p-values for a statistically significant interaction tested using the ANOVA test.

Table 7-8 Interaction Analysis for three clinical outcomes with individual biomarkers and presence of depressive symptoms as predictors.

	SBP Categories	BMI Categories	Total Cholesterol Categories
All-cause death	p-value = 0.11	p-value = 0.70	p-value = 0.05
All-cause hospital admission	p-value = 0.07	p-value = 0.35	p-value = 0.51
Composite major adverse cardiovascular outcome (MACE)	***p-value = 0.03***	p-value = 0.06	p-value = 0.97

In the risk prediction of major adverse cardiovascular outcome, the interaction between SBP categories and the presence of depressive symptoms was statistically significant (p-value=0.03). The observed statistical interaction between SBP categories and depressive symptoms was further analysed in greater detail. Patients were sub-divided based on various combinations of the two extremes of SBP categories and the presence or absence of depressive symptoms at baseline. Patients with low SBP (80-119) only, with very high SBP (160-240) only and depressive symptoms only at baseline had 10%, 19% and 17% higher risk respectively of a major adverse cardiovascular event as compared to those without extremes of SBP and no depressive symptoms. In comparison, patients with both low SBP and depressive symptoms at baseline had 36% higher risk while patients with both very high SBP and depressive symptoms had the highest increased risk of 83% as compared to those in the reference SBP group without depressive symptoms (see Figure 7-7).

Figure 7-7 Forest plot showing interaction between depressive symptoms and extremes of systolic blood pressure with the risk of first major adverse cardiovascular event (289).
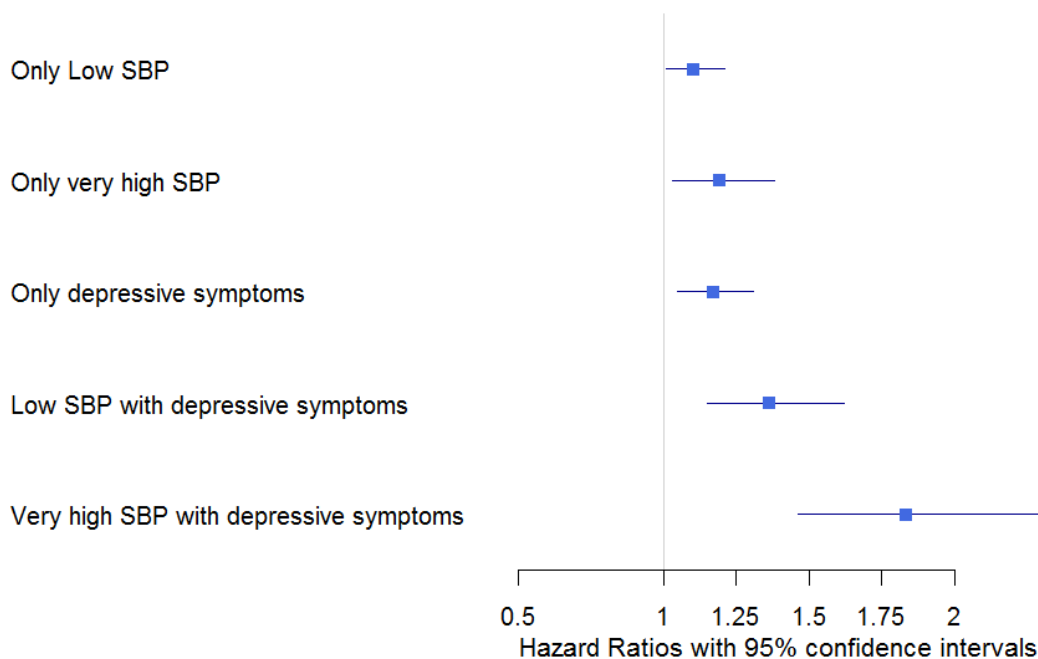
In the multivariable sub-group analysis of the five SBP categories, a non-linear trend was observed in the association between presence of depressive symptoms and risk prediction of a major adverse cardiovascular outcome at four years (see Table 7-9).   There was no evidence of an association between the presence of depressive symptoms and adjusted risk of major adverse cardiovascular outcome for the sub-group of patients with reference SBP (130-139) at baseline. The presence of depressive symptoms was associated with significantly higher risk of major adverse cardiovascular outcome for patients in all other baseline SBP categories. Patients with very high SBP and concurrent depressive symptoms had the highest event rate of 17.7%; moreover the change in the adjusted risk with the addition of depressive symptoms was highest for the sub-group of patients with very high SBP at 55% (see Table 7-9).

> **Key Findings (Individual biomarkers-Interaction Analysis):**
>
> - **SBP and the presence of depressive symptoms were found to have a statistical significant interaction in risk prediction of MACE.**
>
> - **Presence of depressive symptoms compounded the risk of MACE, especially in patients with very high SBP (160-240).**

Table 7-9 Presence of depressive symptoms and the risk of first major adverse cardiovascular outcome (MACE) based on SBP categories.

	Systolic Blood Pressure Categories				
	Low 80-119 n=5711	Tightly controlled 120-129 n=6864	Reference 130-139 n=8389	High 140-159 n=8624	Very High 160-240 n=2514
Not Depressed Event Rate	492/4376 (11.2%)	555/5464 (10.1%)	730/6806 (10.7%)	776/7028 (11.0%)	278/2001 (13.8%)
Depressed (HADS-D≥8) Event Rate	192/1335 (14.3%)	171/1400 (12.2%)	161/1583 (10.1%)	226/1596 (14.1%)	91/513 (17.7%)
HR with 95% CI Depressed vs. Not Depressed (*adjusted)	*1.23 (1.01 to 1.49)*	*1.34 (1.10 to 1.63)*	0.94 (0.77 to 1.14)	*1.29 (1.08 to 1.53)*	*1.55 (1.18 to 2.03)*
p-value	<0.001	<0.001	0.54	<0.001	<0.001

7.3.4 Sensitivity Analysis for subset of patients with diabetes

7.3.4.1 Analysis with AI score for subset of patients with diabetes

Sensitivity analysis was performed for the subset of patients with diabetes (N=18,453). In patients with diabetes, there were 2412 patients who died (13.0%), 11,745 patients (63.6%) had at least 1 hospital admission and 1808 patients (9.7%) suffered from a major adverse cardiovascular outcome (cardiovascular death/ admission due to MI/stroke/heart failure). 3580 (19.4%) were found to have depressive symptoms at baseline, based on HADS-D$\geq$8. The z-score method of calculating AI score was used for sensitivity analysis. The range of AI score (z-score method) was 0.5 to 12.93; while the mean was 3.85 and median was 3.59.

Table 7-10 shows that AI score had a significant association with all of the 3 adverse clinical outcomes in the subset of patients with diabetes, after adjusting for depressive symptoms and the 5 other previously described potential confounders. The effect sizes observed for AI score in the final survival models for the diabetes subset were very similar to the ones observed for the entire sample. The effect sizes for depressive symptoms were comparatively smaller in the diabetes sample to those observed in the whole sample.

The AI score did not have a significant interaction with presence of depressive symptoms in risk prediction of all-cause mortality (p-value = 0.82), all-cause hospital admission (p-value = 0.68) or composite major adverse cardiovascular outcome (p-value = 0.31), using the ANOVA test for interaction analysis.

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<u>Key Findings (AI score analysis-Diabetes Subset):</u>

- **AI score had a significant association with the 3 adverse clinical outcomes in the diabetes subset, with similar effects sizes to the ones observed in the total sample.**

- **No statistically significant interaction observed between AI score and depressive symptoms in the risk prediction of any of the 3 adverse clinical outcomes, in the diabetes subset.**

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Table 7-10 Final multivariable Cox's proportional hazards for the three clinical outcomes in diabetes subset with AI score and depressive symptoms as predictors.

	All-cause Death number of events=1498 HR with 95% CI	All-cause Hospital Admissions number of events=6996 HR with 95% CI	Composite major adverse cardiovascular outcome (MACE) number of events=1184 HR with 95% CI
AI score (z-score method)	1.12 (1.08 to 1.15)	1.04 (1.02 to 1.05)	1.12 (1.08 to 1.16)
Presence of depressive symptoms (HADS-D≥8)	1.38 (1.22 to 1.56)	1.22 (1.15 to 1.30)	1.18 (1.03 to 1.36)
Age	1.07 (1.06 to 1.07)	1.02 (1.02 to 1.02)	1.04 (1.03 to 1.05)
Sex-female	0.77 (0.69 to 0.85)	0.99 (0.95 to 1.04)	0.87 (0.77 to 0.98)
Socio-economic status-affluent group	0.72 (0.64 to 0.80)	0.88 (0.84 to 0.93)	0.77 (0.68 to 0.88)
Number of cardiovascular co-morbidities 2 vs. 1	1.37 (1.22 to 1.53)	1.39 (1.32 to 1.47)	2.79 (2.43 to 3.20)
3 vs. 1	2.07 (1.74 to 2.46)	1.79 (1.62 to 1.97)	4.72 (3.90 to 5.72)
Initiation of anti-depressants-treated vs. non-treated	0.88 (0.66 to 1.18)	1.36 (1.21 to 1.52)	0.83 (0.59 to 1.16)
Concordance (SE)	0.726 (0.007)	0.613 (0.004)	0.732 (0.008)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.			

7.3.4.2 Sensitivity Analysis with Individual Biomarkers (HbA1c) for subset of patients with diabetes

The final multivariable Cox regression models for the 3 adverse clinical outcomes were also analysed using the five individual biomarkers, depressive symptoms and the five confounders as described before (see Table 7-11). High HbA1c (7.5 to 18 DCCT) values at baseline were associated with a 33% higher risk of mortality, 9% higher risk of hospital admission and 53% higher risk of composite major adverse cardiovascular outcome over 4 years, as compared to reference HbA1c (6.5 to 7.4 DCCT) in the subset of patients with diabetes. There was no statistically significant difference observed in the risk of the 3 adverse clinical outcomes between the reference HbA1c category and low HbA1c (3 to 6.4 DCCT).

Table 7-11 Multivariable Cox's proportional hazards for the three clinical outcomes with individual peripheral biomarkers (categorical) and depressive symptoms as predictors in diabetes subset.

		All-cause Death 1512 events HR with 95% CI	All-cause Hospital Admissions 7040 events HR with 95% CI	Composite major adverse cardiovascular outcome (MACE) 1199 events HR with 95% CI
Systolic Blood Pressure in mm Hg	80-119	1.13 (0.96 to 1.33)	*1.09 (1.01 to 1.18)*	*1.20 (1.00 to 1.44)*
	120-129	1.03 (0.88 to 1.20)	1.06 (0.99 to 1.14)	1.01 (0.85 to 1.20)
	130-139	1	1	1
	140-159	1.12 (0.98 to 1.29)	1.05 (0.98 to 1.12)	1.15 (0.98 to 1.35)
	160-240	1.14 (0.93 to 1.40)	*1.10 (1.00 to 1.21)*	*1.42 (1.14 to 1.77)*
Diastolic Blood Pressure in mm Hg	40-79	0.97 (0.76 to 1.25)	1.01 (0.91 to 1.12)	0.97 (0.74 to 1.29)
	80-84	0.84 (0.64 to 1.09)	0.99 (0.89 to 1.11)	0.87 (0.65 to 1.17)
	85-89	1	1	1
	90-99	0.71 (0.50 to 1.02)	0.89 (0.78 to 1.03)	0.71 (0.49 to 1.05)
	100-130	1.28 (0.81 to 2.04)	1.02 (0.83 to 1.25)	1.23 (1.75 to 2.01)
BMI in kg/m2	15-18.5	*1.99 (1.14 to 3.47)*	1.17 (0.78 to 1.76)	1.29 (0.53 to 3.15)
	18.5-25	1	1	1
	25-30	*0.74 (0.65 to 0.85)*	*0.86 (0.80 to 0.93)*	0.92 (0.78 to 1.08)
	30-55	*0.73 (0.63 to 0.83)*	*0.90 (0.84 to 0.97)*	0.94 (0.79 to 1.10)
Total Cholesterol in mmol/l	2-5	1	1	1
	5-10	0.97 (0.83 to 1.12)	1.01 (0.95 to 1.08)	*1.17 (1.00 to 1.37)*
HbA1c in DCCT	3-6.4	1.13 (0.99 to 1.29)	0.99 (0.93 to 1.06)	1.12 (0.96 to 1.30)
	6.5-7.4	1	1	1
	7.5-18	*1.33 (1.18 to 1.51)*	*1.09 (1.03 to 1.15)*	*1.53 (1.33 to 1.76)*
Presence of depressive symptoms (HADS-D≥8)		*1.39 (1.23 to 1.57)*	*1.23 (1.16 to 1.30)*	*1.21 (1.05 to 1.38)*
Age		*1.06 (1.06 to 1.07)*	*1.02 (1.02 to 1.02)*	*1.04 (1.03 to 1.05)*
Sex-female		*0.78 (0.70 to 0.87)*	0.99 (0.95 to 1.04)	*0.85 (0.76 to 0.96)*
Socio-economic status-affluent group		*0.69 (0.61 to 0.78)*	*0.87 (0.83 to 0.92)*	*0.76 (0.67 to 0.86)*
Number of cardiovascular co-morbidities (vs. 1)	2	*1.38 (1.23 to 1.54)*	*1.39 (1.32 to 1.47)*	*2.80 (2.44 to 3.22)*
	3	*2.11 (1.77 to 2.51)*	*1.79 (1.62 to 1.97)*	*4.71 (3.89 to 5.71)*
Initiation of anti-depressants- treated vs. non-treated		0.88 (0.65 to 1.18)	*1.35 (1.22 to 1.52)*	0.81 (0.58 to 1.13)
Concordance Statistics (Standard Error)		0.727 (0.007)	0.613 (0.004)	0.736 (0.008)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure. Significant results in bold.				

HbA1c categories did not have a significant interaction with the presence of depressive symptoms (HADS-D≥8) in risk prediction of all-cause hospital admission (0.08) and MACE (p-value = 0.08), however the interaction was statistically significant in risk prediction of all-cause death (p-value =0.04). The statistically significant interaction between HbA1c categories and depressive symptoms in risk prediction of all-cause death was analysed in further detail.

Patients with diabetes were further sub-divided based on different combinations of the three HbA1c categories and presence or absence of depressive symptoms at baseline. Patients with low HbA1c (3 to 6.4) only, with high HbA1c (7.5 to 18) only and depressive symptoms only at baseline had 18%, 27% and 37% higher risk respectively of all-cause death as compared to those with reference HbA1c (6.5 to 7.4) and no depressive symptoms. In comparison, patients with both low HbA1c and depressive symptoms at baseline had 34% higher risk (see Figure 7-8) of all-cause death while patients with both high HbA1c and depressive symptoms had the most elevated risk of all-cause death at 108% as compared to those in the reference HbA1c group without depressive symptoms.

Key Findings (HbA1c Analysis in Diabetes Subset):

- **High HbA1c was associated with a higher risk of the 3 adverse clinical outcomes in the diabetes subset, after adjusting for potential confounders and other biomarkers in multivariable analysis.**

- **Statistically significant interaction was observed between HbA1c and the presence of depressive symptoms in the risk prediction of all-cause death, in the diabetes subset.**

- **Presence of depressive symptoms compounded the risk of all-cause mortality in patients with high HbA1c (7.5-18) category, in the diabetes subset.**

Figure 7-8 Forest plot showing interaction between depressive symptoms and HbA1c at baseline with the risk of all-cause mortality in patients with diabetes.

Hazard Ratios with 95% confidence intervals
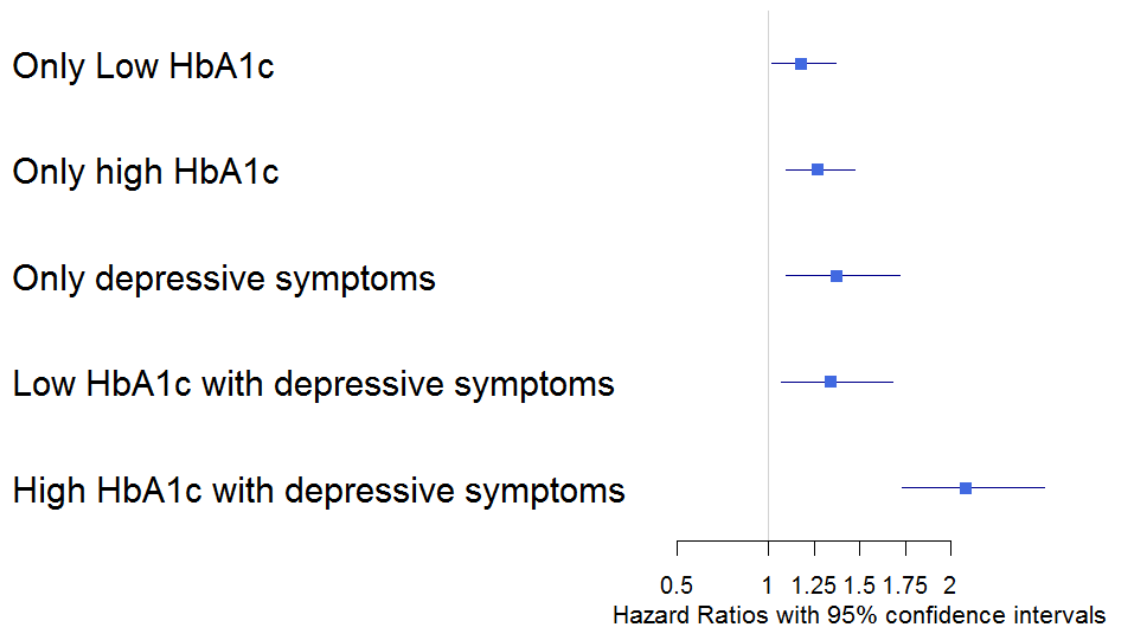
7.4 Discussion

7.4.1 Summary of Findings

In DepChron patients, the presence of depressive symptoms (HADS-D≥8) was associated with an increased risk of all 3 adverse clinical outcomes – all-cause death, all-cause hospital admission and composite major adverse cardiovascular outcome (cardiovascular death or admission due to MI/stroke/HF) at 4 years in patients with existing cardiometabolic disease (CHD or stroke or diabetes). A composite AI score calculated using mainly peripheral cardiometabolic biomarkers was also found to have a significant association (direct linear) with the 3 adverse clinical outcomes described above. These results were adjusted for the effects of 5 potential confounding factors namely, age, sex, socio-economic status, number of cardiovascular comorbidities and initiation of antidepressants within six months.

Among the individual biomarkers, different types of non-linear association was observed between SBP values at baseline and the risk of all-cause hospital admission and major adverse cardiovascular outcome at four years. Patients with very high and low SBP at baseline were observed to have a significantly higher adjusted risk of a major adverse cardiovascular outcome than those with SBP in the reference range. On the contrary, DBP was not a significant predictor in the multivariable analysis for any of the 3 adverse clinical outcomes. Among the metabolic biomarkers, an inverse linear relationship was observed between BMI at baseline and the risk of 3 adverse clinical outcomes. High BMI (overweight and obese categories) was associated with low risk while low BMI was associated with higher risk of all 3 adverse clinical outcomes, when compared to the reference BMI categories. In the subset of patients with diabetes, high HbA1c was associated with higher risk of all 3 adverse clinical outcomes as compared to reference HbA1c at baseline. These results were adjusted for the effects of 5 potential confounding factors, as described above.

AI score did not have a significant interaction with the presence of depressive symptoms in risk prediction of any of the three clinical outcomes. There was a statistically significant interaction noted between SBP and depressive symptoms in risk prediction of MACE. The presence of depressive symptoms compounded

the risk of MACE at four years in all SBP categories higher and lower than the reference category, especially in patients with very high SBP (160-240). In the diabetes subset, HbA1c had a statistically significant interaction with depressive symptoms in risk prediction of all-cause mortality. In patients with high HbA1c (7.5-18) at baseline, presence of concurrent depressive symptoms at baseline compounded the risk of all-cause mortality at 4 years.

Notably, cardiovascular comorbidities had comparatively larger effect sizes with significantly higher risk of all three adverse clinical outcomes with each increase in the number of co-morbid conditions among CHD, stroke and diabetes.

7.4.2 Strengths

This study has some key strengths:

1. The data came from a large, community based sample reflecting real life clinical practice.

2. Electronic data linkage enabled successful follow-up for the vast majority of patients in the cohort.

3. The follow-up duration of four years was sufficient to study the clinical outcomes under investigation.

7.4.3 Limitations

There are several limitations with this study.

1. Only a minority of the patients in the sample had depression screening recorded despite incentivisation. Consequently, there may be important differences between patients with known depression status and those whose depression status was unknown that were not recorded in our data.
2. Complete information on biobehavioural factors such as smoking status and levels of physical activity was not available, and this is likely to influence the prevalence of depressive symptoms and the clinical outcomes considered (252,253).

3. Information on cardiac related medications was not available for these patients.

4. Only single assessments of biomarker values and depressive symptoms were available for the start of the study. The biomarker values and depressive symptoms may have changed over the course of the 4 year follow-up duration but this information was not available.

5. The information on how depressive symptoms were managed was incomplete. The dataset only had information on which patients were initiated on antidepressants. However, it was not known how many patients were referred for other forms of depression treatment, such as psychological therapies.

6. Finally, the overall accuracy of depression screening in this study was reliant on HADS-D which is a self-reported measure and has accuracy related drawbacks when used for assessing depressive symptoms in patients with cardiometabolic disease in a primary care setting (114,256,257). This has been discussed previously in Chapter 5.

7.4.4 Comparison of Findings with Existing Literature

In DepChron patients, the presence of depressive symptoms at baseline was associated with higher risk of all 3 adverse clinical outcomes- all-cause death, all-cause hospital admissions and MACE. The association between presence of depressive symptoms and higher risk of adverse clinical outcomes in patients with pre-existing cardiometabolic disease has been reported previously in the literature (108,110,139). Similarly, the relationship of higher AI score with higher all-cause mortality and higher cardiovascular events has been reported in older and ageing populations (28,233). However, DepChron is different from these studies, as only a few cardiometabolic biomarkers were used for AI score calculation, while other studies in this area have used a much wider selection of biomarkers (28,233). The selection of available biomarkers for AI score calculation was limited in DepChron as it was a routinely collected data. This is also one of the key differences between DepChron and other AI score studies where the analyses was based on research datasets (28,233).

A SBP J curve was observed in this study in risk prediction of major adverse cardiac events which has also been reported extensively in various other studies

(290–294). With regards to DBP, our study did not find the evidence of a J-shaped curve; furthermore DBP at baseline was not a significant predictor of adverse cardiovascular outcomes. Our study findings are contrary to the results observed in the SPRINT trial but there are important differences such as the study design and setting, and also the SPRINT trial excluded patients with diabetes and previous stroke which were included in our study (295). There are other studies which have reported similar findings of better predictive power of SBP over DBP in predicting cardiovascular outcomes (296,297). An inverse-linear relationship was observed in DepChron patients between BMI categories at baseline and risk of adverse clinical outcomes. The existence of a similar "obesity paradox" has been extensively discussed in the literature for patients with cardiometabolic conditions (270,298,299).

In DepChron, a statistically significant interaction was observed between depressive symptoms and systolic blood pressure in risk prediction of cardiovascular events. There is evidence supporting the view that depression may be an independent risk factor for hypertension (300). Similarly, prospective studies of patients with hypertension have found that hypertension is an independent predictor of new-onset depression (301).   Depression is associated with aberrant inflammatory responses (302) and inflammation plays a key role in the pathophysiology of hypertension (303). So, there is a possibility that aberrant inflammatory mechanisms might link the two apparently separate classes of disorder (namely, depression and hypertension).

There is also evidence to suggest that the presence of depressive symptoms may lower the BP (304) and also lower BP is associated with poor mental health in these patients (305). However, this is the first study, to our knowledge, which investigates the interacting relationship between depressive symptoms and BP values in the risk prediction of adverse clinical outcomes in patients with pre-existing cardiometabolic disease.

7.4.5 Potential Implications

There are two potential implications of the findings from our study. Firstly, secondary prevention in cardiometabolic disease management could be targeted

at patients with depressive symptoms and extremes of SBP as they tend to have a "compounded" risk of adverse outcomes. These findings need to be replicated in other datasets and prospectively before making this recommendation. Secondly, the relationship between depressive symptoms and BP need to be studied in greater detail, particularly for patients with cardiometabolic disease who are likely to be on medications which will lower their BP. Similarly, further research is also needed to study the relationship between depressive symptoms and the extremes of HbA1c values for patients with diabetes. These relationships, if proven in other studies, may have two potential implications for clinical practice in secondary prevention of patients with cardiometabolic disease. Firstly, patients with extremes of SBP and HbA1c could be targeted for depression screening, as we know that presence of depressive symptoms can compound their risk of adverse clinical outcomes. Secondly, the management of patients with cardiometabolic disease and co-morbid depression should have "a greater focus" on SBP and HbA1c control, in order to reduce the risk of adverse clinical outcomes.

7.4.6 Conclusion

A composite AI score (calculated using only secondary cardiometabolic biomarkers) and depressive symptoms at baseline were independent predictors of all-cause mortality, all-cause hospital admission and major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) at 4 years in patients with existing cardiometabolic disease. There was no significant interaction between AI score and depressive symptoms in risk prediction of these clinical outcomes, so AI score may not have a role in risk stratification of patients presenting with depressive symptoms with pre-existing cardiometabolic disease.

SBP at baseline showed a J-shaped curve with a higher risk of adverse clinical outcomes in patients with very high and low SBP; DBP at baseline did not have a significant effect in risk prediction of any of the 3 adverse clinical outcomes. There was a statistically significant interaction between depressive symptoms and SBP in the risk prediction of MACE. The presence of depressive symptoms compounded the risk of a MACE event in patients with SBP lower or higher than the reference category at baseline. In patients with diabetes, presence of

depressive symptoms compounded the risk of all-cause death for patients with high HbA1c values. These relationships between depressive symptoms and cardiometabolic markers may have a potential role in secondary prevention of cardiometabolic disease patients, but further research is needed in this area.

In the next chapter, the findings from longitudinal analysis in the Psobid dataset are presented.

Chapter 8 Longitudinal Analysis in Psobid dataset

8.1 Chapter Summary

This chapter presents results from the longitudinal analysis of clinical outcomes in Psobid patients. General and cardiovascular clinical outcomes for depressive symptoms in general population (Psobid cohort) for a follow-up period of 7 years are described. The objective of this analysis is to study the prognosis of depressive symptoms in general population, in relation to physical health outcomes. This addresses research questions 3 and 4 in Psobid dataset. These research questions are:

Research Question 3 (RQ3):

What is the association, if any, between a composite AI score at baseline and the risk of future adverse health outcomes such as vascular events, hospitalisation and mortality in participants with depressive symptoms recruited from general population (Psobid cohort)?

Research Question 4 (RQ4):

What is the association, if any, between individual allostatic load biomarkers at baseline and the risk of future adverse health outcomes, such as vascular events, hospitalisation and mortality in participants with depressive symptoms recruited from general population (Psobid cohort) and how does it compare with the relationship between composite AI score at baseline and risk of adverse outcomes?

The following sections describe: the methods for data collection of clinical outcomes and statistical analysis; the association of AL biomarkers (composite AI score and individual biomarkers) and depressive symptoms with the risk of adverse clinical outcomes (general and cardiovascular) at 7 years; the statistical analyses of the interaction of depressive symptoms with allostatic load biomarkers (composite AI score and individual biomarkers) in risk prediction of these outcomes; and how the results compare with the existing literature as well as strengths, limitations and possible implications of the findings.

8.2 Methods

8.2.1 Data Collection for Clinical Outcomes

Electronic data linkage using the Information Services Division (ISD), Scotland data linkage services was used to study the clinical outcomes. The follow-up period was approximately 7 years between 31st May 2007 and 31st March 2014. The process of electronic data linkage was completely anonymised and no patient identifiable information was accessible at any stage. The process of data linkage has been already described in detail in Chapter 7. The ISD used patient CHI numbers to link the Psobid dataset with the general hospitalization, cancer and death registries. After extracting the information on clinical outcomes for Psobid patients, the CHI numbers were removed from the data by ISD and the data was forwarded for analysis. General and cardiovascular health outcomes were studied using the International System of Disease Classification- 10th Edition (ICD-10) (15).

General Health Outcomes:

1.   All-cause Death

2.   All-cause Hospitalization

3.   Any new incidence of cancer

Cardiovascular Health Outcomes with respective ICD-20 codes are as follows:

1.   Admission due to myocardial Infarction (MI) - I21

2.   Admission due to stroke – I61-I64

3.   Admission due to heart failure (HF): I50

4.   Death due to cardiovascular causes: I00- I99.

Major adverse cardiovascular outcome (MACE) was used as the composite outcome variable, and similar to the analysis in DepChron it included events

such as cardiovascular mortality or admission due to MI/stroke/HF. Patients were censored if they died due to reasons other than cardiovascular causes. Only the first cause of death and the first cause of hospitalization were taken into account to improve accuracy, in line with the approach adopted by previously published studies using data linkage (284-286).

8.2.2 Clinical Variables

Two different criteria for detecting presence of depressive symptoms were used, as assessed by continuous and cut-off scale of GHQ-28. A cut-off score of GHQ-28≥5 was used, as recommended by the national guidelines for case identification of depressive symptoms in a community setting (20).

The AI score was calculated using with the four different methods, which have been described in detail in Chapter 4. These methods were: 1. Method 1- Clinical cut-off 2. Method 2- Sample distribution cut-offs (25th and 75th percentile) 3. Method 3- Sample distribution cut-offs (10th and 75th percentile). 4. Method 4- z-score.

8.2.3 Statistical Analysis-AI score as predictor variable

Time to event analysis was used to study the association of the 2 predictors: a) AI score (4 different methods) and b) presence of depressive symptoms (GHQ-28 ≥5); with the risk of general health outcomes and composite major adverse cardiovascular outcome in Psobid patients. Various Cox proportional hazards regression analyses were performed for the four clinical outcomes under consideration. The four clinical outcomes were: all-cause mortality; all-cause hospital admission; MACE (cardiovascular death or admission due to MI/stroke/HF); and incidence of new cancer. The results are presented as hazard ratios (HR) with 95% confidence intervals (CI) and Harrell's Concordance statistics (with standard errors) using the "survival" package from R (288).

Multivariable analysis was undertaken adjusting for the following confounders: age (as a continuous variable), sex, socio-economic status (affluent and deprived), participant's smoking status (current vs. former smoker vs. non-smoker), and number of existing cardiometabolic conditions (coronary heart disease/stroke, diabetes, and hypertension) with a range from 0-3. The majority

of potential confounders were the same as those used in the DepChron analysis apart from few differences. Differences included that there was no control group in DepChron as far as cardiometabolic conditions were concerned, i.e. all patients in DepChron had at least one of the three cardiometabolic conditions. In addition, stroke and CHD were categorized as one problem in the Psobid dataset at the time of data collection so it was not possible to differentiate who had stroke and who had CHD among Psobid participants. Also, information on initiation of antidepressants was not available in Psobid, which was available in DepChron. On the other hand, information on the participant's smoking status was available in Psobid, which was not available in DepChron.

Cox regression models were fitted to each of the four clinical outcomes with both AI score and depressive symptoms added together as predictors, along with the potential confounders. Confounders that were not statistically significant were excluded from the final multivariable analysis. The results of these models were presented with HR with 95% CI and Harrell's Concordance statistics (with standard errors).

If AI score or depressive symptoms were found to have a statistically significant relationship with a clinical outcome in multivariable analysis, a Kaplan-Meier plot was constructed to visualise the results of this relationship.

To understand the relationship between AI score and depressive symptoms in risk prediction of clinical outcomes, if any, an analysis of variance (ANOVA) test to check for interaction between the AI score and presence of depressive symptoms was undertaken. The results were presented as p-values with a p-value less than 0.05 regarded as statistically significant. In the event of a significant interaction, a sub-group analysis was also performed to further study the nature of the interaction.

8.2.4 Statistical Analysis-Individual Biomarkers

Time to event analysis was used to study the association between the 12 individual biomarkers (SBP, DBP, BMI, total cholesterol, HDL cholesterol, triglycerides, creatinine, glucose, highly sensitive CRP, IL-6 and fibrinogen) as continuous measures and the risk of general and cardiovascular adverse outcomes in Psobid patients. WHR was categorised into raised WHR (>0.9 for females, 0.95 for males) and non-raised WHR. Cox proportional hazards regression analysis, unadjusted and adjusted for potential confounding factors, was performed and the results are presented with hazard ratios and 95% confidence intervals and Harrell's Concordance (C-statistics) with standard errors.

Multivariable analysis adjusting for the same confounders: age (as continuous variable), sex, socio-economic status (affluent and deprived), number of existing cardiometabolic conditions (coronary heart disease/stroke, diabetes, and hypertension) with a range from 0-3, and smoking status (current or former smoker vs. non-smoker) was performed. Cox regression models were fitted to each of the four clinical outcomes with peripheral biomarkers found to have a significant effect in univariable analysis and potential confounders with significant results. The results of these models are presented with HR with 95% CI and C-statistics (with standard errors).

In the event of a significant association between peripheral biomarkers (as continuous variables) and clinical outcome in multivariable analysis, the analysis was repeated using clinically relevant categories for respective biomarkers, where applicable. SBP was divided into five categories: very high (160-240), high (140-159), reference (130-139), tightly controlled (120-129), and low (80-119). I also used 5 categories for DBP: very high (100-130), high (90-99), reference (85-89), tightly controlled (80-84) and low (40-79). BMI was classified into 4 categories: normal: 18.5-25, underweight 15-18.5, overweight 25-30, obese 30-55 (245). Total cholesterol levels was divided into two categories (not raised vs. raised: >5 mmol/l (244)). HDL cholesterol was divided into two categories, low HDL cholesterol (<1.2 mmol/l) and not low HDL cholesterol. For

triglycerides and fasting glucose, the cut-offs for raised values were 2 and 6 mmol/l respectively. For raised creatinine, the cut-off values selected were >120 umol/l for males and >110 umol/l for females.

If a peripheral biomarker was found to have a statistically significant relationship with any of the adverse clinical outcomes in multivariable analysis, a Kaplan-Meier plot was constructed to visualise the results of this relationship.

To understand the relationship between individual biomarkers and depressive symptoms in risk prediction of clinical outcomes, if any, an ANOVA test was constructed to check for interaction between the individual biomarkers and presence of depressive symptoms. The results were presented as p-values with a p-value less than 0.05 regarded as statistically significant. In the event of a significant interaction, a sub-group analysis was undertaken to further study the nature of interaction. In the sub-group analysis, the study sample was divided on the basis of individual biomarker categories. In each sub-group, a Cox proportional hazards regression analysis was performed to study the risk of clinical outcomes with presence of depressive symptoms at baseline, adjusting for potential confounders. The results of the interaction were visualised with the help of a forest plot for HRs with 95%.

8.2.5 Comparing predictive power of AI score against individual peripheral biomarkers

The strength of the associations observed between 4 AI scores and adverse clinical outcomes were compared with that of the peripheral biomarkers and adverse clinical outcomes, for those results which were statistically significant in multivariable analysis. Only those confounders which were found to have a statistically significant result were used. Harrell's Concordance statistics (with standard errors) was used to compare different multivariable models with AI scores and peripheral biomarkers.

8.3 Results

8.3.1 Clinical Outcomes

Electronic data linkage between the Psobid dataset and hospital discharge, cancer registry and mortality records was successful for all 666 participants. The results for GHQ-28 were recorded for 639/666 (95.9%) participants in the Psobid dataset. 183 participants (27.4%) had depressive symptoms based on the criteria of a raised GHQ-28 (≥5).

Among Psobid participants (n=666), 30 participants (4.5%) were dead at the end of the seven year follow-up period. 418 participants (62.7%) had experienced at least one all-cause hospital admission, while 52 participants (7.8%) had been diagnosed with at least one new cancer. Finally, 31 participants (4.6%) had experienced at least one major adverse cardiovascular event at the end of 7 years.

8.3.2 Statistical Analysis- AI Score as Predictor

Table 8-1 shows the results of univariable analysis for the association between AI score (four different methods), presence of depressive symptoms (as assessed by GHQ-28≥5) and four different clinical outcomes. Presence of depressive symptoms did not have a significant association with any of the four clinical outcomes studied in the Psobid participants.

There was no significant association between the risk of all-cause death over seven years and any of the AI scores in Psobid participants. The risk of a composite major adverse cardiovascular event (cardiovascular death/admission due to MI/HF/stroke) and risk of all-cause hospital admissions in Psobid participants was significantly higher with higher AI score at baseline (all 4 methods). Finally, none of the AI scores had any significant association with the risk of incidence of new cancer over seven years in the Psobid participants.

Overall, AI score was found to have a significant association with only two clinical outcomes (MACE and all-cause hospital admission), while depressive symptoms did not have a significant association for any clinical outcomes. The effect sizes for AI score were not comparable to each other as the range for the 4 AI scores were different. Multivariable analysis was conducted for only two clinical outcomes- all cause hospital admission and MACE. For the final multivariable analysis, the clinical cut-offs and the z-score method AI scores were chosen on the basis of their better C-statistics values.

Table 8-1Univariable Cox proportional hazards for four clinical outcomes with AI score and depressive symptoms (GHQ-28≥5) as predictors.

Clinical Outcomes		AI score- Method 1- clinical cut-off (Range 0 to 10) Available N = 570	AI score-Method 2- 25th/75th percentile (Range 1 to 12) Available N=570	AI score-Method 3- 10th/90th percentile (Range 1 to 9) Available N=570	AI score – Method 4- z score method (Range 3.7 to 22.3) Available N=570	Presence of depressive symptoms (GHQ-28≥5) (Yes against No) Available N=639
All-cause death Number of events = 30	Hazard Ratio (HR)	1.18	1.20	1.21	1.14	2.02
	95% Confidence Intervals (CI)	0.98 to 1.42	0.97 to 1.47	0.94 to 1.57	0.99 to 1.31	0.94 to 4.32
	p-value	0.07	0.07	0.12	0.06	0.06
	C statistics- (SE)	0.639 (0.062)	0.604 (0.062)	0.608 (0.062)	0.626 (0.065)	0.581 (0.043)
All-cause hospital admission Number of events = 418	HR	1.06	1.05	1.06	1.05	1.12
	95% CI	1.01 to 1.11	1.00 to 1.10	0.99 to 1.14	1.01 to 1.09	0.90 to 1.38
	p-value	0.01	0.04	0.05	0.007	0.29
	C-statistics (SE)	0.532 (0.016)	0.527 (0.016)	0.537 (0.016)	0.553 (0.016)	0.509 (0.012)
Composite major adverse cardiovascular outcome (MACE) Number of events = 31	HR	1.49	1.24	1.47	1.25	0.79
	95% CI	1.25 to 1.77	1.02 to 1.49	1.18 to 1.82	1.13 to 1.39	0.33 to 1.85
	p-value	<0.001	0.02	<0.001	<0.001	0.59
	C-statistics (SE)	0.755 (0.057)	0.634 (0.057)	0.702 (0.057)	0.722 (0.06)	0.522 (0.042)
Incidence of new cancer Number of events = 52	HR	0.99	0.93	0.87	0.97	1.53
	95% CI	0.85 to 1.15	0.79 to 1.10	0.69 to 1.10	0.84 to 1.11	0.86 to 2.72
	p-value	0.91	0.43	0.26	0.68	0.14
	C-statistics (SE)	0.507 (0.048)	0.529 (0.048)	0.55 (0.047)	0.515 (0.049)	0.546 (0.032)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; MACE=Cardiovascular death or admission due to MI/stroke/heart failure.						

8.3.2.1 The influence of Confounders

The potential effects of five confounders were considered on the adverse clinical outcomes. They were: age (as a continuous variable), sex, socio-economic status (affluent and deprived), number of existing cardiometabolic conditions (coronary heart disease/stroke, diabetes, and hypertension) with a range from 0-3, and smoking status (current or former smoker vs. non-smoker). In the next part of analysis, the statistical significance of the impact of five confounders on each of the two adverse clinical outcomes considered for multivariable analysis was checked. For all-cause hospital admissions, sex (p-value=0.52) and smoking status (p-value=0.65) were not significant predictors and hence removed from the final analysis. For composite major adverse cardiovascular event, statistical association with age (p-value=0.15), smoking status (p-value=0.15) and socio-economic status (p-value=0.54) was not significant.

8.3.2.2 Multivariable Analysis

Table 8-2 shows that for risk prediction of all-cause hospital admission over seven years, age, socio-economic status and cardiovascular co-morbidity (1 against 0 and 2 against 0) had a significant association in multivariable analysis. In other words, if the participants had any one or two of the three conditions-MI/previous stroke or diabetes or hypertension at baseline, they were 35% and 66% respectively more likely to experience a hospital admission during 7 years as compared to those participants without these conditions. Participants selected from a socio-economically deprived background were 26% more likely to experience a hospital admission over seven years, as compared to those from an affluent background. AI score did not have a significant association with these outcomes.

Table 8-2 Multivariable Cox's proportional hazards for all-cause hospital admissions

Available N = 565, available number of events =341. C-Statistics = 0.607 (SE = 0.016) for AI score (clinical cut-off method). C-Statistics = 0.613 (SE = 0.016) for AI score (z-score method).			
	HR	95% CI	p-value
Age	1.03	1.01 to 1.04	<0.001
Socio-economic status-deprived group	1.26	1.00 to 1.58	0.04
Cardiovascular co-morbidity 1 (vs.0)	1.35	1.03 to 1.78	0.02
2 (vs. 0)	1.66	1.05 to 2.61	0.02
3 (vs. 0)	1.36	0.57 to 3.24	0.47
The 2 AI scores (clinical cut-off and z-score method), were added individually and not together to the multivariable analysis.			
AI score (clinical-cut off method)	0.99	0.94 to 1.06	0.95
AI score (z-score method)	1.02	0.93 to 1.70	0.18
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; Only significant confounders included in the final analysis.			

Male sex and cardiovascular co-morbidity (2 and 3 against 0) were found to have a significant association with the risk of major adverse cardiovascular event over seven years in Psobid participants, in the multivariable analysis (see Table 8-3). The risk of a composite cardiovascular event was observed to be very high (up to approximately 5 and 16 times higher) among participants with cardiovascular co-morbidity. The association of AI score was found to be to be statistically significant for the clinical cut-off method (p-value = 0.015), but the association was not significant with the z-score method (p-value = 0.066). One unit increase in AI score (clinical cut-off method) at baseline was associated with approximately 30% higher risk of experiencing a major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) over seven years.

Table 8-3 Multivariable Cox's proportional hazards for major adverse cardiovascular event (cardiovascular death/admission due to HF/stroke/MI)

	Hazard Ratio	95% Confidence Intervals	p-value
Available N = 565, available number of events = 25. C-Statistics = 0.795 (SE = 0.058) for AI score (clinical cut-off method). C-Statistics= 0.783 (SE = 0.058) for AI score (z-score method).			
Sex-male	2.69	1.12 to 6.48	0.02
Cardiovascular co-morbidity 1 (vs.0)	1.88	0.66 to 5.29	0.22
2 (vs. 0)	5.18	1.63 to 16.41	0.005
3 (vs. 0)	16.92	3.76 to 75.98	<0.001
The 2 AI scores (clinical cut-off and z-score method), were added individually and not together to the multivariable analysis.			
AI score (clinical-cut off method)	1.31	1.05 to 1.64	0.015
AI score (z-score method)	1.12	0.99 to 1.27	0.066
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error; Only significant confounders included in the final analysis.			

In summary, AI score (using the clinical cut-off method) at baseline was found to have a significant association with only one outcome - major adverse cardiovascular event over seven years in Psobid participants, in multivariable analysis. The z-score method AI score did not have a significant association with any clinical outcomes, in multivariable analysis. With the exception of all-cause hospital admissions, the available event rate was very low for the other 3 outcomes analysed.

8.3.2.3 Kaplan-Meier plots for AI score and clinical outcomes

The absolute event rate for major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) was significantly higher for participants with raised AI score- the clinical cut-off method (defined as >5; 75th percentile value) at baseline as compared to participants with non-raised AI score (p-value = 0.005). Figure 8-1 shows that nearly 10% of participants with raised AI score at baseline had experienced at least one major adverse cardiovascular event over 7 years as compared to approximately 4% of participants without raised AI score at baseline.

Figure 8-1 Kaplan-Meier plot comparing the rate of major adverse cardiovascular event and AI scores at baseline

AI score and Major Adverse Cardiovascular Event at 7 years
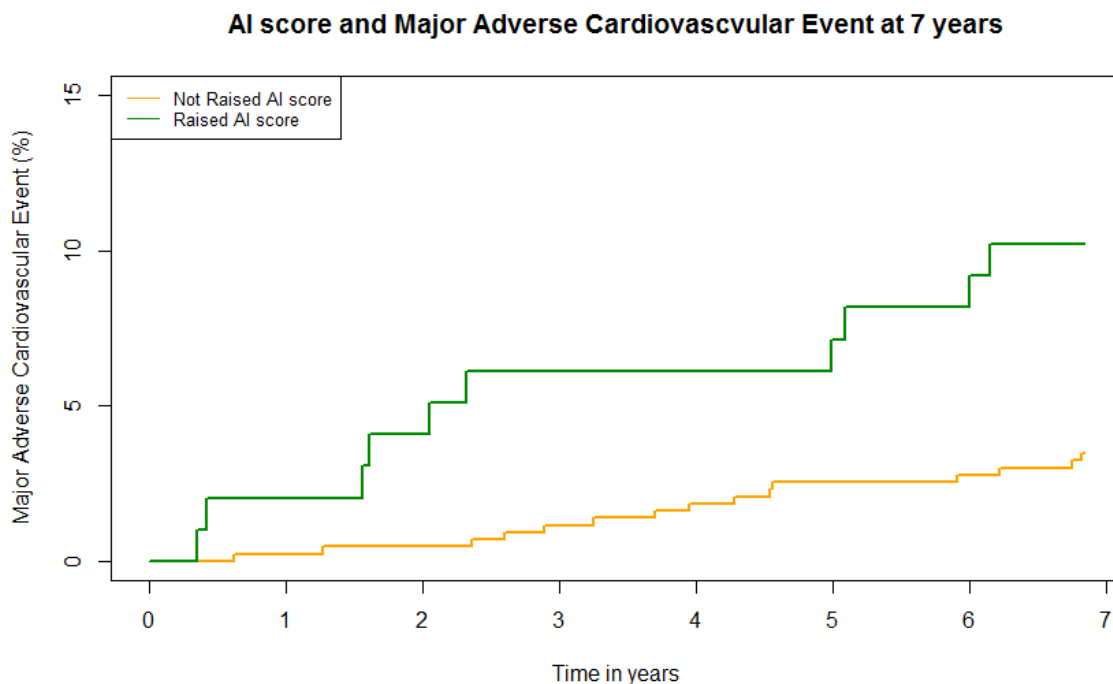
Legend: Raised AI score= defined as >5; 75th percentile value; AI score calculated with clinical cut-off method

8.3.2.4 Interaction between AI score and depressive symptoms

Considering the low event rate, an interaction analysis was performed between AI score and depressive symptoms using ANOVA, but without using the confounders for the individual events. There was no significant interaction between AI score (clinical cut-off method) and depressive symptoms (GHQ-28≥5) in risk prediction of any of the four clinical outcomes. The corresponding p-values were as follows: all-cause death (p-value = 0.47), all-cause hospital admission (p-value = 0.62), major adverse cardiovascular outcome (p-value = 0.30) and incidence of new cancer (p-value = 0.65).

The results were unchanged with no significant interaction found between AI score (z-score method) and depressive symptoms (GHQ-28≥5) in risk prediction of any of the four clinical outcomes. The corresponding p-values were as follows: all-cause death (p-value = 0.61), all-cause hospital admission (p-value = 0.75), major adverse cardiovascular outcome (p-value = 0.42) and incidence of new cancer (p-value = 0.66).

Key Findings (AI score and depressive symptoms as predictor variables):

- **Presence of depressive symptoms did not have a significant association with any of the four adverse clinical outcomes considered.**

- **AI score (clinical cut-off method) HR 1.31 had a significant impact on risk prediction of major adverse cardiovascular event (cardiovascular death or admission due to MI/HF/stroke) over seven years. This result remained significant after adjusting for statistically significant potential confounders.**

8.3.3 Statistical Analysis- Individual Biomarkers as Predictors

8.3.3.1 All-cause death as an outcome variable

Table 8-4 shows six out of 12 biomarkers had a significant association with the risk of all-cause death over seven years. They were: raised WHR, total cholesterol, HDL cholesterol, fasting glucose, creatinine and IL-6.

Table 8-4 Univariable Cox's Proportional for all-cause death with individual biomarkers as predictors.

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics-Concordance (SE= standard error)
Systolic Blood Pressure in mm Hg	4	90 to 202	1.01	0.99 to 1.03	0.07	0.596 (0.053)
Diastolic Blood Pressure in mm Hg	4	52 to 119	1.01	0.98 to 1.04	0.31	0.554 (0.053)
Waist Hip Ratio (raised vs. not raised)	**7**	**0.64 to 1.31**	**2.70**	**1.31 to 5.56**	**0.007**	**0.62 (0.043)**
Body Mass Index in kg/m2	4	16.3 to 51.1	1.05	0.99 to 1.11	0.07	0.6 (0.053)
Total Cholesterol in mmol/l	**21**	**2.4 to 8.6**	**0.43**	**0.29 to 0.64**	**<0.001**	**0.733 (0.055)**
High Density Lipoprotein Cholesterol in mmol/l	**21**	**0.4 to 3.0**	**0.23**	**0.07 to 0.74**	**0.01**	**0.641 (0.055)**
Triglycerides in mmol/l	21	0.3 to 10.8	0.94	0.63 to 1.40	0.78	0.475 (0.055)
Fasting Blood Glucose in mmol/l	**54**	**3.2 to 19.8**	**1.17**	**1.03 to 1.33**	**0.01**	**0.57 (0.06)**
Creatinine in umol/l	**28**	**55.2 to 159.6**	**1.02**	**1.00 to 1.05**	**0.049**	**0.611 (0.055)**
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	1.06	0.98 to 1.16	0.11	0.612 (0.056)
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.31**	**1.13 to 1.51**	**<0.001**	**0.704 (0.056)**
Fibrinogen in g/l	33	1.1 to 6.4	1.42	0.87 to 2.31	0.15	0.587 (0.055)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 31. Significant results in Bold.						

For multivariable analysis, the six biomarkers with a statistically significant result in univariable analysis were included. As seen with the results in the previous section, only age and sex (male vs. female) were included as confounders as other confounders did not have a statistically significant result.

Only total cholesterol and IL-6 had statistically significant effects of prediction of all-cause death in multivariable analysis (see Table 8-5).

Table 8-5 Multivariable Cox's Proportional Hazards for all-cause death with individual biomarkers as predictors

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics-(SE)
Waist Hip Ratio (raised vs. not raised)	7	0.64 to 1.31	1.90	0.91 to 3.98	0.084	0.774 (0.053)
Total Cholesterol in mmol/l	**21**	**2.4 to 8.6**	**0.51**	**0.35 to 0.74**	**<0.001**	**0.819 (0.055)**
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	0.45	0.14 to 1.47	0.19	0.779 (0.055)
Fasting Blood Glucose in mmol/l	54	3.2 to 19.8	1.13	0.98 to 1.30	0.08	0.77 (0.06)
Creatinine in umol/l	28	55.2 to 159.6	0.99	0.95 to 1.02	0.67	0.772 (0.055)
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.19**	**1.02 to 1.39**	**0.02**	**0.791 (0.056)**
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 31. Significant results in Bold. Confounders= Age and Sex.						

To further understand the relationship between the two biomarkers (total cholesterol and IL-6 with significant results in multivariable analysis) and all-cause mortality, the possibility for a non-linear association was checked by adding a quadratic term to the multivariable analysis as described above. The quadratic terms for the two biomarkers did not have a significant association with the risk of all-cause death: total cholesterol (p-value = 0.67) and IL-6 (p-value = 0.08).

The multivariable analysis was repeated using clinical categories for total cholesterol i.e. raised total cholesterol (defined as >5.0 mmol/l). Psobid participants with raised total cholesterol at baseline had an 88% (HR 0.12, 95% CI 0.03 to 0.40, p-value = <0.001) lower risk of all-cause mortality over 7 years, as compared to participants with total cholesterol <5 at baseline.

8.3.3.2 All-cause hospital admission as an outcome variable

In univariable analysis with all-cause hospital admissions as an outcome variable, five individual biomarkers were found to have a significant effect on risk prediction among Psobid participants: BMI, fasting glucose, highly sensitive CRP, IL-6 and fibrinogen (Table 8-6). Notably, all 3 inflammatory markers were found to have a statistically significant result.

Table 8-6 Univariable Cox's Proportional Hazards for all-cause hospital admissions with individual biomarkers as predictors

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics- (SE)
Systolic Blood Pressure in mm Hg	4	90 to 202	1.00	0.99 to 1.00	0.34	0.513 (0.015)
Diastolic Blood Pressure in mm Hg	4	52 to 119	0.99	0.98 to 1.00	0.75	0.499 (0.015)
Waist Hip Ratio (raised vs. not raised)	7	0.64 to 1.31	1.19	0.97 to 1.45	0.087	0.515 (0.012)
Body Mass Index in kg/m2	**4**	**16.3 to 51.1**	**1.02**	**1.00 to 1.04**	**0.01**	**0.521 (0.015)**
Total Cholesterol in mmol/l	21	2.4 to 8.6	0.93	0.84 to 1.02	0.14	0.510 (0.015)
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	0.84	0.64 to 1.11	0.23	0.524 (0.015)
Triglycerides in mmol/l	21	0.3 to 10.8	1.05	0.96 to 1.14	0.22	0.531 (0.015)
Fasting Blood Glucose in mmol/l	**54**	**3.2 to 19.8**	**1.11**	**1.05 to 1.18**	**<0.001**	**0.508 (0.015)**
Creatinine in umol/l	28	55.2 to 159.6	1.00	0.99 to 1.01	0.81	0.490 (0.015)
Highly sensitive C-reactive protein) in mg/l	**30**	**0.6 to 18.9**	**1.02**	**1.00 to 1.05**	**0.048**	**0.534 (0.015)**
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.13**	**1.07 to 1.19**	**<0.001**	**0.575 (0.015)**
Fibrinogen in g/l	**33**	**1.1 to 6.4**	**1.15**	**1.00 to 1.32**	**0.049**	**0.525 (0.015)**
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 418. Significant results in Bold.						

For multivariable analysis, three potential confounders with a statistically significant result: age, socio-economic status (deprived vs. affluent) and number of cardiovascular co-morbidity (0-3) were included. Table 8-7 shows only fasting glucose and IL-6 were found to have a statistically significant association in multivariable analysis.

Table 8-7 Multivariable Cox's Proportional Hazards for all-cause hospital admissions with individual biomarkers as predictors

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics- (SE)
Body Mass Index in kg/m2	4	16.3 to 51.1	1.00	0.98 to 1.02	0.56	0.607 (0.015)
Fasting Blood Glucose in mmol/l	**54**	**3.2 to 19.8**	**1.08**	**1.00 to 1.17**	**0.03**	**0.603 (0.015)**
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	0.99	0.96 to 1.02	0.91	0.606 (0.015)
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.06**	**1.00 to 1.13**	**0.02**	**0.610 (0.015)**
Fibrinogen in g/l	33	1.1 to 6.4	0.97	0.83 to 1.12	0.71	0.608 (0.015)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 418. Significant results in Bold. Confounders = age, socio-economic status, number of cardiometabolic conditions (0-3).						

The quadratic term for fasting glucose (p-value = 0.21) and IL-6 (p-value = 0.06) did not have a statistically significant in multivariable analysis, implying that both of these biomarkers did not have a non-linear relationship with the risk of all-cause hospital admission.

Clinical relevant categories were used for fasting glucose (raised = >6 mmol/l vs. not raised) and added to the multivariable analysis. The hazard ratio for raised fasting glucose was 1.40 (95% CI 0.97 to 2.02) and the p-value was 0.06. Thus, for the multivariable risk prediction of all-cause hospital admission over 7 years, only IL-6 was found to have a statistically significant association in Psobid participants. The result for glucose was not statistically significant after using clinically relevant categories.

8.3.3.3 MACE (cardiovascular death or admission due to MI/stroke/HF) as an outcome variable

In univariable analysis for major adverse cardiovascular event, only two individual biomarkers- highly sensitive CRP and DBP had a non-significant association. The other ten biomarkers had a significant result (see Table 8-8).

Table 8-8 Univariable Cox's Proportional Hazards for major adverse cardiovascular event with individual biomarkers as predictors

Biomarker	Missing Values	Range	Hazard Ratio	95% Confidence Intervals	p-value	C statistics-Concordance (SE= standard error)
Systolic Blood Pressure in mm Hg	**4**	**90 to 202**	**1.01**	**1.00 to 1.03**	**0.02**	**0.608 (0.052)**
Diastolic Blood Pressure in mm Hg	4	52 to 119	1.00	0.97 to 1.03	0.79	0.510 (0.052)
Waist Hip Ratio (raised vs. not raised)	**7**	**0.64 to 1.31**	**9.18**	**3.76 to 22.4**	**<0.001**	**0.749 (0.042)**
Body Mass Index in kg/m2	**4**	**16.3 to 51.1**	**1.05**	**1.00 to 1.11**	**0.040**	**0.628 (0.052)**
Total Cholesterol in mmol/l	**21**	**2.4 to 8.6**	**0.59**	**0.40 to 0.87**	**0.008**	**0.622 (0.055)**
High Density Lipoprotein Cholesterol in mmol/l	**21**	**0.4 to 3.0**	**0.11**	**0.03 to 0.38**	**<0.001**	**0.682 (0.055)**
Triglycerides in mmol/l	**21**	**0.3 to 10.8**	**1.34**	**1.11 to 1.63**	**0.002**	**0.703 (0.055)**
Fasting Blood Glucose in mmol/l	**54**	**3.2 to 19.8**	**1.23**	**1.11 to 1.36**	**<0.001**	**0.696 (0.058)**
Creatinine in umol/l	**28**	**55.2 to 159.6**	**1.03**	**1.00 to 1.05**	**0.01**	**0.607 (0.055)**
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	1.05	0.96 to 1.14	0.22	0.627 (0.055)
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.34**	**1.16 to 1.53**	**<0.001**	**0.746 (0.055)**
Fibrinogen in g/l	**33**	**1.1 to 6.4**	**1.73**	**1.09 to 2.75**	**0.02**	**0.640 (0.055)**
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 31. Significant results in Bold.						

For multivariable analysis, only sex and number of cardiometabolic conditions were included as confounders, as they had a significant result. Table 8-9 shows only raised WHR and IL-6 were found to have a statistically significant association with the risk of a major adverse cardiovascular event in multivariable analysis. IL-6 had a linear association with the risk of MACE as the

quadratic term for IL-6 was found to have a non-significant result in the multivariable analysis (p-value = 0.054).

Table 8-9 Multivariable Cox's Proportional Hazards for major adverse cardiovascular event with individual biomarkers as predictors

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics-(SE)
Systolic Blood Pressure in mm Hg	4	90 to 202	1.00	0.99 to 1.02	0.35	0.760 (0.052)
Waist Hip Ratio (raised vs. not raised)	**7**	**0.64 to 1.31**	**5.51**	**2.14 to 14.15**	**<0.001**	**0.839 (0.051)**
Body Mass Index in kg/m2	4	16.3 to 51.1	1.01	0.94 to 1.08	0.63	0.750 (0.052)
Total Cholesterol in mmol/l	21	2.4 to 8.6	0.95	0.63 to 1.43	0.83	0.749 (0.055)
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	0.36	0.10 to 1.28	0.11	0.788 (0.055)
Triglycerides in mmol/l	21	0.3 to 10.8	1.23	0.94 to 1.61	0.12	0.800 (0.055)
Fasting Blood Glucose in mmol/l	54	3.2 to 19.8	1.03	0.89 to 1.19	0.67	0.779 (0.058)
Creatinine in umol/l	28	55.2 to 159.6	1.01	0.98 to 1.04	0.36	0.758 (0.055)
Interleukin 6 in pg/ml	**37**	**0.2 to 11.8**	**1.22**	**1.03 to 1.44**	**0.02**	**0.802 (0.055)**
Fibrinogen in g/l	33	1.1 to 6.4	1.36	0.82 to 2.25	0.22	0.778 (0.055)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 31. Significant results in Bold. Confounders= Sex and Number of cardiometabolic conditions (0-3).						

8.3.3.4 Incidence of new cancer as outcome variable

Table 8-10 shows that none of the individual biomarkers had a statistically significant association with incidence of new cancer over 7 years among Psobid participants. Considering the negative results in univariable analysis, multivariable analysis was not undertaken for any of the biomarkers.

Table 8-10 Univariable Cox's Proportional Hazards for incidence of new cancer with individual biomarkers as predictors

Biomarker	Missing Values	Range	HR	95% CI	p-value	C statistics (SE)
Systolic Blood Pressure in mm Hg	4	90 to 202	1.01	0.99 to 1.02	0.12	0.561 (0.04)
Diastolic Blood Pressure in mm Hg	4	52 to 119	1.01	0.99 to 1.04	0.15	0.560 (0.04)
Waist Hip Ratio (raised vs. not raised)	7	0.64 to 1.31	1.00	0.56 to 1.80	0.98	0.50 (0.033)
Body Mass Index in kg/m2	4	16.3 to 51.1	1.00	0.95 to 1.05	0.78	0.524 (0.04)
Total Cholesterol in mmol/l	21	2.4 to 8.6	0.76	0.58 to 1.00	0.054	0.576 (0.041)
High Density Lipoprotein Cholesterol in mmol/l	21	0.4 to 3.0	0.86	0.41 to 1.81	0.70	0.527 (0.041)
Triglycerides in mmol/l	21	0.3 to 10.8	1.01	0.77 to 1.32	0.92	0.516 (0.041)
Fasting Blood Glucose in mmol/l	54	3.2 to 19.8	0.93	0.69 to 1.24	0.62	0.476 (0.044)
Creatinine in umol/l	28	55.2 to 159.6	1.01	0.99 to 1.03	0.21	0.566 (0.042)
Highly sensitive C-reactive protein) in mg/l	30	0.6 to 18.9	0.99	0.91 to 1.08	0.93	0.501 (0.041)
Interleukin 6 in pg/ml	37	0.2 to 11.8	1.04	0.87 to 1.23	0.64	0.544 (0.043)
Fibrinogen in g/l	33	1.1 to 6.4	1.07	0.73 to 1.56	0.72	0.526 (0.041)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 52. Significant results in Bold.						

8.3.3.5 Kaplan Meier Plots for peripheral biomarkers and clinical outcomes

Figure 8-2 shows the higher mortality rate among participants with raised IL-6 (defined as higher than 75th percentile value i.e. IL-6 >2.75 pg/ml) at baseline than the rest of the Psobid participants (p-value <0.001). Approximately 10% of participants with raised IL-6 at baseline were dead at the end of seven years.

Figure 8-2 Kaplan Meier plot showing all-cause mortality against IL-6 values at baseline
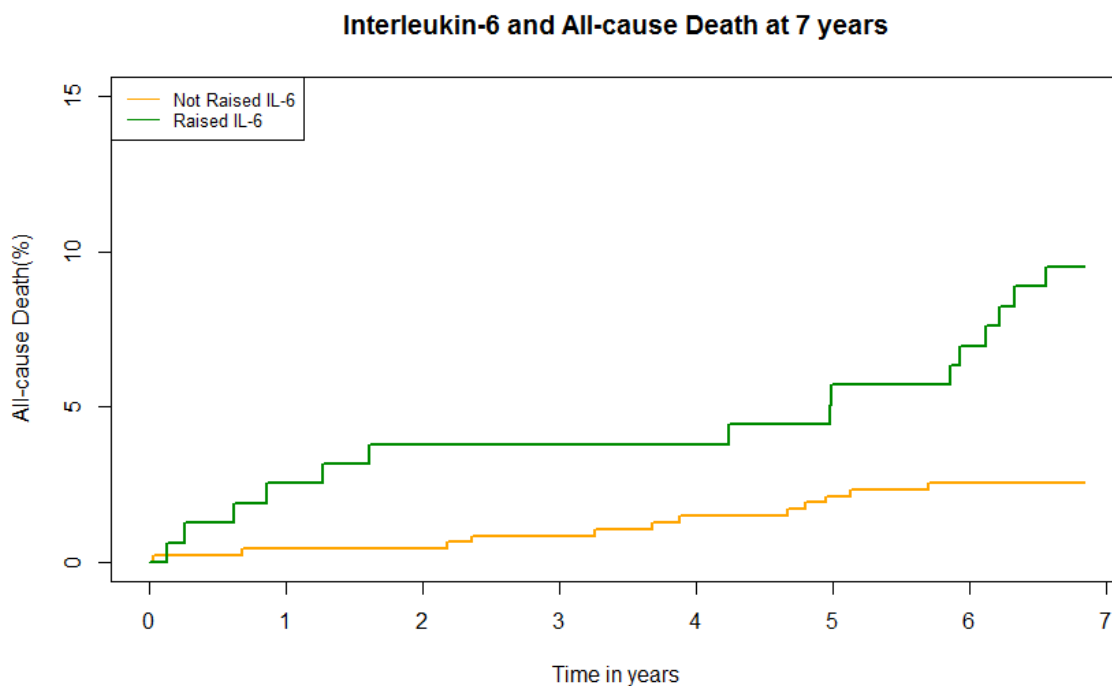
Legend: raised IL-6=defined as higher than 75th percentile value >2.75 pg/ml

In contrast, an inverse linear association was observed between total cholesterol and all-cause death. Figure 8-3 shows that Psobid participants with raised total cholesterol (>5.0 mmol/l) at baseline were less likely to die than the rest of the Psobid participants (p-value <0.001). The mortality rate over seven years was approximately 7% among participants with cholesterol less than 5.0 as compared to approximately 2% rate observed among those with cholesterol higher than 5.0.

Figure 8-3 Kaplan Meier plot showing all-cause mortality with raised total cholesterol (> 5.0) at baseline
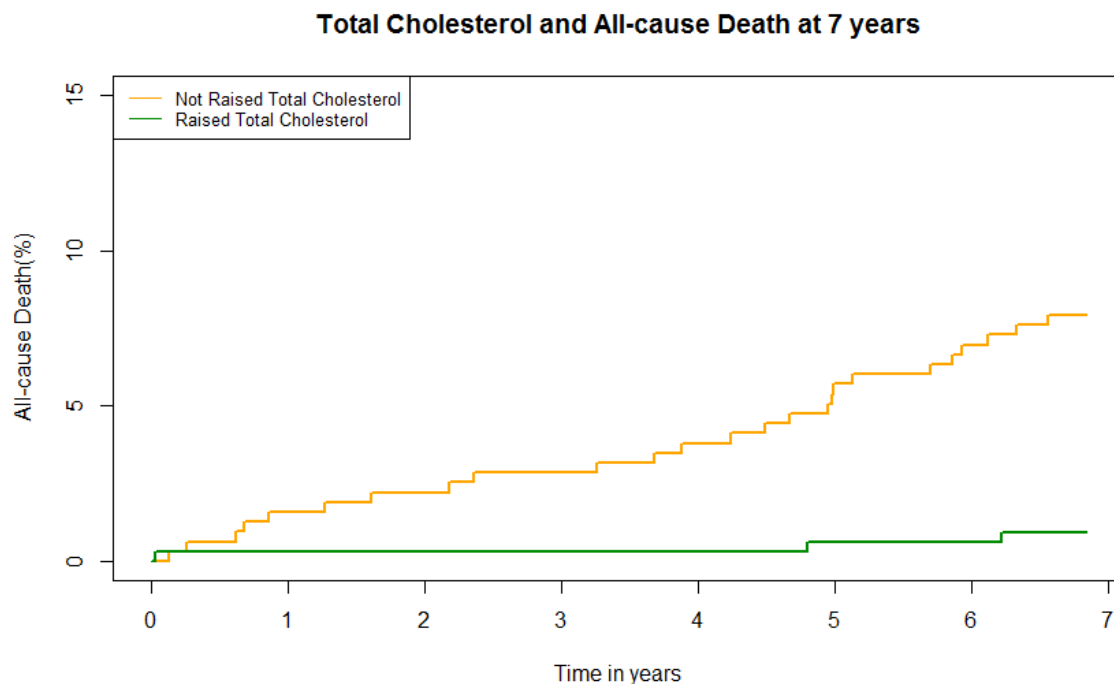
Participants with raised IL-6 at baseline had higher chances (p-value <0.001) of experiencing an all-cause hospital admission during 7 years (Figure 8-4). Participants with raised IL-6 experienced an absolute event rate of roughly 75% over 7 years as compared to approximately 55% event rate observed among those without raised IL-6.

Figure 8-4 Kaplan Meier plot showing all-cause hospital admissions against IL-6 values at baseline
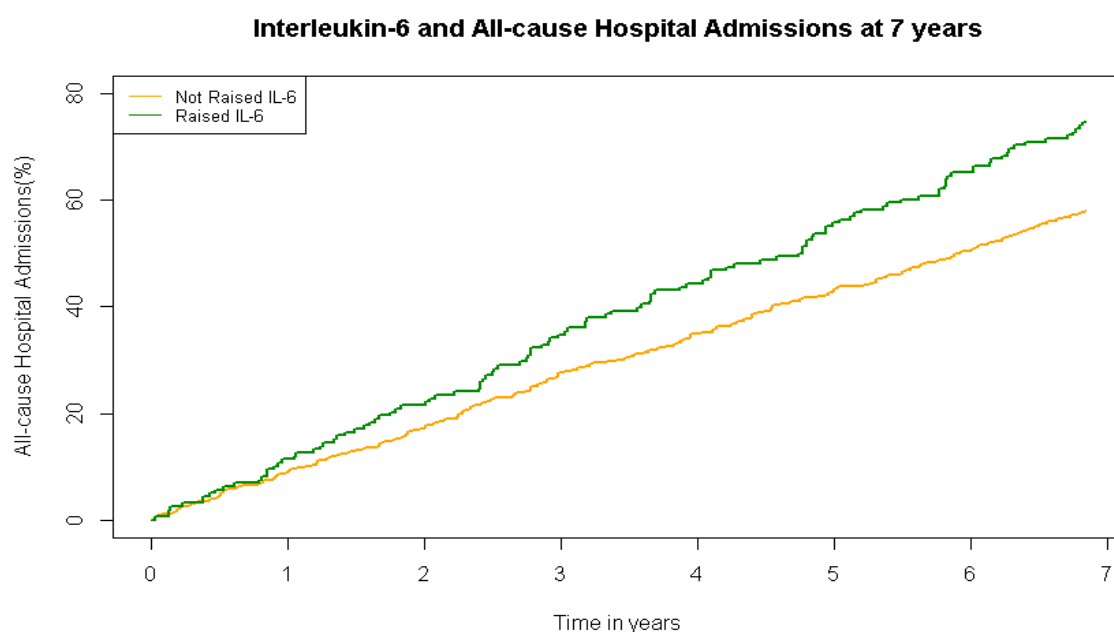
Legend: raised IL-6=defined as higher than 75th percentile value >2.75 pg/ml

Statistically significant higher risk of experiencing at least one major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) was observed among Psobid participants with raised IL-6 (p-value < 0.001) and raised WHR (p-value <0.001) at baseline. The absolute event rates observed for participants with raised IL-6 and those with raised WHR (>0.85 in females, >1.0 in males) at baseline were approximately 10% and 12% respectively (Figure 8-5 and Figure 8-6).

Figure 8-5 Kaplan Meier plot showing major adverse cardiovascular event against IL-6 values at baseline
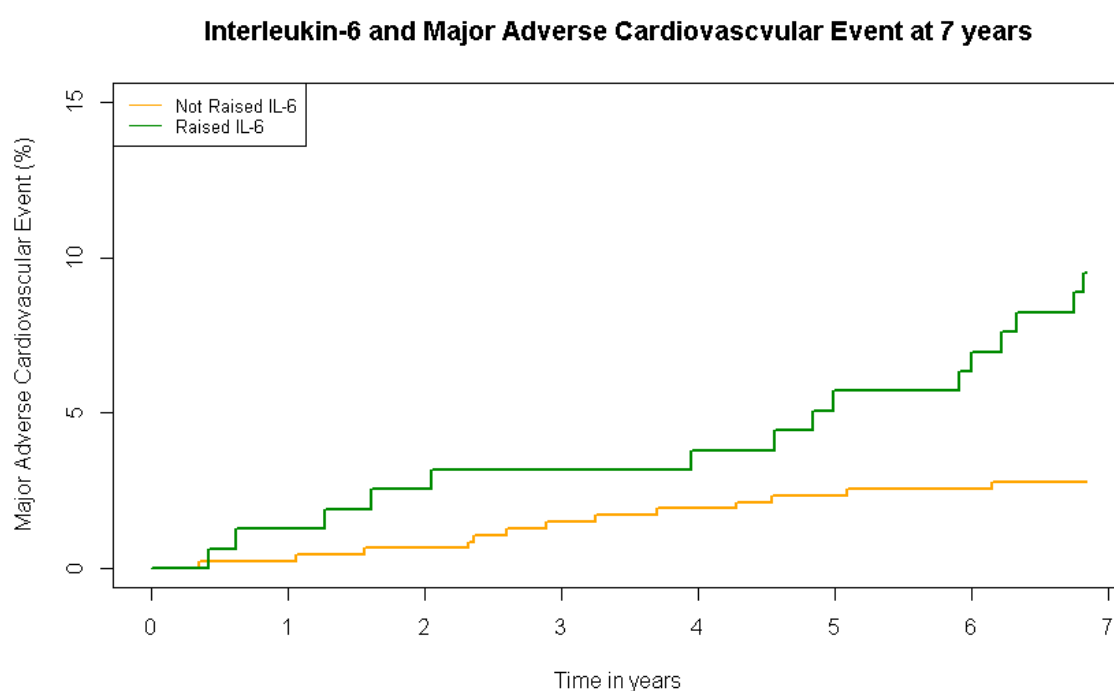
Legend: raised IL-6=defined as higher than 75th percentile value >2.75 pg/ml

Figure 8-6 Kaplan Meier plot showing major adverse cardiovascular event against raised WHR (>0.90 in females, 0.95 in males) at baseline
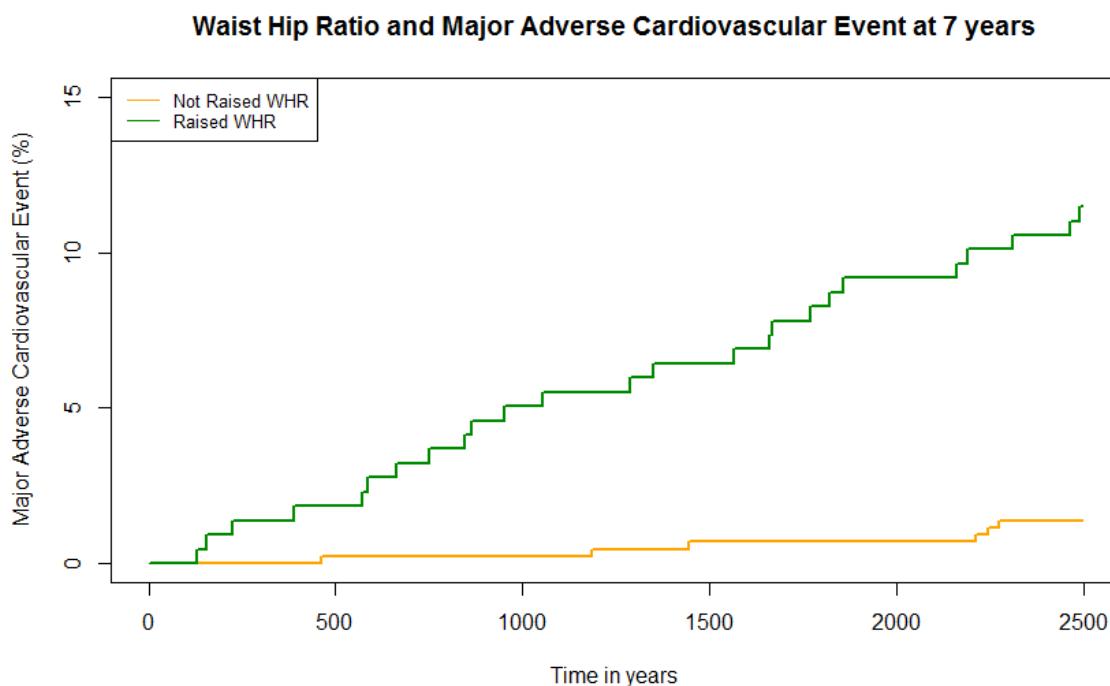
8.3.3.6 Interaction analysis between peripheral biomarkers and depressive symptoms

A statistical interaction analysis between the presence of depressive symptoms and only those peripheral biomarkers which had a significant result in multivariable analysis for any of the 4 clinical outcomes under consideration was performed. Again, as with the analysis with AI score, confounders were not added to the interaction analysis due to the low event rate. These peripheral biomarkers were: IL-6 and total cholesterol (all-cause death), IL-6 (all-cause hospital admission) and IL-6 and WHR (major adverse cardiovascular event). No interaction analysis was undertaken for incidence of new cancer as none of the peripheral biomarkers had a significant association with that outcome.

In summary, there was no significant interaction between the peripheral biomarkers and depressive symptoms for any of the four clinical outcomes under consideration.

Key Findings (Individual Biomarkers as Predictor):

- **IL-6 (continuous) and raised total cholesterol >5.0 (vs. not raised total cholesterol) were found to have a direct linear association with an increased probability of all-cause death at seven years, after adjusting for the effects of potential confounders.**

- **IL-6 (continuous) was also found to have a linear association with increased probability of all-cause hospital admission at seven years, after adjusting for the effects of potential confounders.**

- **IL-6 (continuous) and raised WHR >0.85 in females, >1.0 in males (vs. not raised WHR) were found to have a direct linear association with increased probability of MACE at seven years, after adjusting for the effects of potential confounders.**

- **None of the available peripheral biomarkers had any statistically significant association with incidence of new cancer at seven years among the Psobid participants.**

- **There was no significant statistical interaction between the presence of depressive symptoms and peripheral biomarkers in the risk prediction of any clinical outcomes under consideration among the Psobid participants.**

8.3.4 Comparing predictive power of AI score against individual peripheral biomarkers

The strength of the associations observed between AI scores and clinical outcomes was compared with that of the peripheral biomarkers and adverse clinical outcomes, for those results which were statistically significant in multivariable analysis. AI score had a significant association with the risk of only one clinical outcome in multivariable analysis- MACE (cardiovascular death or admission due to MI/stroke/HF). On the other hand, IL-6 and WHR were observed to have a statistically significant impact in predicting major adverse cardiovascular event in multivariable analysis. Sex and number of cardiometabolic conditions (0-3) were used in the multivariable analysis, as they were the significant confounders. Table 8-11 shows that the model containing WHR had the best C-statistics value, suggesting best fit to the data.

Table 8-11 Comparison of AI scores and peripheral biomarkers as predictors in multivariable Cox's proportional for major adverse cardiovascular event (cardiovascular death/admission due to HF/stroke/MI)

	HR	95% CI	p-value	C-statistics (SE)
AI score- Method 1-clinical cut-off (Range 0 to 10)	1.31	1.05 to 1.64	0.015	0.795 (0.058)
AI score – Method 4- z score method (Range 3.7 to 22.3)	1.12	0.99 to 1.27	0.066	0.783 (0.058)
IL-6 (Range 0.2 to 11.8)	1.22	1.03 to 1.44	0.021	0.802 (0.055)
Raised WHR (vs. not raised WHR) (>0.85 in females; 1.0 in males)	5.51	2.14 to 14.15	<0.001	0.839 (0.051)
HR=Hazard Ratio; CI=Confidence Intervals; C-statistics=Concordance; SE=Standard Error. Number of events = 31. Significant results in Bold. Confounders= Sex and Number of cardiometabolic conditions (0-3).				

Based on these results, a composite AI score did not offer any better predictive value for major adverse cardiovascular event over seven years, when compared to individual peripheral biomarkers such as WHR.

Finally, the combined predictive power of raised WHR and IL-6 was checked by adding both of these biomarkers together along with sex and number of cardiometabolic conditions in a multivariable Cox' proportional hazards, with

MACE as the outcome variable. In this model, IL-6 did not have a statistically significant relationship (HR 1.13, 95% CI 0.95-1.35, p-value=0.15) while raised WHR was still found to have a statistically significant relationship (HR 5.13, 95% CI 1.83-14.38, p-value<0.01) with the risk of having a MACE event over seven years. The C-statistics (Concordance=0.865, se=0.055) of the model using WHR and IL-6 together (along with confounders) was marginally better than the one used for only WHR with confounders, implying that this model has marginally better predictive power for a MACE event over seven years.

Key Findings (Comparing Predictive Power for a MACE event):

- **Individual biomarkers such as WHR and IL-6 had a better predictive power for a MACE event over seven years than a composite AI score in a multivariable Cox Regression, after adjusting the effects of potential confounders.**

- **Using WHR and IL-6 together marginally improved the predictive power of the model for a MACE event over seven years; however the association of IL-6 with MACE was no longer statistically significant when used together with WHR.**

8.4 Discussion

8.4.1 Summary of Findings

The observed event rate among Psobid participants for the 4 adverse clinical outcomes under study were as follows: 30 participants (4.5%) were dead, 418 participants (62.7%) had experienced at least one all-cause hospital admission, 52 participants (7.8%) had been diagnosed with at least one new cancer and 31 participants (4.6%) had experienced at least one major adverse cardiovascular event at the end of seven years. Major adverse cardiovascular event was defined as a combination of cardiovascular death and admission to hospital due to MI or stroke or HF. Presence of depressive symptoms identified by GHQ-28 at baseline did not have a significant association with any clinical outcomes over 7 years among Psobid participants.

A composite AI score (all 4 methods) at baseline had a significant association with the risk of major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) and all-cause hospital admissions over 7 years. However, only the association of AI score (clinical cut-off) with major adverse cardiovascular event remained significant in multivariable analysis.

A continuous measure of IL-6 observed at baseline had a significant association with the risk of 3 clinical outcomes- all-cause mortality, all-cause hospital admissions and major adverse cardiovascular event. These associations remained significant after adjusting for potential confounders.

Psobid participants with raised total cholesterol (values >5.0 mmol/l) had lower risk of all-cause death over 7 years as compared to those with non-raised total cholesterol values at baseline. This result remained statistically significant in multivariable analysis.

Presence of raised WHR (defined as >0.85 in females and >1.0 in males) at baseline was associated with higher risk of major adverse cardiovascular event over seven years among Psobid participants. These results were adjusted for potential confounders in a multivariable analysis.

There was no significant interaction between presence of depressive symptoms and AI score (all 4 methods) in risk prediction of any of the 4 adverse clinical outcomes under consideration. There was no significant interaction between presence of depressive symptoms and the 12 peripheral biomarkers in risk prediction of any of the adverse clinical outcomes under consideration.

A model containing a composite AI score did not offer any better predictive power for MACE, when compared to a model containing individual peripheral biomarker such as raised WHR. These results were adjusted for 5 potential confounders: age, sex, socio-economic status (deprived vs. affluent), number of cardiometabolic conditions (0-3) and smoking status (current or former smoker vs. never smoked). However, only significant confounders were included in the multivariable analysis owing to low event rates.

8.4.2 Strengths

1. The rate of data completion in Psobid was very good (>90% participants) for GHQ-28.

2. The study participants were well characterized with detailed information available on demographics and availability of both primary and secondary allostatic load biomarkers.

3. A 100% electronic data linkage was achieved for Psobid participants using ISD services.

8.4.3 Limitations

1. The participants were chosen from the most deprived and the least deprived areas to highlight the impact of socio-economic status, which is also a strength of the study as well as a limitation. Socio-economic status is a continuum and hence the results of this study cannot be generalized to the whole population.

2. The response rate for study participation was 25%. Hence there is a possibility of a response bias.

3. GHQ-28 was used for estimating the prevalence of depressive symptoms. GHQ-28 was originally designed to screen for minor psychological distress (not restricted to depressive symptoms) (231). However, GHQ-28 has been validated for screening of depressive symptoms in the general population against MDD; GHQ-28 has been reported to have a sensitivity of 79.2% and specificity of 79.6% against a composite international diagnostic instrument for DSM-IV criteria for major depressive disorder (277).

4. A major limitation of this analysis is the low number of adverse clinical outcomes or event rate observed which limited the power of the analysis.

5. There were only single assessments available for biomarker values and depressive symptoms at the start of the study for Psobid participants. The biomarker values and depressive symptoms may have changed over the course of the seven year follow-up duration but this information was not available.

6. In this analysis, IL-6 values at baseline were found to be a significant predictor of three adverse clinical outcomes. IL-6 is not used in routine clinical practice. Hence, there are unlikely to be any clinical implications from these results at the present time.

8.4.4 Comparison of Findings with Existing Literature

The relationship between AI score and all-cause mortality and cardiovascular events has been extensively studied in older and ageing populations, but it has not been studied in detail in middle-aged populations like the Psobid participants (28,233). The third national health and nutritional examination survey (NHANES III) in the U.S. studied the relationship between AI score and all-cause mortality in more than 13,000 adults older than 25 years (306). The study used single measurements of 9 peripheral biomarkers (albumin, CRP, total cholesterol, HDL cholesterol, HbA1c, WHR, SBP, DBP and heart rate) and a single cut-off method to calculate a composite AI score. The study showed that participants with high AI score (>2 and >3) were more likely to die at follow-up (median follow-up 8.7 years) as compared to those with AI score ≤ 1 at baseline (306). These findings are contrary to those observed in our study, although there

are some key differences between the NHANES III study and Psobid. The biomarkers used by that NHANES II were different to those used in Psobid and also the participants were relatively older in Psobid with lower observed event rates (306).

Seeman et al. showed an association between higher AI score (calculated using the single cut-off method) at baseline and incident CVD (defined as MI or stroke or a new diagnosis of hypertension or diabetes) at 2.5 years in 736 elderly participants (age 70-79) from the New England region of U.S. (208). Psobid is one of the first studies to show an association between AI score and cardiovascular events in a middle-aged population.

Psychological distress measured by the General Health Questionnaire (GHQ) has been linked with an increased risk of all-cause mortality in various studies before. Data from the health survey for England using more than 60,000 participants showed a dose-response relationship between psychological distress measured by GHQ-30 and all-cause mortality with a mean follow-up of 8.2 years (307). Similarly, other studies have also shown an association between higher risk of all-cause mortality with higher GHQ-30 at baseline (308,309). All of these three studies have been sampled from the general population and they have all used GHQ-30. Interestingly, the other studies have also shown a strong association of GHQ-30 with cardiovascular deaths (308,309), while in Psobid participants there was no significant association observed between depressive symptoms and the risk of all-cause death and MACE (caveat being the low event rate in Psobid which could be a contributory factor for the observed results).

In Psobid participants, IL-6 was associated with an increased risk of all-cause mortality. There have been three studies investigating the relationship between IL-6 and all-cause mortality (310). Two out of these three studies have shown a positive association (311,312), while one study did not report a significant relationship (313). Overall, the results from meta-analysis of these studies did not report a significant association between IL-6 and all-cause mortality (310). This is contrary to the results observed in Psobid. Notably, the participants in these three studies were comparatively older than the Psobid participants (310). The relationship between IL-6 and cardiovascular events (cardiovascular death or MI) has been studied more extensively in 17 different population studies (314).

Based on the results of meta-analysis of 17 studies, an increase in IL-6 values by 1 standard deviation was associated with 61% higher risk of cardiovascular events (adjusted odds ratio 1.61, 95% 1.42 to 1.83) (314). In comparison, with each unit increase in IL-6, the risk of cardiovascular event increased by 22% in Psobid participants (adjusted HR 1.22, 95% CI 1.03 to 1.44). The standard deviation for IL-6 was 1.5 in Psobid participants.

Carriere and colleagues observed that low total cholesterol (<4.95 mmol/l in males and <5.23 mmol/l in females) values were associated with higher risk of all-cause mortality at 5 years, when compared to the reference total cholesterol values (4.95 to 6.18 mmol/l in males and 5.23 to 6.61 mmol/l in females) in more than 1400 French residents >60 years of age (315). However, this association was no longer significant with longer follow-up, i.e. 5 to 9 years. In contrast, a statistically significant relationship was observed in Psobid participants between low total cholesterol (<5 mmol/l) and higher risk of all-cause mortality over 7 years.

In Psobid participants, a strong association was observed between baseline WHR and risk of major adverse cardiovascular event over 7 years. A meta-analysis of 15 studies and 258,114 participants showed that a 0.10 increase in WHR was associated with 50% adjusted higher risk of cardiovascular event (316). The observed effect size was much larger in Psobid, with approximately 25 times higher risk of cardiovascular event with every 0.10 increase in WHR values. The meta-analysis did not perform a regression using categorical values of WHR.

8.4.5 Potential Implications

There are three potential implications for future research. Firstly, a composite AI score did not offer any better accuracy in predicting cardiovascular events, when compared with individual biomarkers such as WHR, especially in this middle aged population. Secondly, in spite of strong evidence of a relationship between IL-6 and adverse clinical outcomes, the clinical applicability of IL-6 remains uncertain. The focus of future research should be the possibility of identifying a clinically relevant cut-off for IL-6, which may aid its clinical use. Similarly, WHR measurement is time consuming and it may be difficult to implement in an average 10 minute GP consultation. Finally, there was no

significant interaction observed between depressive symptoms and peripheral biomarkers (individual or composite) in predicting clinical outcomes in Psobid. This is contrary to the results of significant interaction of depressive symptoms and cardiovascular biomarkers observed in the DepChron dataset. The differences in the observed results could be due to differences in the two datasets, such as, study setting, age-group, and prevalence of underlying health conditions.

8.4.6 Conclusion

Presence of depressive symptoms was not associated with any of the measured clinical outcomes at seven years follow-up in Psobid participants. A composite AI score was shown to have an association with the risk of major adverse cardiovascular events in middle aged participants sampled from the general population. However, the strength of this association was not any stronger than the relationship observed between WHR and cardiovascular events. IL-6 was found to have a statistically significant relationship with the risk of adverse health outcomes such as all-cause mortality and all-cause hospital admissions, as well as risk of major adverse cardiovascular events.

The longitudinal analysis in Psobid has suggested that the hypothesis of usefulness of peripheral biomarkers in risk prediction of clinical outcomes in patients with depressive symptoms may not be applicable to middle aged participants selected from the general population. In the next chapter, the results of developing a risk scoring system for predicting clinical outcomes in patients with depressive symptoms using demographics and peripheral biomarker values are presented.

Chapter 9: Development of a Risk Scoring System for Adverse Clinical Outcomes in Patients with Depressive Symptoms

9.1 Chapter Summary

In this chapter, the development of a scoring system based on the risk of adverse clinical outcomes in patients with depressive symptoms is presented. The objective of this analysis is to study the role of peripheral biomarkers in prognosis of depressive symptoms in relation to physical adverse health outcomes. This addresses the research question 5 in DepChron and Psobid datasets.

Research Question 5 (RQ5):

What is the accuracy of a risk scoring system developed using patient demographics, allostatic load biomarker values and severity of depressive symptoms, in predicting adverse health outcomes in patients with depressive symptoms?

DepChron

DepChron patients had existing cardiometabolic disease, hence major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) was chosen as the outcome of interest.

The purpose of this analysis was to develop a risk prediction model for secondary cardiovascular events using demographic factors, peripheral biomarker values and depressive screening results in patients with existing cardiometabolic disease. The sample was the subset of patients in DepChron who underwent depression screening (n=35537).

Psobid

In Psobid, it was not possible to perform this analysis as the observed event rate for different clinical outcomes was low (all-cause death: 30, major adverse

cardiovascular event: 31 and incidence of new cancer: 52). As a rough guide, 10 events are required for every predictor variable in a model (317). Incidence of new cancer possibly had a sufficient number of events, but neither depressive symptoms nor any of the allostatic load biomarkers was found to have a significant association with that outcome (see Chapter 8). Hence, this analysis was not undertaken in the Psobid population. Incidence of hospital admissions also had a sufficient number of events, however information on other factors such as polypharmacy and information on previous admission was missing in Psobid cohort, which are likely to influence rate of hospital admissions and thus this analysis was not performed.

In the following section, the methods and results for developing a risk scoring system in the DepChron dataset are described. Finally, the strengths and limitations of the findings are discussed and compared with existing literature.

9.2 Methods

9.2.1 Developing a Risk Prediction Model

In the development of a risk prediction model, the statistical methods described by Streyerberg in his book "Clinical Prediction Models" were adopted (318)(319,320). MACE (cardiovascular death and admission due to MI/Stroke/HF) was used as the outcome variable. This was a development and validation study using resampling with bootstrapping, Type 1B based on TRIPOD statement classification of risk prediction studies (30)

The series of seven steps used in creating a risk prediction model have been summarized here.

The first step involved managing the observations with missing values. Considering the relatively big sample size in DepChron, the observations with missing values were excluded. The DepChron patients with missing depression scores were observed to a have a higher rate of MACE events- see Table 7.1 in Chapter 7. Excluding patients with missing values means that patients at higher risk of MACE for unknown reasons were excluded. The second step involved coding the predictor variables appropriately. The predictor variables considered were: age (categorized into 3 groups: 18-44, 45-64 and 65-90), gender, socio-economic status (affluent based on SIMD1-5 vs. deprived based on SIMD 6-10), number of co-morbid cardiometabolic conditions (1-3 of diabetes, stroke and CHD), SBP at baseline (very high=160-240, high=140-159, reference=130-139, tightly controlled=120-129, and low=80-119), DBP at baseline (very high=100-130, high=90-99, reference=85-89, tightly controlled=80-84 and low=40-79), BMI at baseline (reference=18.5-25, underweight=15-18.5, overweight=25-30, obese=30-55), and total cholesterol at baseline (reference<5.0, raised=>5.0). HADS-D at baseline was categorized into no depressive symptoms (0-7) and presence of depressive symptoms (≥8)(152).

Thus, there were nine potential predictors in the scoring system. These included four cardiovascular biomarkers and they were all categorized based on relevant clinical guidelines (245)(244)). There is no consensus among various guidelines published internationally for optimal BP targets in patients with existing cardiometabolic disease (241–244). BP was divided into five different categories based on clinical judgement to improve the interpretability of results. The values for individual biomarkers were restricted to a clinically plausible range based on both clinical judgement and the findings of general population studies, as in the analysis in previous Chapters. SBP measurements were restricted to a range between 90 to 240 mm Hg and DBP to a range between 50 to 130 mm Hg (236,237). Similarly, BMI was restricted to a range between 15 to 55 (238), total cholesterol to 2-10 (239).

The third step was called model specification, which involved examination of clinical relevance of the selected outcome and predictor variables. DepChron patients had existing cardiometabolic conditions; hence the outcome variable selected was clinically relevant for this population. The nine predictors selected were also clinically relevant however there were some notable absences. The information on smoking status, disease severity and cardiovascular medication was not available and this is discussed further in the limitations.

The fourth step was called model estimation, which involved selecting the predictors which have a significant impact on the outcome under consideration. A backward stepwise regression analysis was used for choosing the predictors for the final prediction model. This method employs Akaike Information Criterion (AIC), which uses Chi-square value should be less than twice the value of degree of freedom (DF), as a requirement for selection of a predictor (321).

The fifth step was called model performance. In this step, the discriminatory power of the final prediction model was visualised using a cumulative incidence plot. The sample was divided into four risk quartiles based on the predictor values selected in the final model.

The sixth step was called model validation or stability using bootstrapping techniques. The idea behind bootstrap is to use the data of a sample study at hand as a "surrogate population", for the purpose of approximating the sampling

distribution of a statistic; i.e. to resample (with replacement) from the sample data at hand and create a large number of "phantom samples" known as bootstrap samples (322). In step four which has been described above, selection of predictors (out of the nine predictors in total) for the final model was based on backward stepwise regression using the original sample data. In step six bootstrapping, backward stepwise regression was repeated on 200 "phantom samples" using bootstrapping. The choice of the predictors selected from the 200 boot strapping samples was compared with that of the choice of predictors from the original sample selected from step four.

The final step was called model presentation. In this step, the final prediction model was presented using a "nomogram" (a graphical representation of a scale). Each predictor in the final model was given a score based on its strength of influence on the outcome under consideration. The individual points assigned to a variable category were based on the strength of the association between that category and the outcome of interest. Additionally, the event free survival rates for sample patients with different scores were presented against different time intervals during follow-up, in a tabular format.

The "rms" package in *R,* published by Frank Harrell, was used for the entire analysis described above (323).

9.2.2 Area Under Curve for Risk Prediction Model

The performance of the final prediction model in predicting a major adverse cardiovascular event was evaluated using "Area Under Curve (AUC)" graphs. An AUC value of more than 50% suggests that the predictive power of a particular predictor or a group of predictors is better than chance. The AUC values were presented along with 95% confidence intervals, which were calculated using 200 boot strapping samples. The performance of the final model was compared to that of the individual predictors selected in the final model alone using AUC values with 95% CI.

The "pROC" package in *R* was used for this part of the analysis(324).

9.3 Results

9.3.1 Risk prediction model in DepChron patients who underwent depression screening (n=35537)

3939 patients (11%) of the 35537 DepChron patients who underwent depression screening at baseline had suffered a major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) by the end of 4 years of follow-up. Of the 35537 who were screened, 24829 patients (69.8%) were included for the final model prediction and the rest of the patients were excluded either due to extreme values of one of the peripheral biomarkers or due to missing values. In the final selected sample, 2886 patients (11.6%) had experienced an event by the end of 4 years follow-up.

The nine predictor variables with their respective categories and distribution are described in Table 9-1.

Table 9-1 Distribution of predictors considered for MACE risk prediction model in DepChron patients

Predictors considered for the final model development and their distribution	Sample used for model development N=24829
Age Group	
18-44	565 (2.2%)
45-64	6799 (27.4%)
65-90	17465 (70.4%)
Male sex (%)	14527 (58.5%)
Deprived socio-economic status SIMD deciles<=5 (%)	16078 (64.8%)
Number of cardiometabolic conditions- (%)	
One	18215 (73.4%)
Two	5965 (24.0%)
Three	649 (2.6%)
Systolic Blood Pressure-(%)	
130-139 mm Hg Reference	6566 (26.4%)
120-129 mm Hg	5332 (21.5%)
80-119 mm Hg	4444 (17.9%)
140-159 mm Hg	6565 (26.4%)
160-240 mm Hg	1922 (7.8%)
Diastolic Blood Pressure- (%)	
85-89 mm Hg Reference	1424 (5.7%)
80-84 mm Hg	5418 (21.8%)
40-79 mm Hg	16105 (64.8%)
90-99 mm Hg	1467 (5.9%)
100-130 mm Hg	415 (1.8%)
Body Mass Index (%)	
18.5-25 kg/m2 Reference	6091 (24.5%)
15-18.5 kg/m2	278 (1.1%)
25-30 kg/m2	9483 (38.2%)
30-55 kg/m2	8977 (36.2%)
Total Cholesterol in mmol/l (%)	
5-10 mmol/l	4709 (19.0%)
HADS-D Categories	
No Depression	19908 (75.3%)
Presence of Depressive symptoms (≥8)	4921 (24.7%)
MACE=Major adverse cardiovascular event (Cardiovascular death or admission due to MI/stroke/heart failure)	

Using the backward stepwise regression analysis, 8 out of 9 predictors were chosen for the final risk prediction model. Only DBP (p-value=0.48, DF=4) was eliminated from the final model as it did not make a significant impact on predicting the outcome. The final model consisted of 8 predictor variables: SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions (see Table 9-2).

Table 9-2 Final multivariable cox's regression analysis-final model used for risk prediction model specification

		Composite major adverse cardiovascular outcome (MACE) 2920 events HR with 95% CI
Systolic Blood Pressure in mm Hg	80-119	*1.15 (1.03 to 1.29)*
	120-129	1.00 (0.90 to 1.12)
	130-139	1
	140-159	1.07 (0.96 to 1.18)
	160-240	*1.28 (1.11 to 1.49)*
BMI in kg/m2	15-18.5	*1.36 (1.02 to 1.82)*
	18.5-25	1
	25-30	*0.83 (0.76 to 0.91)*
	30-55	*0.83 (0.75 to 0.92)*
Total Cholesterol in mmol/l	2-5	1
	5-10	*1.21 (1.10 to 1.33)*
Presence of depressive symptoms (HADS-D≥8)		*1.22 (1.11 to 1.33)*
Age		*1.05 (1.04 to 1.05)*
Sex-female		*0.81 (0.75 to 0.88)*
Socio-economic status-affluent group		*0.79 (0.73 to 0.86)*
Number of cardiovascular co-morbidities (vs. 1)	2	*1.95 (1.81 to 2.11)*
	3	*3.22 (2.76 to 3.76)*
Initiation of anti-depressants- treated vs. non-treated		0.84 (0.68 to 1.04)
Concordance (SE)		0.692 (0.005)

Figure 9-1 shows the performance of the eight selected predictors together in predicting a major adverse cardiovascular event using a cumulative incidence curve. Patients in the risk quartile four (based on the highest risk values of the 8 predictors) had the highest event rate over four years. Patients in risk quartile four had an approximately 22% event rate at the end of four years as compared to an event rate of approximately 4% observed in patients in risk quartile one (lowest risk values of the 8 predictors).

Figure 9-1 Four risk quartiles based risk prediction model and cumulative incidence of MACE
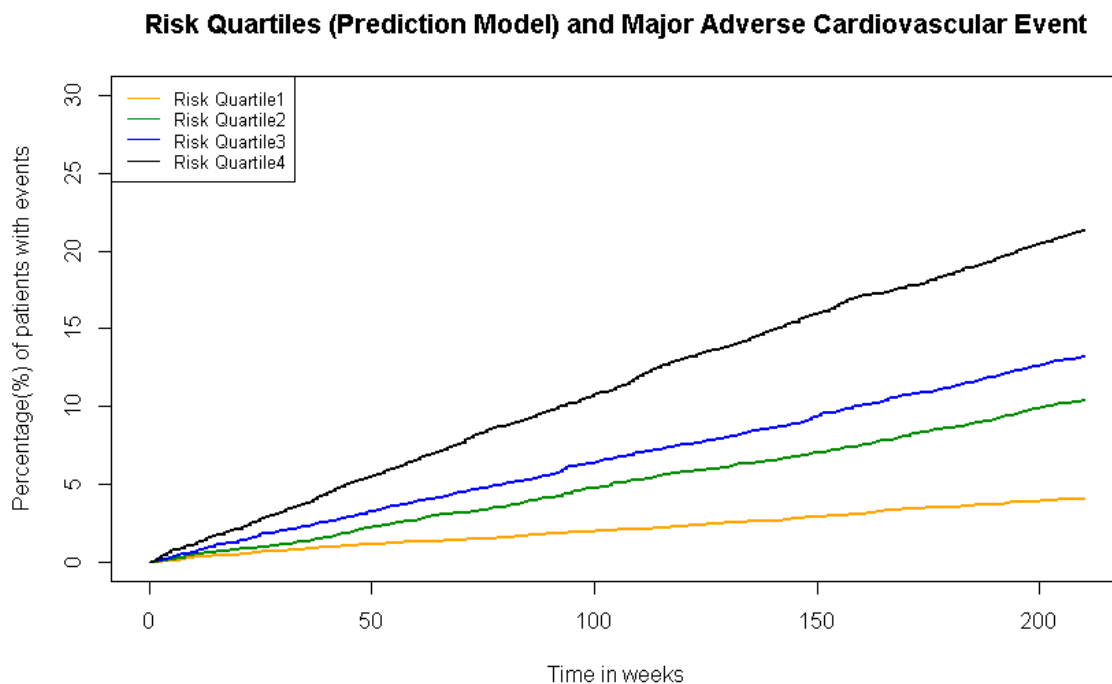
Legend: Risk prediction model= eight predictors-SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions; MACE=Major adverse cardiovascular event (Cardiovascular death or admission due to MI/stroke/heart failure)

In bootstrapping analysis, backward stepwise regression was repeated on 200 re-samples or "phantom samples". Five out of eight predictors were chosen in the final prediction model for all of the 200 boot strap samples: age, SBP, BMI, socio-economic status and number of cardiometabolic conditions. Gender was chosen in 181 samples, total cholesterol in 184 and HADS-D screening result in 188 samples. In total, all eight predictors were chosen in the final risk prediction model in 177 re-samples (88.5%) out of 200. Thus, the boot strapping analysis validated the choice of variables selected in the final risk prediction model for a major adverse cardiovascular event, based on this dataset.

Table 9-3 shows the details of a risk prediction score (range 0-29) based on these 8 predictor values at baseline. The age categories had the highest difference in points between categories (10 points for age group 65-90 against 0 points for age group 18-44), closely followed by number of cardiometabolic conditions (8 points for having all three cardiometabolic conditions, 4 points for having two cardiometabolic conditions and 0 points for having only one cardiometabolic

condition). The lowest differences were observed for socio-economic status, gender, HADS-D screening result and total cholesterol values (1 point each for deprived, male, positive result on depression screening and raised cholesterol categories against 0 for their respective low risk category).

Table 9-3 Risk scoring system for prediction of MACE in DepChron

Predictors	Points	Predictors	Points
SBP 130-139 reference	0	Socio-economic status-deprived	1
SBP 120-129 tightly controlled	0	Socio-economic status-affluent	0
SBP 80-119 low	1	Age group 18-44	0
SBP 140-159 high	1	Age group 45-64	5
SBP 160-240 very high	2	Age group 65-90	10
BMI 18.5-25 reference	2	Gender Female	0
BMI 15-18.5 low	5	Gender Male	1
BMI 25-30 Overweight	1	HADS-D 0-7 No depressive symptoms	0
BMI 30-55 Obese	0	HADS-D 8-21 Presence of depressive symptoms	1
Number of cardiometabolic conditions-1	0	Total cholesterol 2-5	0
Number of cardiometabolic conditions-2	4	Total cholesterol 5-10	1
Number of cardiometabolic conditions-3	8	**Maximum Score=29** **Minimum Score=0**	1
Legend: Risk prediction model= eight predictors-SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions; MACE=Major adverse cardiovascular event (Cardiovascular death or admission due to MI/stroke/heart failure)			

Figure 9-2 shows a "nomogram" or graphical representation of the points in the scoring system based on variable categories and also the event free survival rate based on corresponding scores on the scoring system. The figure shows that an increase in the score on the scale was associated with lower chances of being event free at 1, 2 and 4 years of follow-up.

Figure 9-2 Nomogram of risk scoring system for prediction of MACE in DepChron
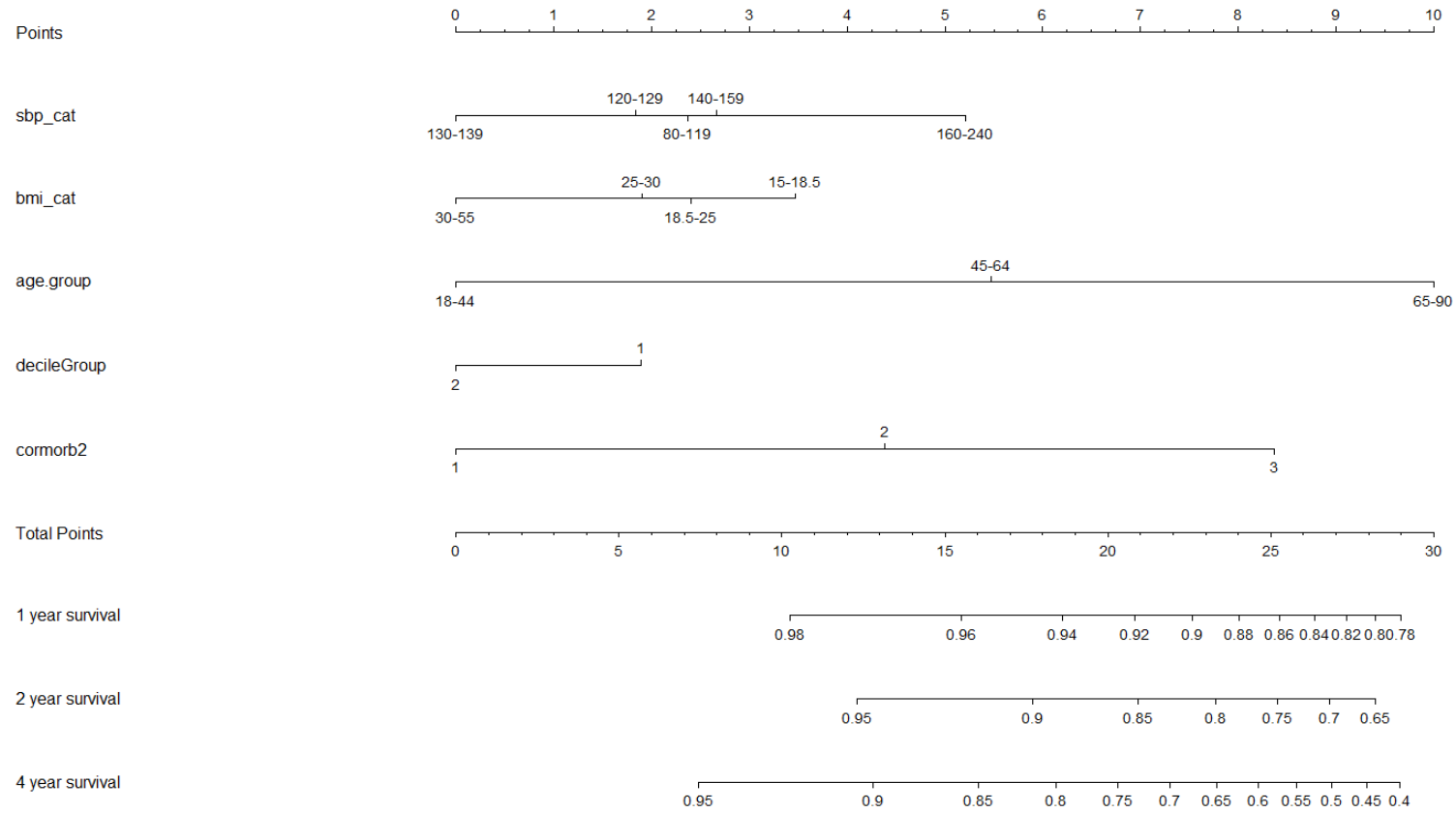
Difference in event free survival rates observed with the different scores on the scoring system is also illustrated in Table 9-4. A higher score implies higher risk of subsequent MACE events. The table shows that with increase in follow-up duration, the event free survival rates reduce significantly for those with very high scores (and higher risk) on the scale. For example, the difference in event free survival at one year was 18% between those with the highest and lowest risk. However, the difference in event free survival at four years increased to 62% between those with the highest and lowest risk.

Table 9-4 Scoring system and event (MACE) free survival rate for the risk prediction model

Points	1 year event free survival	2 year event free survival	4 year event free survival
29	72%	50%	30%
28	74%	55%	32%
27	76%	60%	36%
26	80%	65%	40%
25	82%	70%	45%
24	86%	75%	50%
22	88%	80%	50%
21	90%	82%	65%
20	92%	85%	70%
18	94%	90%	79%
15	96%	92%	84%
11	98%	95%	92%

Key Findings (Risk Prediction Model):

- **A risk scoring system with a range from 0-29 (consisting of eight predictor variables: age, gender, socio-economic status, SBP, BMI, HADS-D screening result, total cholesterol and number of cardiometabolic conditions) for predicting a MACE (cardiovascular death or admission due to MI/stroke/HF) over four years was constructed for DepChron patients who underwent depression screening (n=35537).**

- **These results for the choice of predictor variables were validated for 200 "phantom samples" or re-samples using bootstrapping**

- **Higher scores on the scoring system were associated with higher risk of experiencing a MACE over four years. .**

9.3.2 Area Under Curve for the risk prediction model for DepChron patients who underwent depression screening (n=35537)

Figure 9-3 shows the AUC graph for the risk prediction model (eight predictors: SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions) with 95% confidence intervals, with MACE as the outcome. The AUC value of 56.7% suggests that the model had limited accuracy with reduction in specificity as sensitivity increased.

Figure 9-3 AUC for risk prediction model for MACE at four years
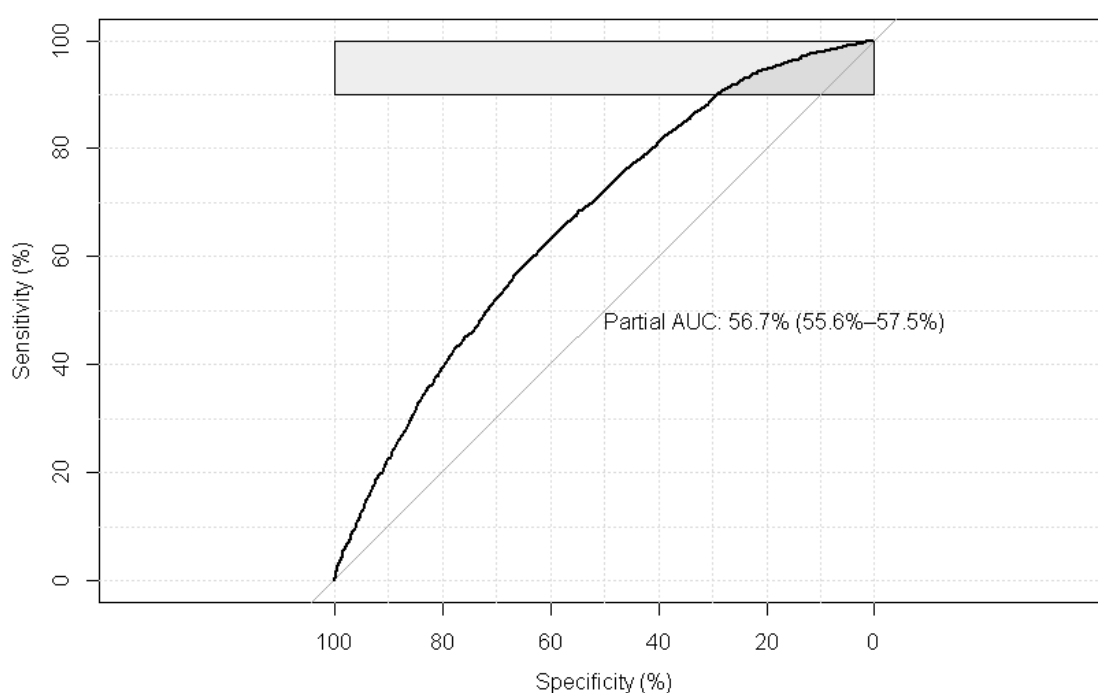
 Legend: Risk prediction model= eight predictors-SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions; MACE=Major adverse cardiovascular event (Cardiovascular death or admission due to MI/stroke/heart failure)

Finally, the AUC value from the final model was compared to that of the AUC value for each of the eight predictors in the risk scoring system-HADS-D screening result, SBP, BMI, total cholesterol, age, gender, socio-economic status and number of cardiometabolic conditions (see Figure 9-4 to Figure 9-11). Age had the best individual AUC value among the eight predictors at 53.4% (see Figure 9-8) while number of cardiometabolic conditions was second best at 50.9% (see Figure 9-11). Among the peripheral biomarkers, BMI had the best AUC value at 50.4% (see Figure 9-6). All of the individual AUC values apart from age were less than 51%, which suggests that the individual predictors were not

substantially better at predicting the outcome than chance. Thus, using these eight predictors together in a risk scoring system did improve the AUC value as compared to using them individually, though the improvement was modest.

Figure 9-4 AUC for HADS-D screening result for MACE at four years
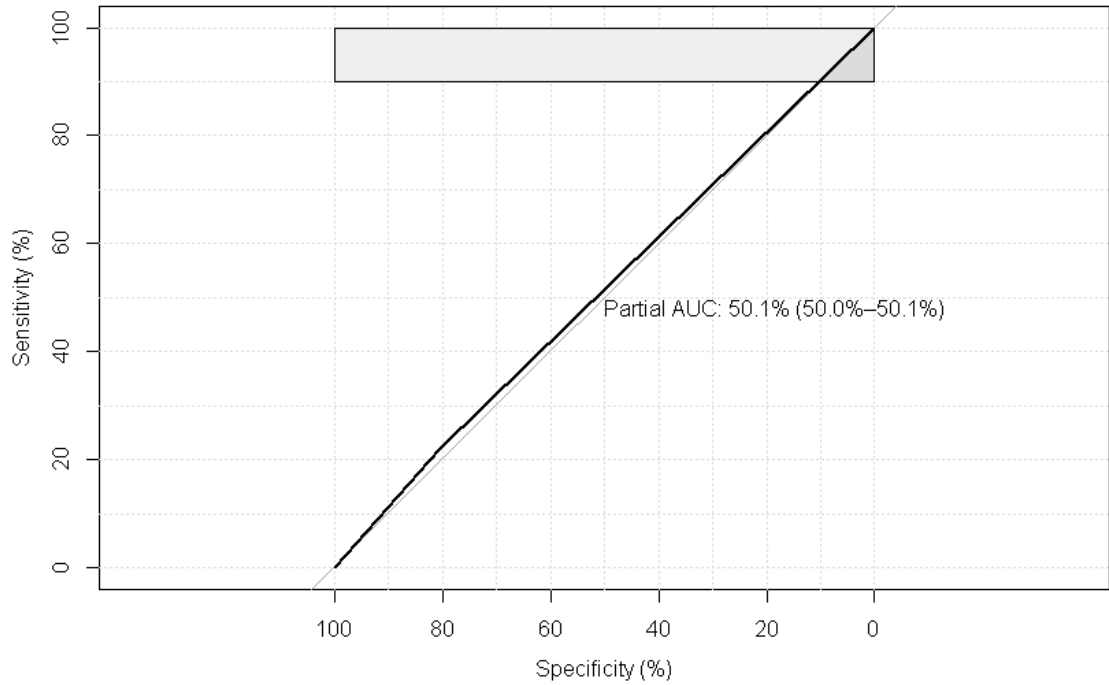
Figure 9-5 AUC for SBP for MACE at four years
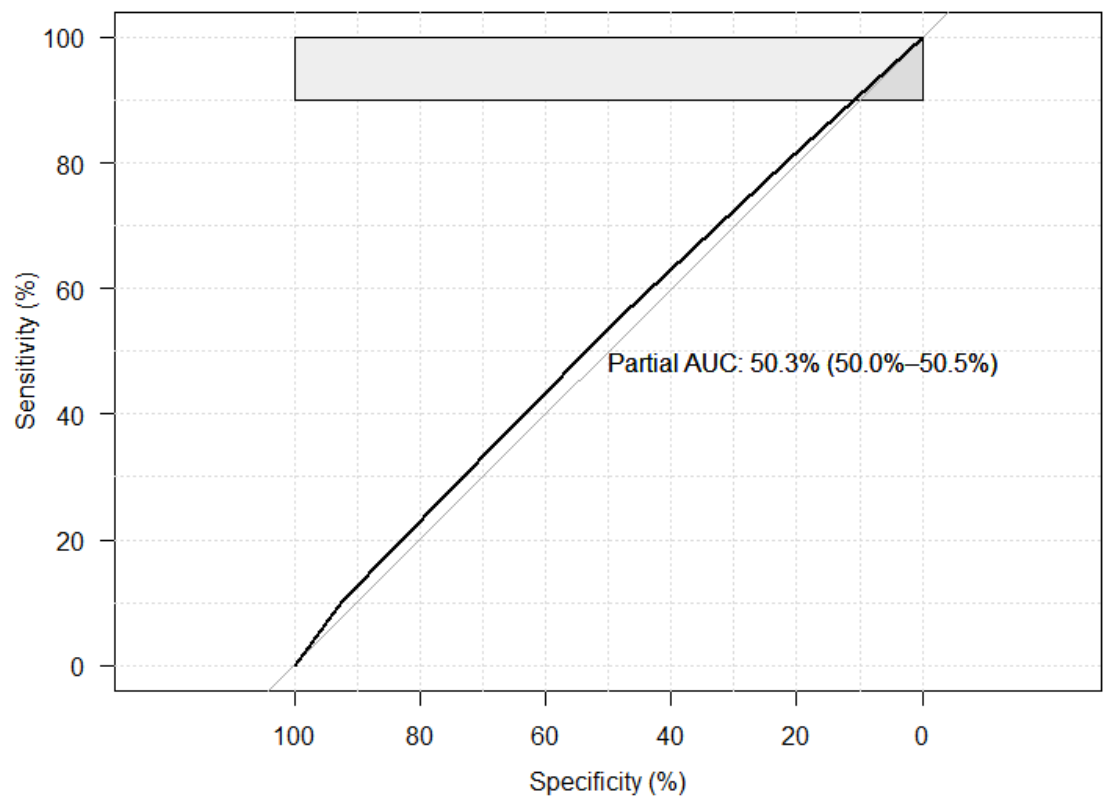
Figure 9-6 AUC for BMI for MACE at four years
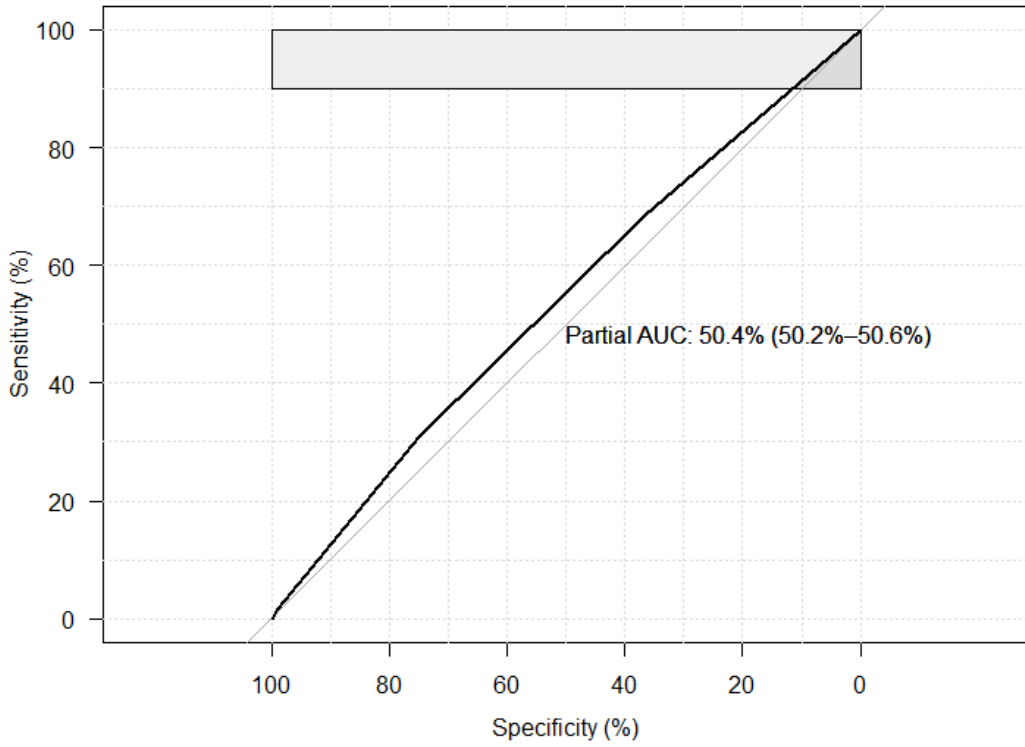
Figure 9-7 AUC for total cholesterol for MACE at four years
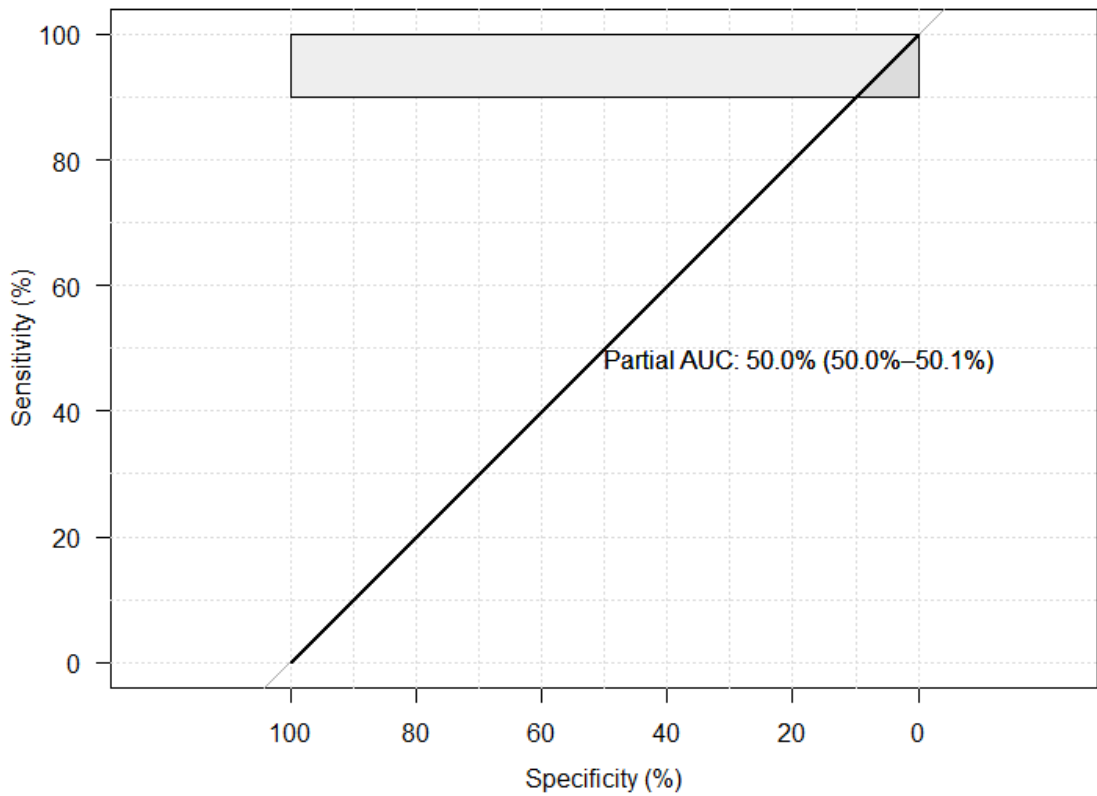
Figure 9-8 AUC for age for MACE at four years
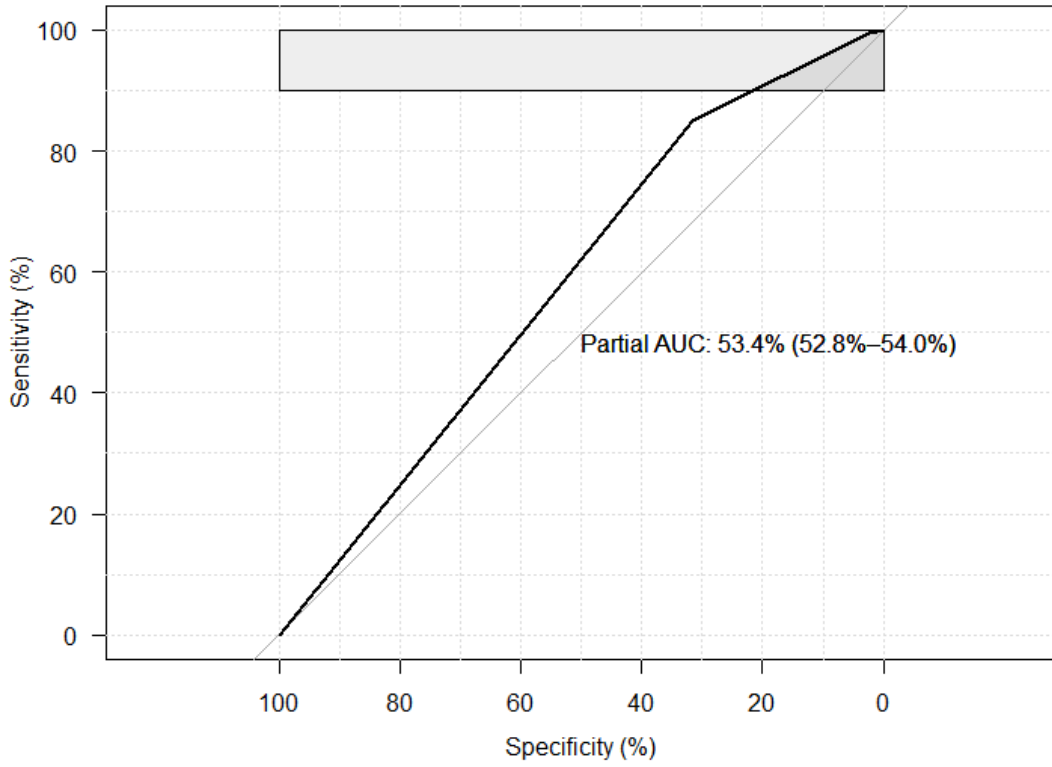
Figure 9-9 AUC for gender for MACE at four years
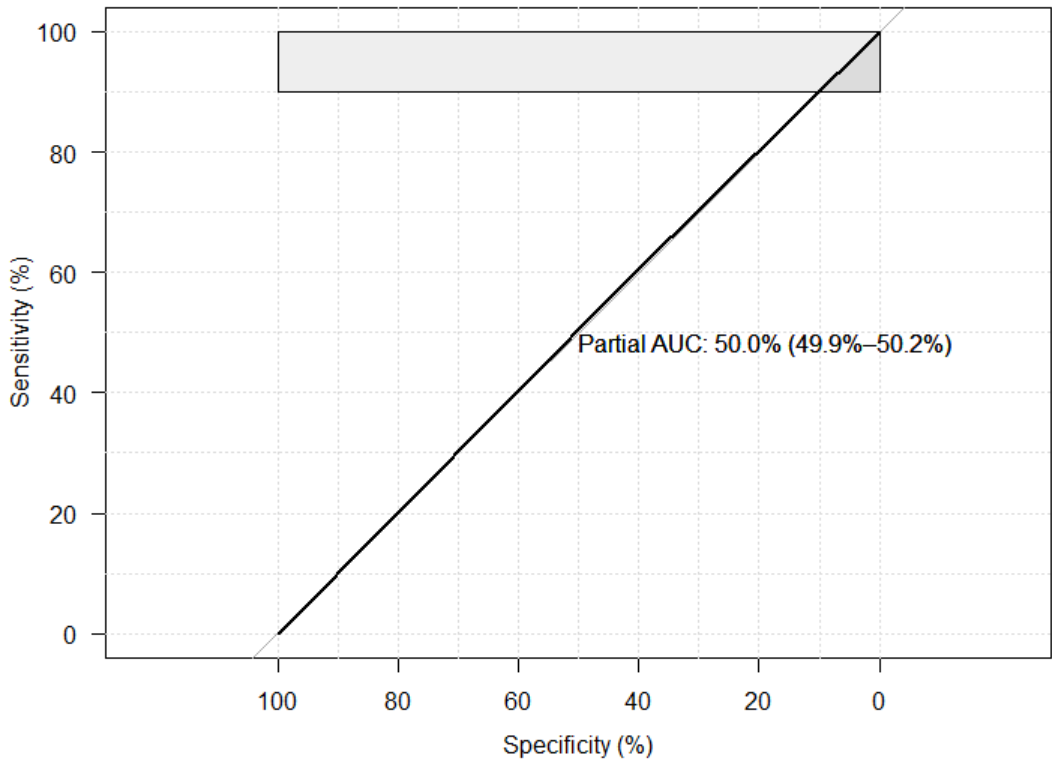
Figure 9-10 AUC for socio-economic status for MACE at four years

Partial AUC: 50.4% (50.2%–50.6%)
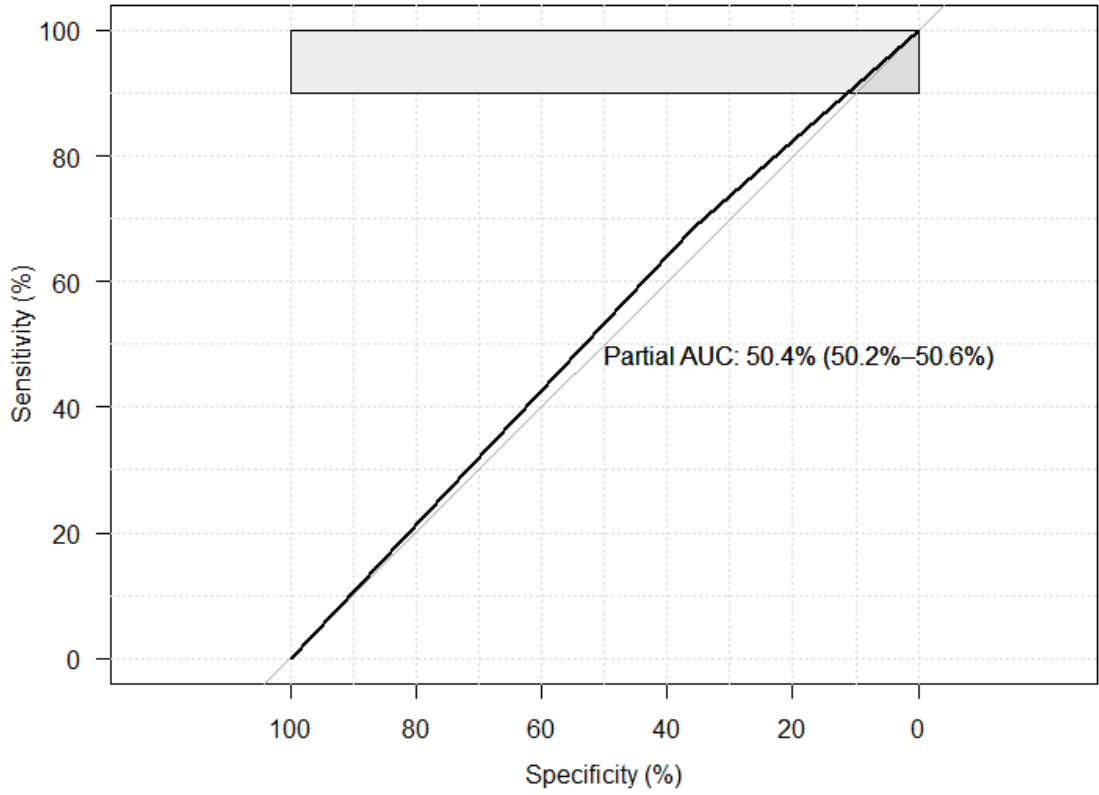
Figure 9-11 AUC for number of cardiometabolic conditions for MACE at four years

Partial AUC: 50.9% (50.8%–51.0%)
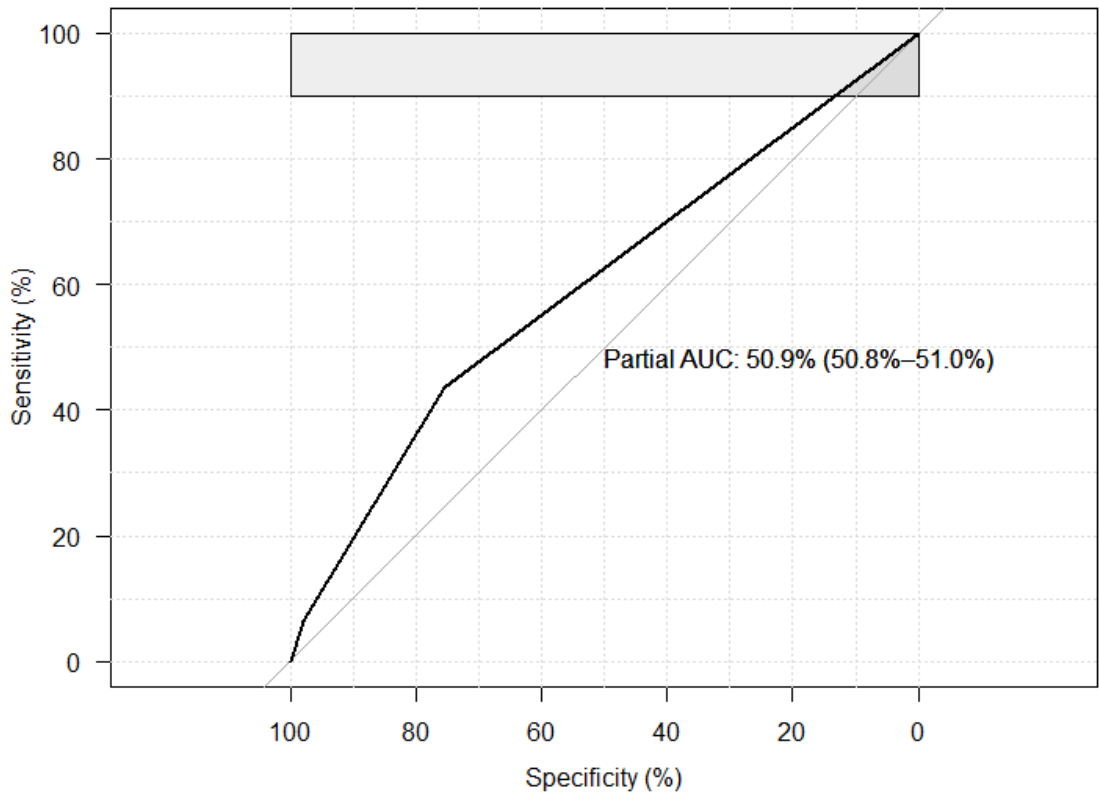
Key Findings (AUC analysis):

- **A model using eight predictors (SBP, BMI, total cholesterol, age group, gender, socio-economic status, HADS-D screening result and number of cardiometabolic conditions) was found to have a modest AUC value (56.7%, 95% CI 55.6%-57.5%) in risk prediction of MACE at four years.**

- **The AUC value of the combined model was better than AUC values of the eight individual predictors, in risk prediction of MACE at four years.**

9.4 Discussion

9.4.1 Summary of Findings

A risk scoring system (range=0-29) for predicting a major adverse cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) over 4 years was constructed for DepChron patients who underwent depression screening (n=35537). The risk scoring system consisted of eight predictor variables (age, gender, socio-economic status, SBP, BMI, HADS-D screening result, total cholesterol and number of cardiometabolic conditions). DBP was considered but excluded from the final model due to having a statistically non-significant association with the outcome. Four out of the eight predictors selected were potentially modifiable: SBP, BMI, HADS-D screening result and total cholesterol.

A higher score on the scoring system at baseline was associated with higher risk of major adverse cardiovascular event at 1, 2 and 4 year intervals. The AUC value of the scoring system was 56.7%, which implied poor predictive ability. The AUC value of the scoring system using eight predictors together was better than that of the eight predictors used individually.

9.4.2 Comparison of Findings with Existing Literature

There are very few studies investigating the predictors of long term prognosis for patients who have experienced a myocardial infarction or stroke. The PREDICT score was devised to calculate the risk of six year all-cause mortality in patients hospitalized with a myocardial infarction (325). The variables included in the PREDICT score were age, kidney function, ECG findings, history of heart failure, admission day and history of shock at the time of hospitalization. In another study, the predictors for one year all-cause mortality in elderly patients hospitalized with acute myocardial infarction were investigated (326). The factors with the strongest association with mortality were older age, urinary incontinence, assisted mobility, presence of heart failure or cardiomegaly any time before discharge, presence of peripheral vascular disease, body mass index <20 kg/m2, renal dysfunction (defined as creatinine >2.5 mg/dl or blood urea nitrogen >40 mg/dl) and left ventricular dysfunction (left ventricular ejection fraction <40%). A systematic review found only one risk score for long term prediction of cardiovascular and general health outcomes in patients with

existing CHD (327). The GRACE risk score based on nine factors independently predicted death and the combined end point of death or myocardial infarction in the period from admission to six months after discharge: age, development (or history) of heart failure, peripheral vascular disease, systolic blood pressure, Killip class, initial serum creatinine concentration, elevated initial cardiac markers, cardiac arrest on admission, and ST segment deviation. The reported C-statistics was 0.73 for death or myocardial infarction from admission to six months after discharge, which is higher than that of the model proposed in this analysis (328). Finally, the data from the Heart and Soul study proposed a model consisting of N-terminal pro-type brain natriuretic peptide, high-sensitivity cardiac troponin T, urinary albumin: creatinine ratio, and current smoking for 5-year secondary CV event and reported a C-index of 0.65 (329).

A systematic review found a few studies investigating the use of prediction models for calculating the long term risk of recurrent stroke but these studies mainly used data at the time of acute presentation with stroke or transient ischaemic attack (TIA) (330). The majority of the scoring systems included in this systematic review were either of shorter duration or calculated for patients presenting with a TIA and hence not comparable to DepChron findings (330). The Oxford TIA score predicted five year risk of a cardiovascular event (combined risk of myocardial infarction or stroke) with a AUC value of 65% (95% CI 62% to 68%) in patients presenting with acute stroke or TIA using eight clinical factors at the time of presentation: age, sex, affected region (amaurosis fugax as well as carotid and vertebrobasilar), frequency of TIA, residual neurological deficits, peripheral vascular disease, and left ventricular hypertrophy (331). The Essen Stroke Risk Score (ESRS) was derived from the CAPRIE (clopidogrel vs aspirin in patients at risk of ischemic events) trial in patients with vascular disease with a mean follow-up of 1.9 years (332). The study used eight clinical predictors to calculate the risk of recurrent stroke: age, hypertension, diabetes mellitus, myocardial infarction, other cardiovascular disease, peripheral artery disease, smoking status, and history of TIA or stroke; and the reported AUC value was 61% (95% CI 54% to 69%). (332).

This is the first study to my knowledge which involved calculating a risk scoring system for predicting a major adverse cardiovascular event for patients with existing cardiometabolic disease in a community setting.

9.4.3 Strengths of Findings

1. The method used for developing the scoring system was robust and has been validated in the published literature (318,320,333–336).

2. The clinical outcome and the various predictors considered for developing the risk prediction models were relevant for the sample under study.

3. The data was collected from a community setting as a part of routine practice hence likely to reflect real life clinical practice and had a large sample size. The event per variable ratio was appropriate after excluding missing values.

9.4.4 Limitations of Findings

There are various limitations of this dataset which have been highlighted previously in chapters 5 and 7. The limitations of this particular analysis were:

1. There was no external validation of the findings. The accuracy of risk scoring systems has to be investigated in different patient samples. In addition, the overall AUC value of the risk prediction model was poor.

2. Information on smoking status at the time of the start of study was missing from this data. Smoking status is likely to influence the outcome considered i.e. major adverse cardiovascular event and could be an important component of a risk scoring system for predicting secondary cardiovascular events in patients with existing cardiometabolic disease (332). In addition, information on disease severity and duration, polypharmacy and presence of chronic kidney disease was missing, which may have influenced the outcome considered.

3. Information on other relevant co-morbid conditions such as heart failure and peripheral vascular disease was missing. These are likely to be important predictors of the outcome considered (325,326,331,332).

9.4.5 Potential Implications

There are various national guidelines recommending lifestyle and pharmacological interventions in patients with existing cardiometabolic disease

for secondary prevention (244,246,337). However, adherence to pharmacological and lifestyle measures remains sub-optimal (338,339). Moreover, evidence has suggested that better adherence may lead to better outcomes in this population (340).

Improving treatment adherence for patients with cardiometabolic disease is a huge challenge for health care resources considering the increase in survival rates post MI and stroke (341). Better risk stratification based on prognosis may potentially lead to better resource allocation by increasing monitoring for patients with the highest risk of secondary events (334,336). This in turn, may lead to improvement in clinical outcomes; however further research is needed in this area. For example, currently in UK primary care, a comprehensive annual health assessment is offered to all patients with cardiometabolic disease, based on QOF incentives (342,343). This disease management programme does not take account of a patient's risk of having secondary cardiovascular outcomes when offering monitoring (342,343). In other words, this is a "one size fits all" approach. Further research is warranted to explore the benefits, if any, of modifying the level of monitoring offered on the basis of a patient's individual risk of suffering adverse clinical outcomes.

9.4.6 Conclusion

In a cohort of patients with existing cardiometabolic disease (CHD, previous stroke or diabetes), it was possible to construct a scoring system based on baseline demographic and clinical variables collected in the community to predict the risk of a secondary cardiovascular event (cardiovascular death or admission due to MI/stroke/HF). The risk scores constructed had limited accuracy. The risk score needs further external validation in other patient groups and may need modification by including other relevant predictors missing from this data to improve accuracy. Risk stratification based on prognosis may have the potential to improve resource allocation to the highest risk patients, and this in turn may lead to better clinical outcomes.

In the final chapter of this thesis, the overall findings are summarized and key strengths and limitations as well as potential implications for future research are discussed.

Chapter 10 Discussion: Key Findings, Implications, Summary of Strengths and Limitations, and Conclusion

10.1 Chapter Summary

In this chapter, the main findings of this thesis and its potential clinical and research implications are discussed. A summary of key strengths and limitations of the whole body of work is followed by reflections on development of personal skills and learning during the course of writing this thesis and an overall conclusion.

10.2 Key Findings and Comparison with Literature

Literature Review Findings

The reviews showed that blood based peripheral biomarkers were statistically significant in predicting six different clinical outcomes in participants with depressive symptoms. Outcomes related to both mental health (depressive symptoms) and physical health were statistically associated with pre-treatment levels of peripheral biomarkers; however only two studies investigated outcomes related to physical health. Twelve different biomarkers related to five different biological systems were found to have a statistically significant association with clinical outcomes in patients with depressive symptoms, while 24 biomarkers were found to have no statistical association with clinical outcomes studied. Despite extensive research on the relationship of biomarkers and depressive symptoms, the research was of generally limited quality and clinical utility.

Depression and cardiometabolic disease

DepChron patients had a high prevalence of depressive symptoms (19.9%). In addition, DepChron patients with co-morbid depressive symptoms were more likely to suffer from all-cause mortality, all-cause hospital admissions and major adverse cardiovascular events over four years as compared to those without depressive symptoms, after adjusting for potential confounders (see Chapter 7). This resonates with findings from the published literature which suggests that patients with cardiometabolic disease are more likely to have co-existing depressive symptoms than the general population (105–107) and that patients with cardiometabolic disease and depressive symptoms are more likely to have adverse clinical outcomes as compared to those without depressive symptoms(127,128,278,344).

Role of peripheral biomarkers in depression risk assessment

Analysis of the DepChron dataset suggested that SBP and HbA1c may have a potential role in risk stratification of patients with cardiometabolic disease and co-morbid depressive symptoms (see Chapter 7). There was a statistically significant interaction noted between SBP and depressive symptoms in risk

prediction of major adverse cardiovascular outcome (p-value=0.03). Patients with low SBP only, with very high SBP only and depressive symptoms only at baseline had 10%, 19% and 17% higher risk respectively of a MACE as compared to those without extremes of SBP and no depressive symptoms. In comparison, patients with both low SBP and depressive symptoms at baseline had 36% higher risk while patients with both very high SBP and depressive symptoms had the highest increased risk of 83%. There was also a statistically significant interaction between HbA1c and depressive symptoms in risk prediction of all-cause mortality for patients with diabetes (p-value=0.04). Patients with low HbA1c (3 to 6.4) only, with high HbA1c (7.5 to 18) only and depressive symptoms only at baseline had 18%, 27% and 37% higher risk respectively of all-cause death as compared to those with reference HbA1c (6.5 to 7.4) and no depressive symptoms. In comparison, patients with both low HbA1c and depressive symptoms at baseline had 34% higher risk while patients with both high HbA1c and depressive symptoms had the most elevated risk of 108% as compared to those in the reference HbA1c group without depressive symptoms. This is the first study, to my knowledge, which investigates the interacting relationship between depressive symptoms and BP and HbA1c values in the risk prediction of adverse clinical outcomes in patients with pre-existing cardiometabolic disease.

AI score, depression and clinical outcomes

Among DepChron patients (majority of older age group >65 and with existing cardiometabolic conditions), there was a cross-sectional association between AI score calculated with primarily cardiometabolic biomarkers and depressive symptoms (see Chapter 5). Among Psobid participants (aged 35-65 and chosen from general population), a cross-sectional association was not observed between AI score and depressive symptoms (see Chapter 6). In DepChron and Psobid, AI score using multiple biomarkers did not have a stronger association with depressive symptoms than its individual biomarkers (see Chapter 5 and 6). In various cross-sectional studies involving mainly elderly populations, depression has been observed to have a significant association with AI score (216,217,219), which was contradictory to the non-significant relationship observed in the Psobid participants. In addition, AI score did not have any statistical interaction in predicting adverse physical outcomes in patients presenting with depressive

symptoms in the DepChron and Psobid cohorts (see Chapter 7 and 8).On the basis of the analysis in this thesis, using an algorithm of multiple biomarkers (such as AI score), instead of using single peripheral biomarkers, did not offer any added utility in predicting adverse physical health outcomes in patients with depressive symptoms. Again, this is in contrast with some of the previously published studies reporting an association between AI score with various adverse physical adverse health outcomes (233)(28).

10.3 Discussion - Implications of Findings

The findings of this programme of work have potential implications for:

1. The studies of relationship between depressive symptoms and peripheral biomarkers and depressive symptoms
2. The management of patients presenting with depressive symptoms and co-morbid cardiometabolic disease.

10.3.1 Relationship between depressive symptoms and peripheral biomarkers

General practitioners are faced everyday with the challenge of differentiating "normal distress" caused by difficult life circumstances from a "pathological" depressed state, for patients presenting with depressive symptoms, in clinical practice. Risk stratification for depressive symptoms is important because it is likely to guide management recommendations for the patient. We now have evidence to suggest that merely counting depressive symptoms reported by the patient may not be good enough to formulate management plans, due to a lack of accuracy and heterogeneity in presentation (33,345). So this leads to the question: What are the possible alternatives for assessing patients presenting with depressive symptoms?

An alternative strategy could be to stratify patients presenting with depressive symptoms based on their risk of adverse clinical outcomes, and such a strategy has been used widely in other areas of medicine (320,335,336). In future, more work is needed in the area of outcomes based risk stratification of depressive symptoms, particularly investigating the role of peripheral biomarkers. In this thesis, only physical health outcomes were assessed in the two cohorts. Future research needs to investigate the role of peripheral biomarkers in risk stratification of depressive symptoms with a focus on mental health outcomes such as recovery or remission of depressive symptoms. Secondly, the findings from this thesis need to be replicated in different patient populations, ideally in prospective studies where more information on potential confounders such as disease severity and duration and lifestyle factors (such as smoking status) are known, to validate the observed association between peripheral biomarkers and adverse physical outcomes noted in the DepChron data. Future research in this

area could potentially lead to the use of peripheral biomarkers in defining the risk of both physical and mental adverse health outcomes in patients presenting with depressive symptoms, which in turn might improve their risk stratification in clinical practice. The important caveat being that the findings of DepChron and Psobid are based on depressions screening rather than a clinical diagnosis of MDD.

The reasons for the observed findings of this thesis between AI score, depressive symptoms and adverse clinical outcomes remain unclear and needs further research. Future research on the usefulness of AI score in clinical practice should focus on its relationship with depressive symptoms in a middle aged population, as this has not been studied extensively (216,217,219). Secondly, in both cohorts, some of the individual biomarkers were found to have a stronger association with adverse clinical outcomes than AI score. The clinical utility for AI score may depend on its predictive power for adverse clinical outcomes, over and above individual biomarkers. Further research is needed to compare the predictive power of AI score for adverse clinical outcomes against that of individual biomarkers to determine whether there really is added value from using AI score as has been previously (233)(28).

10.3.2 Management of depressive symptoms in patients with cardiometabolic disease

There are two important implications for research and practice, based on the findings from this programme of work. Firstly, findings from DepChron have suggested or raised the possibility that there may be practical means of identifying those cardiometabolic disease patients who are more likely to have co-existing depressive symptoms, who might benefit from "targeted" depression screening. Depression screening has been recommended for patients with cardiometabolic disease, in view of the high prevalence of depression, by the American Heart Association (112); however there is no evidence to date that routine depression screening for patients with cardiometabolic disease leads to any improvement in depressive symptoms or cardiovascular outcomes (114,115). In contrast to the findings of this thesis, there is some evidence to suggest that blanket depression screening for all cardiometabolic disease patients has been

found to have a "low yield" of positive results (346), and in turn may not be cost effective. The UK Quality and Outcomes Framework (QOF), an annual reward and incentive programme for primary care, offered financial incentives to primary care practitioners for routine depression screening for all patients with coronary heart disease and diabetes, between 2006/07 and 2013/14 (149); these financial incentives have subsequently been withdrawn since 2013/14 (150). NICE (National Institute for Health and Care Excellence) recommends that depression screening or 'case finding' in patients with chronic disease should only be targeted towards those who are believed to be 'high risk' (148). Findings form analysis of the DepChron dataset (see Chapter 5); suggest that cardiometabolic disease patients with abnormal biomarker values (such as SBP and HbA1c) are at 'high risk' of having co-morbid depressive symptoms. Further research is needed in this area, to validate these findings in other patient populations, before making any clinical recommendations but clearly this is an important area for further investigation. If these findings are validated in other cohorts, the next step for research in this area would be developing and testing an intervention which would target those identified through depression screening as 'high risk' (based on their biomarker values) cardiometabolic disease patients.

Secondly, it was possible to construct a scoring system based on baseline demographic and clinical variables collected in the community to predict the risk of a secondary cardiovascular event (cardiovascular death or admission due to MI/stroke/HF) in the DepChron cohort, although with limited accuracy (see Chapter 9). The analysis of the DepChron data suggested that patients with multiple cardiometabolic conditions, who also had a combination of high SBP and depressive symptoms were at the highest risk of having a secondary cardiovascular event (see Chapter 9). Currently in the UK, patients with existing cardiometabolic disease are usually offered a yearly review appointment with the practice nurse in their GP surgery (149), not taking into account their risk of secondary cardiovascular events. Cardiometabolic disease patients who are at higher risk of secondary cardiovascular events may benefit from increased monitoring of some of their modifiable risk factors such as SBP or more intensive efforts at engagement if they do not attend or adhere to therapies. It will be important to try to replicate the analyses undertaken using the DepChron

dataset in other datasets where potential confounders are better characterised and missing data is less of a problem. For example, these findings could be replicated in other large datasets such as UK Biobank (http://www.ukbiobank.ac.uk/about-biobank-uk/) or Generation Scotland (http://www.generationscotland.org). Again, it is not possible to make any clinical recommendations based solely on the findings from DepChron but this work does indicate that further research is needed in this area.

10.4 Summary of Key Strengths and Limitations

This programme of work has a number of key strengths which are summarized here. The analyses were performed in two different patient cohorts with different demographic profiles and characteristics. The DepChron dataset had a very large sample size and provided us with data collected from routine care, reflecting clinical practice. The second dataset, Psobid, provided access to a wide range of peripheral biomarkers, allowing a comprehensive calculation of AI score. Electronic data linkage was successful for the majority of the sample for both datasets. Two validated but different instruments were used for depression assessment in the two datasets; this latter issue can be viewed as strength but also a limitation.

Important limitations have been described in detail within each section but some overarching limitations were as follows. For both datasets, there was only a single measurement of the peripheral biomarkers and depression status available for both patient cohorts.   These factors could have changed over the course of follow-up and it would have been preferable to have information on these data available at multiple time points. In general practice clinical setting, usually an approach consisting of multiple assessments over a period of time is used in making diagnostic and prognostic assessments of patients presenting with depressive symptoms. An important limitation in DepChron was the large number of patients with missing values which limited the scope of the analyses. Importantly, information about potential confounders such as smoking status, disease duration and severity, use of psychological therapies and cardiovascular medications were missing from DepChron participants.

In addition, although the Psobid dataset had a broader range of biomarkers to study, the number of clinical outcomes observed among Psobid participants over the follow-up duration was low, which limited the intended statistical analysis. Finally, neuroendocrine biomarkers frequently used in AI score calculation were not available in either dataset.

10.5 Skills Development and Learning

This project has enabled me to develop an understanding of some key statistical skills. I have learned to use logistic and linear regression modelling and presenting for studying cross-sectional association. I have also developed an understanding of using survival analysis methods such as cox regression analysis for cohort studies. Finally, I have learned the statistical techniques of developing a risk prediction model and checking its accuracy using methods such as Area under Curve. I have developed an understanding of using 'R', which is an open source statistical software. These statistical methods are likely to be very useful to me in future while pursuing a career in academic medicine.

In addition to learning valuable statistical skills, I have acquired an understanding of the principles of conducting an epidemiological study. For example, sample size and the quality of data are important factors to be considered while selecting a dataset to answer a research question. DepChron was a large dataset with poor data completion rate, which is often the case for data collected as a part of routine clinical practice. Psobid was a smaller dataset with good data completion rate, which is more likely in a research project dataset. This thesis involved secondary data analysis which meant huge savings in the cost and the time involved for the project. However, there was a need to compromise on the number and the type of biomarkers used for AI score calculation, as it was a secondary data analysis. Importantly, external validation of findings is an important step for any epidemiological study, which I was unable to do with the resources available during the course of this fellowship. However, I am planning to repeat these analyses using other datasets in the near future.

10.6 Future Research Implications

Further research should focus on studying the interaction of peripheral biomarkers with depressive symptoms in predicting clinical outcomes, and risk stratification of patients with cardiometabolic disease and co-morbid depression. Potential future research studies would include:

• Replicating the findings of DepChron in different datasets, preferably in datasets which do not have some of the key limitations that have been described concerning the DepChron dataset. Importantly, re-examination in a dataset with good quality data on potential confounders such as lifestyle factors (e.g. smoking status), disease severity and cardiovascular medications would be useful.

• The potential datasets could be large existing cohort studies, for example, UK Biobank cohort UK Biobank cohort (http://www.ukbiobank.ac.uk/about-biobank-uk/) and Generation Scotland (http://www.generationscotland.org), or it could be secondary data analysis of trial datasets available from NIH (National Institute of Health) data repository (e.g. ENRICHD https://biolincc.nhlbi.nih.gov/studies/enrichd/) or elsewhere.

If the results found in DepChron were replicable in other datasets then consideration could be given to intervention development such as:

• Developing an intervention for targeting depression screening for those patients with cardiometabolic disease who are noted to have either extremes of cardiovascular biomarker values (for e.g. blood pressure, BMI and HbA1c), as they are at higher risk of having concurrent depressive symptoms.

• Testing a disease management programme for patients with cardiometabolic disease where the level of monitoring offered to a patient is determined by their individual risk of suffering from adverse cardiovascular outcomes and exploring cost effectiveness of this intervention.

• Developing an intervention for patients with cardiometabolic disease and co-morbid depressive symptoms where the management approach focuses

equally on cardiovascular risk reduction and improvement in depressive symptoms. The majority of intervention studies in this area have focussed on managing depressive symptoms and have failed to show any benefit in reducing cardiovascular outcomes. A management approach which focuses on cardiovascular risk reduction as well may lead to an improvement in cardiovascular outcomes in patients with cardiometabolic disease and co-morbid depressive symptoms.

Clearly any of the above type of intervention studies would necessitate extensive user involvement to examine views of the acceptability and feasibility of such approaches and to aid intervention development.

10.7 Conclusion

Depressive symptoms were found to have a cross-sectional association with individual peripheral biomarkers both in patients with cardiometabolic disease and middle-aged participants recruited from the general population. Depressive symptoms were also associated with a higher risk of adverse clinical outcomes in patients with cardiometabolic disease. AI score calculated with different statistical formulations did not add any additional usefulness in predicting concurrent depressive symptoms or clinical outcomes at follow-up, over and above its individual constituent biomarkers, in either of the patient cohorts.

SBP had a significant interaction with depressive symptoms in predicting cardiovascular events in patients with cardiometabolic disease; HbA1c had a significant interaction with depressive symptoms in predicting all-cause mortality in patients with diabetes. Peripheral biomarkers may have a role in predicting clinical outcomes in patients with depressive symptoms, especially for those with existing cardiometabolic disease. The scoring system for predicting a major adverse cardiovascular event in patients with depression and cardiometabolic disease showed limited predictive ability.

The findings from this thesis have improved the understanding of the relationship between cardiovascular biomarkers, depressive symptoms and adverse clinical outcomes in patients with cardiometabolic disease. This has the potential to lead to further research in this sphere with the potential to change clinical practice by improving our understanding of the risk stratification of patients with depressive symptoms, especially in patients with existing cardiometabolic disease, which may in turn influence future management.

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Appendix 1

Risk assessment and predicting outcomes in patients with depressive symptoms: a review of potential role of peripheral blood based biomarkers

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Depression is one of the major global health challenges and a leading contributor of health related disability and costs. Depression is a heterogeneous disorder and current methods for assessing its severity in clinical practice rely on symptom count, however this approach is unreliable and inconsistent. The clinical evaluation of depressive symptoms is particularly challenging in primary care, where the majority of patients with depression are managed, due to the presence of co-morbidities. Current methods for risk assessment of depression do not accurately predict treatment response or clinical outcomes. Several biological pathways have been implicated in the pathophysiology of depression; however, accurate and predictive biomarkers remain elusive. We conducted a systematic review of the published evidence supporting the use of peripheral biomarkers to predict outcomes in depression, using Medline and Embase. Peripheral biomarkers in depression were found to be statistically significant predictors of mental health outcomes such as treatment response, poor outcome and symptom remission; and physical health outcomes such as increased incidence of cardiovascular events and deaths, and all-cause mortality. However, the available evidence has multiple methodological limitations which must be overcome to make any real clinical progress. Despite extensive research on the relationship of depression with peripheral biomarkers, its translational application in practice remains uncertain. In future, peripheral biomarkers identified with novel techniques and combining multiple biomarkers may have a potential role in depression risk assessment but further research is needed in this area.

Keywords: peripheral biomarkers, depression, treatment response, risk assessment, outcomes

INTRODUCTION

HETEROGENEITY IN DEPRESSIVE SYMPTOMS

Depression is a heterogeneous disorder with a spectrum ranging from minor/sub threshold to major depressive disorder (MDD) (Rodriguez et al., 2012). According to the latest global disease burden study, depressive disorders (MDD and sub threshold/minor depression) are the leading cause of disability and disease burden globally (Ferrari et al., 2013). The methods currently available for risk assessment and stratification of symptom severity for patients presenting with depressive symptoms rely predominantly on counting the absolute number of depressive symptoms present but there is no universally accepted standardized definition. The Diagnostics and Statistical Manual (DSM)-IV's diagnosis of a MDD requires the presence of at least 5 out of 9 symptoms of depression with significant impairment or distress, while those presenting with at least 2 but less than 5 symptoms and no previous history of MDD are stratified as sub threshold or minor depression (American Psychiatric Association, 2000). The

category of sub threshold depression has been removed from recently published DSM-V (American Psychiatric Association, 2013). On the other hand, the International Classification of Diseases (ICD-10) stratifies depressive symptoms on the basis of the number of depressive symptoms present into mild (4 out of 10), moderate (5 or 6 out of 10) and severe (7 or more out of 10) depressive episode (WHO, 2010). However, this approach has been questioned owing to lack of consensus (Wittchen et al., 2001; Hegerl et al., 2012) and because it ignores the complexity and diversity of depressive symptoms (Goldberg, 2011). The bulk of patients reporting with depressive symptoms are managed in primary care; however the rate of accurate stratification of depressive symptoms in primary care was less than 50% based on a meta-analysis involving more than 50,000 patients (Mitchell et al., 2009). Minor or sub threshold depression has been associated with severe deficits in psychological well-being and quality of life, progression to major disorder and increased mortality (Cuijpers and Smit, 2002; Lyness et al., 2006; Nierenberg et al., 2010),

underlining the need for its early identification and appropriate treatment.

MANAGEMENT OF DEPRESSION

The uncertainty in stratifying depression severity based on symptom count affects subsequent management. A review of treatment guidelines for depression across North America and Europe revealed that "mild MDD and sub threshold depression has the most variance in recommendations"; with suggested approaches ranging from watchful waiting to active treatment with antidepressants (Davidson, 2010). In the last decade, three separate meta-analyses reported that the efficacy of antidepressants is related to the initial severity of depression and they may not be effective in the treatment of mild depression (Khan et al., 2002; Kirsch et al., 2008; Fournier et al., 2010). However, this view has been challenged recently with emerging evidence suggesting that the efficacy of antidepressants in depression may not be related to its initial severity (Gibbons et al., 2012; Fountoulakis et al., 2013). Psychological therapies have been found to be effective in the management of mild depression but they have not been subjected to the same level of scrutiny as pharmacological therapies as yet (Cuijpers et al., 2007). The ambiguity surrounding stratifying the severity of depression based on symptom count and its subsequent management could partially explain why most patients with depression do not receive adequate treatment and many treated patients develop treatment resistance and relapse (Thase, 2006; Nemeroff, 2007). Therefore, different approaches for risk assessment and severity stratification of patients presenting with depressive symptoms are urgently required.

PATHOGENESIS OF DEPRESSION

The etiopathogenesis of depression has been extensively studied over the last five decades with various explanatory mechanisms involving different physiological systems, suggesting heterogeneity (Zunszain et al., 2011). The "monoamine hypothesis of depression" was proposed in the 1960s with early work showing increased levels of plasma tryptophan (serotonin precursor) in patients with major depression (Coppen et al., 1973). Failure to suppress cortisol in response to dexamethasone in patients with depression was the initial finding which supported the role of hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in the pathophysiology of depression (Carroll et al., 1981). The "cytokine hypothesis" suggests that depression is triggered, in part, via inflammatory processes in response to various internal and external stressors, following some seminal work in the early 1990s (Maes et al., 1991). This hypothesis has been further developed to suggest that inflammatory, oxidative and nitrosative stress are causally related to depression and increased translocation of lipopolysaccharide from gram negative bacteria may aggravate these pathways (Maes, 2008). The "neurogenesis hypothesis" of depression proposes that depression is characterized by neurodegeneration and impaired neurogenesis in the brain, in particular the hippocampus region (Sapolsky, 2004). The bi-directional relationship between metabolic syndrome and depression and their common pathophysiological pathways has been reported extensively (McIntyre et al., 2009; Vancampfort et al., 2013). Of course, several hypotheses may overlap or be relevant here.

BIOMARKERS OF DEPRESSION

A biomarker can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Biomarkers Definitions Working Group, 2001). The research into pathogenesis of depression has led to a strong evidence base supporting a cross-sectional relationship between depressive symptoms and a number of different biomarkers pertaining to some of the physiological systems described above, but their role, if any, in predicting clinical outcomes in depression remains unclear (Macaluso et al., 2012). Peripheral biomarkers (blood based) are relatively non-invasive (other than the need for a blood sample) and easier to measure; hence they have a greater potential for translational application into routine clinical practice, when compared to imaging, genetic and CNS biomarkers. Peripheral biomarkers such as those related to HPA axis, inflammatory and monoamine systems may have a role in the diagnosis of depression by identifying a "biological sub-type" of depression, and in prognostication of depression by predicting treatment response, which in turn could help in its severity stratification and management (Fisar and Raboch, 2008; Leuchter et al., 2010; Schmidt et al., 2011). Various inflammatory and oxidative stress biomarkers have been proposed to have a potential role, not only in predicting antidepressant response, but also in enhancing treatment matching and onset prediction in patients with depression (Lopresti et al., 2014). For example, in a study based on a multi-center trial involving depression patients, showed an interaction between antidepressants and C-reactive protein with patients with raised CRP more likely to respond to nortripytline than escitalopram (Uher et al., 2014).

AIMS OF THE REVIEW

To attempt to address this issue, we examine the evidence base exploring the potential role of peripheral biomarkers at baseline in predicting future outcomes in patients with depression. We discuss the potential role of peripheral biomarkers identified using novel and emerging techniques such as proteomics, metabolomics, genetics, and epigenetics in risk assessment and outcome prediction in patients with depressive symptoms. We also review the relationship between depressive symptoms and a composite index score derived using multiple peripheral biomarkers such as allostatic index (AI) and discuss its possible role in future, in management of depression.

METHODS

Two electronic databases (Ovid Medline and Embase) were searched for studies published between 1946 and Jan 2013 using the MESH terms "Biological markers" AND "Depression." All original and review studies using peripheral biomarkers at baseline as a risk assessment tool for predicting future outcomes in patients with depression were included. Clinical outcomes pertaining to both mental health (e.g., depressive symptoms) and physical health (e.g., cardiovascular event) were included. Only studies published in English language were considered for inclusion. Studies related to animal, imaging biomarkers, cerebrospinal fluid biomarkers, and mood disorders other than depression were excluded. Studies which investigated the role of

depressive symptoms and peripheral biomarkers independently in predicting adverse physical outcomes but did not examine the interaction between depressive symptoms and peripheral biomarkers, or in other words did not perform a sub-group analysis in patients with depression, were excluded. Studies that investigated changes in peripheral biomarker levels following treatment for depression and which didn't report any correlation between baseline biomarker levels and depressive symptoms were excluded as the aim of this review was to focus on the use of peripheral biomarkers at baseline or pre-treatment as a predictive tool of clinical outcome (both mental and physical), rather than a change in biomarker level itself. The search strategy returned 1096 studies from two databases after excluding duplicates (see **Figure 1** for details).

Title, abstract and full text screening followed by reference and citation searching and data extraction were carried out independently by two researchers (Bhautesh D. Jani and Gary McLean). The data extraction comprised of study sample size and country, type of study and setting, details of how depression was diagnosed and treated, follow-up duration, biomarkers assessed, clinical outcomes studied and potential bias in the results. The description of methodology used by included studies for biomarker measurement and the source of peripheral biomarker (i.e., serum or plasma or whole blood) was also reviewed in data extraction.

RESULTS

INCLUDED STUDIES AND THEIR CHARACTERISTICS

There was extensive evidence (109 studies) exploring and supporting the cross-sectional relationship between depression and different peripheral biomarkers. However, only a minority of studies ($n = 14$) explored the use of peripheral biomarkers to predict outcomes in patients with depression. Fifteen papers were included for data extraction; which consisted of nine prospective cohort studies (Duval et al., 1996; Perez et al., 1998; Alvarez et al., 1999; Johnston et al., 1999; Lanquillon et al., 2000; Ladwig et al., 2005; Binder et al., 2009; Jokinen and Nordstrom, 2009; Baune et al., 2012), three case-control studies (Arolt et al., 2003; Baldwin et al., 2006; Jang et al., 2008), two randomized controlled trials (Kin et al., 1997; Raison et al., 2013) and one meta-analysis (Ribeiro et al., 1993). Full details of included studies are summarized in **Table 1**.

Sample sizes ranged from 8 to 986 with sample sizes of less than 50 participants in00206 studies (Duval et al., 1996; Alvarez et al., 1999; Johnston et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Baldwin et al., 2006), while three studies had a sample size of 25 or less (Alvarez et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003). Follow-up duration ranged from 4 weeks to 18 years with the follow-up duration being less than 6 months in 9 studies(Duval et al., 1996; Kin et al., 1997; Perez et al., 1998; Alvarez et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Jang et al., 2008; Binder et al., 2009; Raison et al., 2013) while only 5 studies followed their subjects for more than 12 months (Johnston et al., 1999; Ladwig et al., 2005; Baldwin et al., 2006; Jokinen and Nordstrom, 2009; Baune et al., 2012). Six studies used a diagnostic interview technique (Duval et al., 1996; Johnston et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Baldwin et al., 2006; Jang et al., 2008) and seven studies used a depression rating scale (Kin et al., 1997; Perez et al., 1998; Alvarez et al., 1999; Ladwig et al., 2005; Binder et al., 2009; Baune et al., 2012; Raison et al., 2013) while diagnostic method was not specified in one of the included

FIGURE 1 | Flow chart for the systematic review on the role of peripheral biomarkers predicting outcomes in patients with depression.

Table 1 | Summary of included studies.

	Type of study and setting	Sample size at follow-up	Depression diagnosis criteria	Source of Biomarker (Serum/Plasma/whole blood), Biomarkers Assessed (and type of biomarker) *implies statistically significant (*p*-value < 0.05)	Treatment offered	Follow-up duration	Outcomes studied
Alvarez et al., 1999; France	Cohort; psychiatry inpatients	$N = 8$	MADRS ≥ 20	Serum 5 plasma fluoxetine (Neurotransmitter metabolism) Plasma norfluoxetine (Neurotransmitter metabolism) Plasma fluoxetine plus norfluoxetine (Neurotransmitter metabolism) Plasma 5 HT (Neurotransmitter metabolism) Serum 5HT (Neurotransmitter metabolism)	Fluoxetine 20 mg	28 days	Treatment response defined as 50% reduction in MADRS scores from baseline
Arolt et al., 2003; Germany	Case-control; psychiatry inpatients	$N = 25$ (MDD), $N = 25$ (healthy controls)	Composite International Diagnostic Interview for DSM-IV criteria for MDD	Plasma S100 B protein* (Neurotrophic)	Different groups of anti-depressants	28 days	Treatment Response defined as 50% reduction in HDRS from baseline
Baldwin et al., 2006; UK	Case-control; Community	$N = 28$ (MDD), $N = 35$ (healthy controls)	SCID for MDD	HDL cholesterol* (Metabolic) LDL cholesterol (Metabolic) BMI (Metabolic) ESR (Inflammatory) Pre-prandial glucose (Metabolic) Source: Serum/Plasma/Whole blood was not specified	Not specified	3.1 years	Poor outcome of depression based on author described criteria assessed by SCID
Baune et al., 2012; Australia	Cohort; community	$N = 73$	GDS ≥ 6	Serum IL1β (Inflammatory) Serum IL6 (Inflammatory) Serum IL8 * (Inflammatory) Serum IL10 (Inflammatory) Serum IL 12p70 * (Inflammatory) sVCAM-1 (Inflammatory) Serum PAI-1 (Inflammatory) SAA (Inflammatory) Serum TNF-α (Inflammatory) Serum CRP (Inflammatory)	Not specified	23.39 months (average)	Remission of depression symptoms defined as GDS <6

(Continued)

Table 1 | Continued

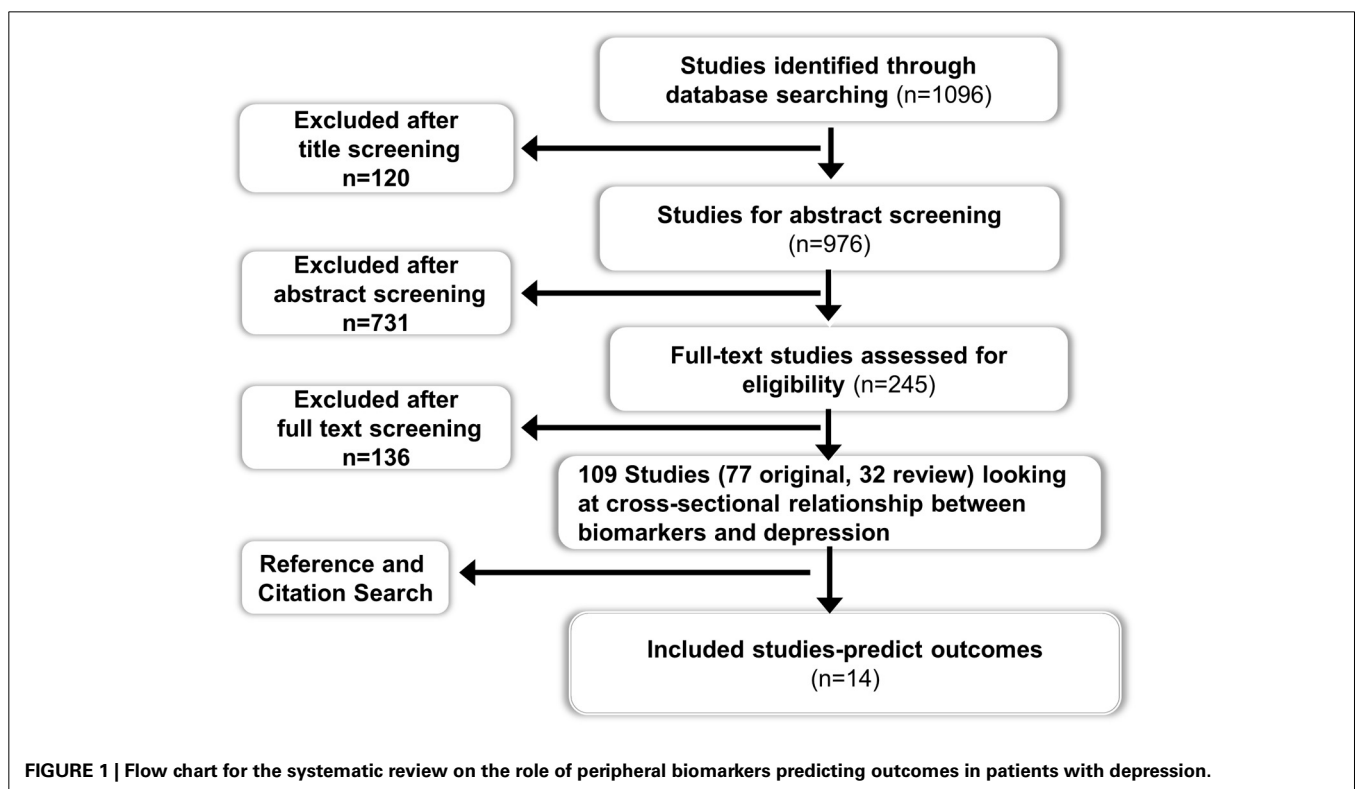|	Type of study and setting	Sample size at follow-up	Depression diagnosis criteria	Source of Biomarker (Serum/Plasma/whole blood), Biomarkers Assessed (and type of biomarker) *implies statistically significant (p-value < 0.05)	Treatment offered	Follow-up duration	Outcomes studied
Duval et al., 1996; France	Cohort study; psychiatry Inpatients	N = 30	Unstructured interview for DSM-IV for MDD	Plasma TSH (Neuroendocrine) Plasma Free T3 (Neuroendocrine) Plasma Free T4 (Neuroendocrine) Plasma TSH response to Protirelin stimulation* **(for outcomes 1 and 2)** (Neuroendocrine) Plasma Free T3 response to Protirelin stimulation (Neuroendocrine) Plasma Free T4 response to Protirelin stimulation (Neuroendocrine)	1.Amitriptyline (n = 13) 2.Fluoxetine (n = 9) 3.Toloxatone (n = 8)	1 month	1. Remission of depression symptoms defined as HDRS<8 2. "Partial Response" (treatment response) defined as HDRS 8–15
Jang et al., 2008; South Korea	Case-control; Psychiatry Outpatients	N = 59 (MDD), N = 34 (healthy controls)	SCID for MDD	Serum S100B protein * (Neurotrophic)	Different groups of anti-depressants	6 weeks	Treatment response defined as 50% reduction in HDRS from baseline
Johnston et al., 1999; UK	Cohort; Psychiatry Outpatients and Inpatients	N = 34	SCID for MDD	Plasma Norepinephrine* (Neuroendocrine) Plasma Cortisol (Neuroendocrine)	Not specified	8 years (average)	Poor Outcome defined by Depression Outcome Scale and Lee and Murray criteria
Jokinen and Nordstrom, 2009; Sweeden	Cohort; Psychiatry Inpatients	N = 346	DSM- IV criteria for all mood disorders, diagnostic method unspecified	Plasma Cortisol* **(for outcomes 1 and 2)** (Neuroendocrine) Plasma Dexamethasone non-suppression* **(for outcomes 1 and 2)** (Neuroendocrine)	Not specified	18 years (average)	1. Death due to natural causes 2. Cardiovascular deaths
Kin et al., 1997; Multi-center	RCT with 3 arms; not specified	N = 70 randomized into 3 arms	HDRS ≥ 18	Plasma dexamethasone non-suppression* **(only in Nortriptyline arm)** (Neuroendocrine)	3 arms: 1. Nortriptyline 75 mg 2.Moclobemide 400 mg 3. Placebo	7 weeks	Treatment Response defined as 50% reduction in HDRS from baseline
Ladwig et al., 2005; Germany	Cohort; Community	N = 975 (only males)	von Zerssen affective symptom check list with a score ≥11	Serum Highly sensitive CRP high risk group > 3 mg/ml* (Inflammatory)	Not specified	7.7 years (average)	1. Myocardial Infarction 2. Sudden cardiac death

(Continued)

Table 1 | Continued

	Type of study and setting	Sample size at follow-up	Depression diagnosis criteria	Source of Biomarker (Serum/Plasma/whole blood), Biomarkers Assessed (and type of biomarker) *implies statistically significant (p-value < 0.05)	Treatment offered	Follow-up duration	Outcomes studied
Lanquillon et al., 2000; Germany	Cohort; Psychiatry inpatients	$N = 24$	SCID for MDD	Whole blood Lymphocyte count (Inflammatory) Whole blood Monocyte count (Inflammatory) Whole blood Ratio lymphocyte/monocyte* (Inflammatory) Whole blood CRP (Inflammatory) Whole blood ESR (Inflammatory) Whole blood IL-6 * (Inflammatory) Whole blood TNF-alpha (Inflammatory)	Amitriptyline in increasing dose	6 weeks	Treatment Response defined as 50% reduction in HDRS and MADRS from baseline
Perez et al., 1998; Spain	Cohort; Psychiatry Inpatients	$N = 83$	HDRS $\geq$ 17	Plasma 5HIAA (Neurotransmitter) Plasma Total Tryptophan (Neurotransmitter) Plasma 5 HT (Neurotransmitter) Platelet 5 HT with high concentration 800 ng/10^9 platelets* **(stronger relationship in SSRI sub-group)** (Neurotransmitter)	Different groups of anti-depressants	6 weeks	Treatment Response defined as 50% reduction in HDRS from baseline
Raison et al., 2013; US	RCT with 2 arms; Community	$N = 60$ randomized into two arms	Treatment resistance Depression diagnosed using Massachusetts General Hospital Staging method for treatment resistance $\geq$ 2	Plasma Highly sensitive CRP high risk group > 5 mg/ml (Inflammatory)	2 arms: 1.Infliximab infusions × 3 2. Placebo	12 weeks	1. Treatment Response defined as 50% reduction in HDRS from baseline 2. Remission of depression symptoms defined as HDRS<8
Ribeiro et al., 1993; US	Meta-analysis with 3 different research questions (RQ1-3)	RQ-1 $N = 1273$	Heterogeneous, including different symptoms scores and interview techniques	Dexamethasone non-suppression* **(for RQ2 only)** Source: Serum/Plasma/Whole blood was not specified for the included studies	Various	Not specified	1. (RQ1) "Treatment Response"

(Continued)

Table 1 | Continued

| RQ-2 N = 412 | Heterogeneous, including different symptoms scores and interview techniques | Various | 1–7 weeks | 2. (RQ2) Response to Placebo |
| RQ-3 N = 411 | Heterogeneous, including different symptoms scores and interview techniques | Various | 1–60 months | 3. Long term outcome of depression based on predefined author criteria |

MADRS, Montgomery Asperg Depression Rating Scale; 5HT, 5 Hydroxytryptamine; HDRS, Hamilton Depression Rating Scale; DSM-IV, Diagnostic and Statistical Manual IV; MDD, Major Depressive Disorder; SCID, Structured Diagnostic Interview for DSM-IV; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; ESR, Erythrocyte Sedimentation Rate; BMI, Body Mass Index; GDS, Geriatric Depression Scale; IL, Interleukin; sVCAM-1, Serum Vascular Cell AdhesionMolecule -1; PAI-1, Plasminogen Activator Inhibitor-1; SAA, Serum Amyloid A; TNF-α, Tumor, Necrotic Factor-alpha; CRP, C Reactive Protein; TSH, Thyroid Stimulating Hormone; RCT, Randomized Controlled Trial; 5-HIAA, 5-Hydroxyindoleacetic acid, RQ, Research Question.

studies (Jokinen and Nordstrom, 2009). The nature of the treatment was specified in nine studies (Duval et al., 1996; Kin et al., 1997; Perez et al., 1998; Alvarez et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Jang et al., 2008; Binder et al., 2009; Raison et al., 2013); the relationship between outcome and baseline depression severity was only taken into account in 5 studies (Duval et al., 1996; Johnston et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Baldwin et al., 2006). The included meta-analysis had a variable sample size and follow-up duration depending on the different research questions considered by the study and the diagnostic methods used were heterogeneous including various symptoms scores and interview techniques (Ribeiro et al., 1993).

BIOMARKERS STUDIED AND METHOD OF COLLECTION

The included studies assessed 36 different peripheral biomarkers at baseline as a predictor of clinical outcomes. These biomarkers were measured in serum or plasma and could be broadly classified as pertaining to inflammatory ($n = 14$), neurotransmitter metabolism ($n = 9$), neuroendocrine ($n = 8$), metabolic ($n = 4$), and neurotrophic ($n = 1$) systems. All included studies assessed statistical significance based on the criteria of having a p-value less than 0.05. Twelve biomarkers were found to be statistically significant in predicting outcomes (summarized in **Figure 2**). Inflammatory (Lanquillon et al., 2000; Ladwig et al., 2005; Baldwin et al., 2006; Baune et al., 2012; Raison et al., 2013) and neuroendocrine (Ribeiro et al., 1993; Duval et al., 1996; Kin et al., 1997; Johnston et al., 1999; Jokinen and Nordstrom, 2009) biomarkers were each assessed in five of the included studies, followed by neurotransmitter (Perez et al., 1998; Alvarez et al., 1999; Johnston et al., 1999) biomarkers in three studies, neurotrophic (Arolt et al., 2003; Jang et al., 2008) biomarker in two studies, while metabolic (Baldwin et al., 2006) biomarkers were assessed in only one study.

The source of peripheral biomarker measurement was plasma in half of the included studies ($n = 7$) (Duval et al., 1996; Kin et al., 1997; Perez et al., 1998; Johnston et al., 1999; Arolt et al., 2003; Jokinen and Nordstrom, 2009; Raison et al., 2013); serum in three studies (Ladwig et al., 2005; Jang et al., 2008; Baune et al., 2012); whole blood (Lanquillon et al., 2000) and mixed (both serum and plasma) (Alvarez et al., 1999) in 1 study each; and not reported in two of the included studies (Ribeiro et al., 1993; Baldwin et al., 2006). Four of the included studies did not describe the procedures of measuring peripheral biomarker in detail (Ribeiro et al., 1993; Duval et al., 1996; Baldwin et al., 2006; Jokinen and Nordstrom, 2009). Four of the included studies describe the anticoagulant used for collecting plasma samples with ethylenediaminetetraacetic acid (EDTA) used by two studies (Perez et al., 1998; Raison et al., 2013); and heparin (Arolt et al., 2003) and sodium citrate (Alvarez et al., 1999) used by one study each.

TYPES OF CLINICAL OUTCOMES STUDIED AND STATISTICAL METHODS

The majority of included studies ($n = 12$) (Ribeiro et al., 1993; Duval et al., 1996; Kin et al., 1997; Perez et al., 1998; Alvarez et al., 1999; Johnston et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Baldwin et al., 2006; Jang et al., 2008; Baune et al., 2012;

FIGURE 2 | Different outcomes in depression and their significant predictors. This Figure describes the various mental and physical health outcomes considered by included studies in the review, the number of studies which examined each outcome, the peripheral biomarkers which were found to have a statistically significant impact in predicting each outcome and the direction of the relationship. DST, Dexamethasone Suppression Test; CRP, C Reactive Protein; IL, Interleukin; 5HT, 5 Hydroxytryptamine; TSH, Thyroid Stimulating Hormone; ↑: higher, ↓: lower. *The study did not specify the source of the biomarker studied (i.e., serum or plasma).

Raison et al., 2013) examined outcomes pertaining to mental health or depressive symptoms, with only two studies assessing physical health outcomes (Ladwig et al., 2005; Jokinen and Nordstrom, 2009). Author defined positive treatment response to anti-depressants with improvement in depressive symptoms [e.g., 50% reduction in depression rating scale Hamilton Depression Rating Scale (HDRS) from baseline] was the commonest outcome considered by nine included studies (Ribeiro et al., 1993; Duval et al., 1996; Kin et al., 1997; Perez et al., 1998; Alvarez et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Jang et al., 2008; Raison et al., 2013). This was followed by other mental health outcomes such as author defined criteria for poor outcome of depressive symptoms ($n = 3$) (Ribeiro et al., 1993; Johnston et al., 1999; Baldwin et al., 2006) for e.g., Lee and Murray operational criteria for outcome in depression; and remission of depression symptoms ($n = 3$) (Duval et al., 1996; Baune et al., 2012; Raison et al., 2013) for e.g., HDRS <8 at follow-up. The physical health outcomes measured were cardiovascular deaths ($n = 2$) (Ladwig et al., 2005; Jokinen and Nordstrom, 2009),

myocardial infarction ($n = 1$) (Ladwig et al., 2005) and death due to natural causes ($n = 1$) (Jokinen and Nordstrom, 2009). The usefulness of statistical models for physical outcomes was not compared against routinely used and evidence backed risk scores such as the Framingham score for cardiovascular events. Biomarkers were shown to be statistically significant in predicting all of the six outcomes considered, including mental and physical outcomes. **Figure 2** summarizes the six different mental and physical health outcomes studied, the number of studies which examined each outcome, the 12 peripheral biomarkers which were noted to be statistically significant in predicting each outcome and the direction of the relationship between the biomarker and the outcome.

The Area Under Curve (AUC) statistic was presented only by 1 study, with AUC statistic for Dexamethasone suppression test (DST) reported as 0.65 for predicting increased incidence of cardiovascular deaths only for the male subset of their sample (Jokinen and Nordstrom, 2009). DST was found to have a significant impact in predicting three different outcomes in

two different studies; which included adverse outcomes such as increased incidence of all-cause mortality and cardiovascular deaths (Jokinen and Nordstrom, 2009), and favorable outcome such as positive treatment response to anti-depressants (Kin et al., 1997). In the included meta-analysis, DST failed to have a significant impact in predicting positive response to anti-depressants but was significant in predicting positive response to placebo (Ribeiro et al., 1993). Elevated levels of serum S100B was the only biomarker which was found to have a statistically significant role in predicting the same clinical outcome (positive treatment response to anti-depressants) in more than one included studies (Arolt et al., 2003; Jang et al., 2008).

PATIENT DEMOGRAPHICS AND ATTRITION RATE

Description of patient demographics, co-morbid conditions and attrition at follow-up for included studies is provided in **Table 2**. Details of the age of participants were not described by three studies (Ribeiro et al., 1993; Kin et al., 1997; Baune et al., 2012); while information on gender distribution was missing from five studies (Ribeiro et al., 1993; Kin et al., 1997; Arolt et al., 2003; Baldwin et al., 2006; Baune et al., 2012). The socio-economic status of participants was very poorly described with only two studies (Ladwig et al., 2005; Raison et al., 2013) characterizing it and only one study (Ladwig et al., 2005) including socio-economic status in their statistical analysis. Patients with pre-existing chronic disease were excluded by the majority of the included studies ($n = 8$) (Duval et al., 1996; Perez et al., 1998; Johnston et al., 1999; Lanquillon et al., 2000; Arolt et al., 2003; Ladwig et al., 2005; Jang et al., 2008; Jokinen and Nordstrom, 2009) and chronic disease status was not considered or described by four of the included studies (Ribeiro et al., 1993; Kin et al., 1997; Alvarez et al., 1999; Baldwin et al., 2006). Of the two studies which included patients with co-existing chronic disease (Baune et al., 2012; Raison et al., 2013), only one study accounted for the number of co-morbidities in their statistical analysis (Raison et al., 2013). The reported participant attrition rate at follow-up varied from 0 to 44%; with two included studies (Ribeiro et al., 1993; Baune et al., 2012) not specifying the details of attrition.

DISCUSSION

SUMMARY OF FINDINGS

Our review shows that blood based peripheral biomarkers were statistically significant in predicting six different clinical outcomes in participants with depression. Outcomes related to both mental health (depressive symptoms) and physical health were statistically associated with pre-treatment levels of peripheral biomarkers; however only two studies investigated outcomes related to physical health. Twelve different biomarkers related to five different biological systems (inflammatory, neuroendocrine, neurotransmitter metabolism, neurotrophic, and metabolic) were found to have a potential role in predicting outcomes of depression. Despite extensive research on the biomarkers of etiopathogenesis of depression, there is limited published research exploring its translational application in clinical practice. Furthermore, the research is of generally limited quality and lacks clinical utility.

The included studies have several methodological problems. The study sample size was small and follow-up duration was short in the majority of included studies. The majority of included studies used questionnaire scores using symptom count for diagnosing depression at baseline, while the gold standard interview technique for depression diagnosis was used only by a minority. Baseline severity of depressive symptoms assessed using symptom count is associated with higher rate of relapse in patients with depression (Ishak et al., 2013) but accounting for the baseline severity of depressive symptoms was only undertaken by a minority of studies. There is a strong evidence base suggesting that depression is two to three time more prevalent in patients with co-existing chronic disease as compared to the general population (Egede, 2007; Moussavi et al., 2007; Mitchell et al., 2013) but the effect of co-morbidity on clinical outcomes was examined by only two studies.

Importantly, the clinical implications of the observed statistical relationships in the included studies were not well explained. The c-statistic or area under receiver operating characteristic (ROC) curve (Cook, 2007), which is regarded as one of the standard methods for evaluating clinical discriminating power of a statistical model, was reported by only one study. The usefulness of statistical models for physical outcomes in the included study was not compared against robustly validated and routinely used risk scores such as the Framingham score for cardiovascular events (D'Agostino et al., 2008). Finally, some of the biomarkers included in this review are complicated to measure and likely to be expensive. The source and method of measurement for biomarkers in the included studies were heterogeneous and this may have an influence on assay levels of the biomarkers measured (Tort et al., 2003; Wong et al., 2008; Yu et al., 2011). The cost implications of doing these tests were not considered in detail in the included studies and this is likely to be a relevant factor when considering their potential use in routine clinical practice. There is some evidence that peripheral biomarkers may have a role in stratifying depression severity by means of predicting various physical and mental health outcomes in depression but further more robust research needs to be done in this area to address the shortcomings of the available evidence.

OUTCOMES BASED APPROACH IN DEPRESSION SEVERITY STRATIFICATION

The use of prediction rules and biomarkers to inform clinical decision making is not a novel concept. It has been used in making management decisions in a wide variety of clinical scenarios such as patients presenting with high cholesterol, atrial fibrillation, chest pain, ankle injury, and intensive care (Reilly and Evans, 2006). In psychiatry, it has been proposed to use this principle for predicting inpatient violence (Abderhalden et al., 2006). Depression contributes to disease burden not only owing to reduction in quality of life and functional productivity, but also due to the increased risk of adverse physical outcomes such as hospitalization and mortality (Ferrari et al., 2013). There is strong evidence showing an association of depression (MDD and mild depression) with increased risk of adverse physical outcomes such as all-cause mortality, cardiovascular disease, hypertension, stroke, diabetes, alzheimer's disease, obesity, and cancer

Table 2 | Patient population and attrition rates in included studies.

Study	Mean Age in years (Standard Deviation, if available) and Sex F, Females; M, Males	Socio-economic status	Co-morbid medical conditions	Participant numbers Number of participants at baseline (B) and follow-up(FU); attrition in percentage
Alvarez et al., 1999	45 (13.8) 6F, 2M	Not described	Not described	10 B 8 FU 20% attrition
Arolt et al., 2003	46.4 (9.8) Not described	Not described	Patients with co-morbid conditions excluded from study	25 B 25 FU No attrition
Baldwin et al., 2006	73.9 Not described	Not described	Not described	50 B 28 FU 44% attrition
Baune et al., 2012	Not described	Not described	Presence/absence of a list of medical conditions noted and entered into statistical analysis	73 B Sample size at follow-up not specified
Duval et al., 1996	39.8 (12.9); 19M, 11F	Not described	Patients with co-morbid conditions excluded from study	30 B 30 FU No attrition
Jang et al., 2008	60.3; 43F, 16M	Not described	Patients with co-morbid conditions excluded from study	59 B 59 FU No attrition
Johnston et al., 1999	47; 24F, 10M	Not described	Patients with co-morbid conditions excluded from study	47 B 34 FU 27.6% attrition
Jokinen and Nordstrom, 2009	52 (16.4); 256F, 126M	Not described	Patients with co-morbid conditions excluded from study	382 B 346 FU 9.4% attrition
Kin et al., 1997	Not described	Not described	Not described	95 B 70 FU 26.3% attrition
Ladwig et al., 2005	57.75 (7.8); 975M	Education status described and entered into statistical analysis	Patients with co-morbid conditions excluded from study	986 B 975 FU 1.1% attrition
Lanquillon et al., 2000	53.5; 15F, 9M	Not described	Patients with co-morbid conditions excluded from study	35 B 24 FU 30.5% attrition
Perez et al., 1998	M 45 (2.9), F 44.9 (2.0); 59F, 24M	Not described	Patients with co-morbid conditions excluded from study	89 B 83 FU 6.7% attrition
Raison et al., 2013	42.5(8.2) placebo group, 44.3 (9.4) intervention group; 40F, 20M	Education and employment status described but not entered into statistical analysis	Notable exclusions- previous history of cancer, history of unstable cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease (determined by Physical examination and laboratory testing). Number of co-morbid medical conditions noted and entered into statistical analysis	60 B 60 FU No attrition
Ribeiro et al., 1993	Not described	Not described	Not described	Not described

(Penninx et al., 2013). Physical adverse outcomes associated with depression attribute to a significant amount of morbidity and mortality (Ferrari et al., 2013; Penninx et al., 2013). Consequently, it is imperative that the risk of adverse physical outcomes

associated with depression should be considered while taking decisions regarding depression severity stratification and subsequent management. Crucially, the clinical utility of biomarkers in predicting physical outcomes in depression, if any, should be

compared and validated against some of the established and available risk scoring systems (e.g., Framingham, D'Agostino et al., 2008) for physical outcomes.

ROLE OF PERIPHERAL BIOMARKERS IN IDENTIFYING DEPRESSION SUBTYPES

The use of peripheral biomarkers in identifying different subtypes of depression has been explored by other studies in the literature. A meta-analysis reviewing the association between HPA axis hyperactivity (Dexamethasone non-suppression) and depression suggested a dose-response relationship, with patients with mild depression showing higher HPA hyperactivity compared to controls but lower than that of patients with MDD (Stetler and Miller, 2011). Peripheral inflammatory markers such as Tumor necrotic factor (TNF)-α and IL (Interleukin)-6, serum neopterin have been shown to have association with melancholic subtypes of MDD (Maes et al., 2012; Dunjic-Kostic et al., 2013). A review of metabolic and neuroendocrine biomarkers (Body mass index BMI, waist-hip ratio, fasting glucose, serum adrenocorticotropic hormone ACTH) in pre-menopausal women with MDD supported their role in identifying three different subtypes of MDD-melancholic, atypical and undifferentiated (Cizza et al., 2012). This suggests that peripheral biomarkers may have a useful role in addressing some of the challenges posed by heterogeneity of depression, with a particular biomarker likely to have a more useful role in a specific subtype of depression. However, before any decisions are made, much better high quality research is needed.

NOVEL BIOMARKERS IN DEPRESSION

In recent years, novel techniques in proteomics, metabolomics, genetics, and epigenetics have led to several new biomarkers being proposed in depression. Proteomic techniques have been used to identify nine differentiating proteins belonging to lipid metabolism and immune system from treatment naïve patients with depression, when compared against healthy controls (Xu et al., 2012). Similarly, metabolomic techniques such as nuclear magnetic response (NMR) based analysis of both urine and plasma have been utilized to identify differentiating proteins related to lipid metabolism and neurotransmitter system with good accuracy in treatment naïve patients with depression, when compared to healthy controls (Zheng et al., 2012a,b). The role of brain-derived neurotrophic gene polymorphisms, glucocorticoid receptor polymorphism and serotonin gene receptor have been studied in diagnosis and prognostification of depression with some encouraging results (Chi et al., 2010; Szczepankiewicz et al., 2011; Uher et al., 2011). Although the findings from genome wide association studies (GWAS) till date in depression have failed to make a major breakthrough, they may have a potential role in stratification of depression and further research is ongoing (Wray et al., 2012; Flint and Kendler, 2014). Thus, these emerging techniques and biomarkers may have a role in diagnosis, identifying specific subtypes of depression and prognostification in depression (Schneider and Prvulovic, 2013).

MULTIPLE BIOMARKERS, ALLOSTATIC LOAD, AND DEPRESSION

The term allostasis refers to the adaptive physiological responses organisms activate when homeostasis is disrupted during acute stress, real or interpreted threats (McEwen and Stellar, 1993). When chronically activated, allostatic mechanisms become physiologically taxing—or an allostatic load (AL)—that consequently increase one's susceptibility to disease (McEwen, 1998). There is some early evidence to suggest that an index comprising multiple biomarkers or AI may exhibit a stronger relationship with depressive symptoms, especially in elderly populations, when compared with examination of individual biomarkers in isolation (Juster et al., 2011). The role of multiple biomarkers in risk assessment and predicting outcomes in patients with depression needs to be explored and compared against the role of individual biomarkers.

LIMITATIONS

Our search strategy was limited to studies published in English language. A variety of other biomarkers such as genetic, imaging and CSF biomarkers may have a role in depression stratification by predicting clinical outcomes (Schneider and Prvulovic, 2013). However, this review considered only peripheral or blood-based biomarkers used in current clinical practice due to their comparative non-invasive nature and ease of measurement. The uncertainty surrounding management decisions in patients with depression in current practice is a particular issue at the time of initial presentation (Davidson, 2010). Hence, this review was focussed on addressing the issue of the use of peripheral biomarkers at baseline or pre-treatment as a predictive tool of clinical outcome (both mental and physical) and not on assessing changes in a peripheral biomarker level following treatment for depression.

FUTURE RESEARCH

There is a need for further research in this area, involving large scale studies with longer duration of follow-up, better characterization of patient populations and inclusion of patients with chronic diseases. An "ideal" scientific process for a biomarker evaluation in clinical risk discrimination has been highlighted in other fields such as cardiovascular disease, a similar approach can be adopted for biomarkers of depression (Welsh et al., 2008). Further epidemiological studies of greater quality which minimize potential bias and evaluate clinical utility are urgently needed. Future studies also need to incorporate other physical health outcomes such as rate of cardiovascular events, incidence of cancer and all-cause mortality associated with depression and compare validity against established benchmarks, along with mental health outcomes related to depression symptoms.

CONCLUSION

Pre-treatment levels of 12 different blood based peripheral biomarkers related to five different biological pathways were found to have a statistically significant relationship with outcomes in patients with depression. Six different outcomes in depression were predicted using these biomarkers, pertaining to both physical and mental health, but the clinical implications remain unclear. It appears likely that peripheral biomarkers may have an important role in helping clinicians to stratify depression severity and to predict clinical outcomes. However, the available evidence has multiple methodological limitations which must be overcome to make any real clinical headway; in particular, interaction between these biomarkers, depressive symptoms and co-morbid physical conditions needs to be explored further.

AUTHOR CONTRIBUTIONS

Literature search was carried out by Bhautesh D. Jani and Barbara I. Nicholl. Title, abstract and full text screening followed by reference and citation searching and data extraction were carried out by Bhautesh D. Jani and Gary McLean. All authors contributed to the manuscript.

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Appendix 2

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RESEARCH ARTICLE   **Open Access**

Revisiting the J shaped curve, exploring the association between cardiovascular risk factors and concurrent depressive symptoms in patients with cardiometabolic disease: Findings from a large cross-sectional study

Bhautesh Dinesh Jani[1], Jonathan Cavanagh[2], Sarah JE Barry[3], Geoff Der[4], Naveed Sattar[5] and Frances S Mair[6*]

Abstract

Background: Depression is common in patients with cardiometabolic diseases but little is known about the relationship, if any, between cardiovascular risk factor values and depressive symptoms in patients with these conditions. The objective of this paper is to study the association between cardiovascular risk factors and concurrent depressive symptoms in patients with three common cardiometabolic conditions: coronary heart disease (CHD), stroke and diabetes.

Methods: We retrospectively reviewed primary care data for N = 35537 with 1 of the above 3 conditions who underwent depression screening using the depressive subscale of hospital anxiety and depression score (HADS-D). We reviewed 4 cardiometabolic risk factors (Systolic Blood Pressure [SBP], Diastolic Blood Pressure [DBP], BMI and total cholesterol) recorded concurrently in all patients and HbA1c in patients with diabetes (n = 18453). We analysed the association between individual risk factor value and a positive HADS-D screening result (>7) using logistic regression.

Results: SBP and BMI were noted to have a non-linear "J-shaped" relationship with the probability of having a positive HADS-D and observed nadirs (levels with the lowest probability) of 148 mm Hg and 30.70 kg/m2, respectively. Total cholesterol and DBP found to have a weaker curvilinear association with concurrent depression symptoms and nadirs of 3.60 mmol/l and 74 mmHg. Among patients with Diabetes, HbA1c was also found to have a "J-shaped" relationship with probability of having a positive HADS-D with an observed nadir of 7.06% DCCT. The above relationships remain significant after adjusting for age, sex, socio-economic status and number of co-morbid conditions.

Conclusion: In patients with cardiometabolic disease, cardiovascular risk factor values at both extremes were associated with higher positive depression screening after adjusting for confounders. These findings have potentially important implications for clinical practice in relation to both risk stratification for depression and approaches to secondary prevention in individuals with cardiometabolic disease and merit further investigation to determine the nature and direction of the observed association.
Please see related article: http://www.biomedcentral.com/1741-7015/12/199.

Keywords: Cardiovascular risk factors, J-curve, Depression, Blood pressure, Body mass index, Total cholesterol, HbA1C, Diabetes, Stroke, Coronary heart disease

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Background

Patients with chronic disease are two to three times more likely to suffer from depression when compared to the general population [1,2]. It is estimated that depression prevalence is 15-25% in patients with cardio-metabolic diseases such as coronary heart disease (CHD), diabetes and stroke [3-5]. Those with cardiometabolic disease who have suffered from depression have been reported to experience increased adverse clinical outcomes and mortality, and poorer functional abilities [4,6-8].

In 2008, the American Heart Association Science Advisory recommended routine depression screening for all patients with CHD [9]. However, there is no evidence to date that routine depression screening for patients with cardiometabolic disease leads to any improvement in depression or cardiac outcomes [10,11]. Moreover, there is some evidence in the UK and US to suggest that routine depression screening for all patients with cardiometabolic disease may struggle to achieve universal coverage [12-14]. In the UK, NICE(National Institute for Health and Care Excellence) recommends that depression screening or 'case finding' in patients with chronic disease should be targeted towards those who are believed to be 'high risk' [15]; but further research is needed to define who is at 'high risk'.

The relationship between depression and traditional cardiometabolic disease risk factors such as obesity, hypertension, hyperlipidaemia and raised HbA1c have been studied extensively in the general population. Depression is noted to have a significant positive association with obesity in the general population, with a stronger association noted in females [16,17]. In addition, evidence from longitudinal studies show that depression may have a bi-directional relationship with obesity [18]. Results from a meta-analysis of prospective cohort studies shows that depression increases the risk of hypertension incidence in the community [19]. A contradictory relationship has been observed between depression and hyperlipidaemia in elderly men and women in the community; with increased prevalence of depressive symptoms observed with low levels of high density lipoprotein cholesterol (higher atherogenic risk) in women and with low levels of low density lipoprotein cholesterol (lower atherogenic risk) in men [20]. In a prospective study of older adults in the general population, the probability of depression increased with raised HbA1c [21]. However, most of the evidence in this area has come from general population studies and there is a paucity of research in those with known cardiometabolic diseases who are likely to be subjected to treatment to reduce these risk factors.

Little is known about the relationship between cardiovascular risk factors and depressive symptoms in those with cardiometabolic disease. The aim of this project is to address this gap by studying the relationship, if any, between a range of cardiovascular risk factors (specifically SBP, DBP, total cholesterol, HbA1c and BMI) and depressive symptoms in those with three cardiometabolic conditions, namely, stroke, diabetes and CHD.

Methods

Ethics statement

We received approval from the West of Scotland research ethics committee to undertake this work. The work involved retrospective analysis of a large routinely collected dataset which was completely annonymised and the research team did not have access to patient identifiers, hence individual patient consent was not obtained. NHS Greater Glasgow and Clyde Enhanced Services data group, which was the authorised "guardian" of this data set, granted the permission to analyse the data.

Study design and setting

The data reported in this paper comes from the West of Scotland, with a population of circa 1.8 million served by two different health boards. The local health boards oversee a programme of incentivised depression screening in chronic disease as part of a wider chronic disease management programme of 'Local Enhanced Services' (LES). These are contractual arrangements at a local health board level with family practices where incentivisation is offered to primary care practitioners on certain indicators of chronic disease management. However, there are no penalties for non-adherence. In the areas under investigation in our study, family practices were paid under the LES scheme to carry out a comprehensive annual health assessment, which included depression screening, for patients with three common cardiometabolic conditions, CHD, diabetes and stroke. The annual health assessment was usually carried out by a practice nurse and lasted approximately one hour. The protocol for health assessment was specific for each of the three diseases but included monitoring of blood pressure (BP), total cholesterol, body mass index (BMI) and in those with diabetes, HbA1c. The assessment included detailed history taking, various physical examinations and blood tests.

Participants

We restricted our analysis to adults aged from 18 to 90 and health assessments recorded between 01/04/2008 to 31/03/2009. A total of 125,143 patients were listed as having CHD, diabetes or stroke in the year 2008–09, the "DepChron" dataset [14], described in a previous publication. Of the total sample, 10,670 (8.5%) patients were under treatment for depression and were thus exempt from screening. The remaining 114,473 (91.5% of total sample size) patients were eligible for depression screening. However, the uptake of depression screening was

poor and only undertaken in 35,537 (31.1% of those eligible) and 78,936 (68.9%) were not screened.

Measurement of clinical risk factors and outcome variable

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were recorded in mm Hg and BMI in kg/m^2 determined from height and weight measurements. A blood sample was collected by the practice nurse at the time of assessment; the result for total cholesterol was reported in mmol/l and HbA1c was reported in Diabetes Control and Complications Trial (DCCT) units.

We restricted the values for cardiovascular risk factors to a clinically plausible range based on both our clinical judgement and the findings of general population studies. SBP measurements were restricted to a range between 90 to 240 mm Hg and DBP to a range between 50 to 130 mm Hg [22,23]. Similarly, BMI was restricted to a range between 15 to55 [24], total cholesterol to 2–10 [25] and HbA1C to 3-18% [26]. Observations in the data which were outside these range were excluded from the analysis. The depression subscale of HADS (HADS-D) gives a total score of 0 to 21, and a threshold of >7 was used to define the presence of depressive symptoms, as endorsed by national guidelines [27]. The area based Scottish Index of Multiple Deprivations (SIMD) was used as a measure of socioeconomic status [28].

Statistical analysis

We used multiple logistic regression with the outcome variable as the prevalence of a positive screening for depression (defined as HADS-D >7). We used five separate regression models to examine the impact of each individual cardiovascular risk factor (SBP, DBP, total cholesterol, BMI, HbA1c) on the odds of a raised HADS-D. We entered quadratic terms for each clinical measure into regression models to allow for a non-linear relationship. We entered age (18–64 vs. 65–90), sex (male vs. female) and socio-economic status (deprived: SIMD deciles 1–5 vs. affluent: SIMD deciles 6–10) into all of the models as binary variables. We also included the number of comorbid conditions (range 1–3, representing a combination of one or more of the three cardiometabolic disease under investigations: CHD, stroke or diabetes) into all regression models as a categorical variable. We present the results as a graph of the predicted probability of a raised HADS-D against corresponding values of the clinical risk factor. We calculated the turning point for each risk factor using the formula min = −b/2a where "a" represents coefficient of quadratic term and "b" represents coefficient of linear term.

We used the R statistical software, version 3.0.2 for statistical analysis [29].

Supplementary and sensitivity analyses

The screened population was a subset of the whole dataset and the majority of the patients eligible for depression screening did not have HADS-D recorded due to poor uptake of depression screening. We compared the demographic features and distribution of clinical risk factors in both the screened population and the total population. We tested for interactions of each clinical risk factor with age, gender, number of comorbid condition and deprivation status for each of the corresponding regression models to check for potential effect modification. We also tested for cubic terms in each of the five regression models for five clinical measures. Sensitivity of the results to excluded values was assessed by repeating the analyses with all available patients.

We also performed multiple linear regression analysis with HADS-D as a continuous scale. We used five separate regression models to examine the impact of each individual cardiovascular risk factor (SBP, DBP, total cholesterol, BMI, HbA1c) on HADS-D as a continuous scale after excluding extreme values for each clinical measure as defined above. Quadratic and cubic terms for each clinical measure and other predictor variables, such as age, sex, socio-economic status and number of co-morbid conditions were added to the linear regression model as described above. The turning point or the "nadir" was calculated using the same formula described in the preceding section.

Results

Sample size and characteristics

N = 35,537 (32.5% of total population) patients with one of the three chronic cardiometabolic diseases CHD, previous stroke and diabetes had results of depression screening with HADS-D recorded (see Figure 1). The demographic characteristics and cardiovascular risk factor distribution between the screened and total population were similar (please see "Additional file 1-Additional Analysis"). The HADS-D was positive (>7) for 7080 patients (19.9%). The demographic characteristics of the study sample are described in Table 1.

The distributions of the five cardiovascular risk factors in the study sample such as sample mean, standard deviation, missing values and the observations outside the plausible range considered and observed nadirs are described in Table 2.

Blood pressure, total cholesterol, body mass index and depression

SBP was found to have a "J-shaped" relationship with the probability of having a positive result with HADS-D screening, based on a regression model using all of the screened population with at least one of the three chronic diseases. The nadir or the minimum level of SBP with the

Figure 1 Study sample size and recruitment.
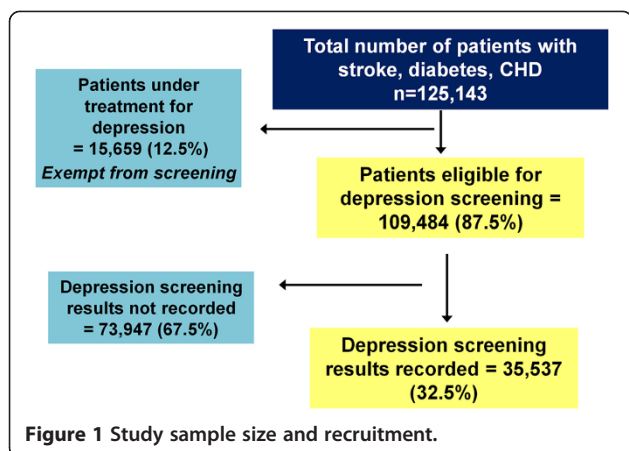
least probability of having a positive screening result with HADS-D was found to be 148 mm Hg (see Figure 2). DBP was found to have a "J-shaped" relationship with the probability of having a positive result with HADS-D screening, based on a regression model using all of the screened population with at least one of the three chronic diseases. However, the shape of the J-curve was shallow for DBP when compared with SBP. The nadir for DBP with the least probability of having a positive screening result with HADS-D was found to be 74 mm Hg. This observed relationship between SBP, DBP and depressive symptoms remained significant after adjusting for age, sex, number of comorbid conditions and socio-economic status.

BMI was found to have a non-linear relationship with the probability of having a positive result with HADS-D screening, based on a regression model using all of the screened population with at least one of the three cardio-metabolic conditions. The nadir or the minimum level for BMI was found to be 30.70 kg/m^2 (see Figure 2). Total Cholesterol was found to have a non-linear relationship

with the probability of having a positive result with HADS-D screening (HADS-D > 7), based on the same regression model described above. However, the shape of the curve was less pronounced with lower values of total cholesterol and wider confidence intervals when compared to SBP and BMI. The nadir or the minimum level for total cholesterol was found to be 3.60 mmol/l (see Figure 2). The relationship between BMI and total cholesterol with probability of having HADS-D positive remained significant after adjusting for age, sex, number of comorbid conditions and socio-economic status.

HbA1C and depression
HbA1c was found to have a non-linear "J-shaped" relationship with the probability of having a positive result with HADS-D screening (HADS-D > 7), based on a regression model using only patients with diabetes (n = 18,453, missing = 2775, excluded = 2). The shape of the curve was more similar but the confidence intervals were slightly wider, when compared to SBP, DBP and BMI. The nadir or the minimum level for HbA1c was found to 7.06% DCCT (54 mmol/mol IFCC) (see Figure 3). This relationship also remained significant after adjusting for age, sex, number of comorbid conditions and socio-economic status.

Supplementary and sensitivity analysis
There were no significant cubic terms for any of the cardiovascular risk factors. There were significant interactions between DBP and sex (p-value = 0.01) and BMI and age (p-value = 0.009) (please see "Additional file 1-Additional Analysis"). Hence, we calculated the nadirs separately for these groups with significant interactions. The nadirs for DBP were 78 mm Hg for males and 63 mm Hg for females respectively. The nadirs for BMI were 32.12 kg/m2 for those aged18-64 years and 29.54 kg/m2 for those 65–90 years respectively. The shape of the curve was unchanged for DBP and BMI after doing sub-group analysis for sex and age respectively (please see "Additional file 1-Additional Analysis"). The results were unchanged after including extremes of clinical values outside the clinically plausible range described above (please see "Additional file 1-Additional Analysis").

The five cardiovascular risk factors had a non-linear relationship in the respective linear regression models, after adjusting for age, sex, socio-economic status and number of co-morbid conditions. The observed nadirs for SBP, DBP, total cholesterol and BMI were 145 mm Hg, 78 mm Hg, 3.41 mmol/l and 30.25 kg/m2 respectively. The observed nadir for HbA1c in patients with diabetes was 6.21 DCCT (44.4 mmol/mol IFCC). The value for HADS-D increased with increase in value of these clinical measures above their respective nadirs but

Table 1 Patient demographics of the study sample

	Demographics	DepChron (n=35,537)
Age group	*Missing*	11
	18-64	11553 (32.52%)
	64-90	23973 (67.48%)
Gender	*Missing*	18
	Male	20658 (58.16%)
	Female	14861 (41.84%)
Deprivation status	*Missing*	732
	Deprived	22726 (65.30%)
	Affluent	12079 (34.70%)
Number of comorbid condition	*Missing*	0
	One	27356 (76.99%)
	Two	7410 (20.85%)
	Three	771 (2.16%)

Table 2 Study sample (n = 35537) distribution for the five clinical risk factors, missing values, the number of extreme observations outside the usually observed clinically plausible values, analyzed data and observed nadirs

Clinical measure (range included)	Mean (SD)	N missing	Exclusions	N analyzed	Observed nadirs
Systolic BP (90–240)	133 (17.54)	3398	n <90 = 110	32029	148 mm Hg
			n >240 = 0		
Diastolic BP (50–130)	74 · 57 (10.32)	3398	n <50 = 165	31972	74 mm Hg
			n >130 = 2		
Body mass index (15–55)	28 · 95 (6.02)	5398	n <15 = 29	30042	30.70 kg/m2
			n >55 = 68		
Total cholesterol (2–10)	4 · 31 (1.05)	4226	n <2 = 50	31244	3.60 mmol/l
			n >10 = 17		
HbA1c (3–18)	7 · 52 (1.68)	2775	n <3 = 2	15676	7.06 DCCT
			n >18 = 0		

Legend: BP = Blood Pressure; n = 18453 for HbA1c.

it increased with decrease in value below these observed nadirs. There were no significant cubic terms. The results of each linear regression are presented in detail in "Additional file 2- Linear Regression with HADS-D as continuous measure".

Discussion

In a large, community based sample of patients with CHD, previous stroke, or diabetes depressive symptoms assessed using depression screening were found to have a nonlinear association with five routine cardiovascular risk factors of disease management. The relationships were 'J-shaped' with high levels of SBP and BMI associated with greater levels of concurrent depressive symptoms, but with the lowest levels also associated with increased prevalence of depressive symptoms. DBP and total Cholesterol had a similar but weaker relationship with depression. In patients with diabetes, a "J-shaped" relationship was again observed between HbA1c levels and depressive symptoms. These associations remained significant after adjusting for demographic factors such

as age, sex, number of comorbid conditions and socio-economic status; including or excluding clinical observations with extreme values and using HADS-D as continuous scale.

Previous evidence studying the relationship between cardiovascular risk factor values and depressive symptoms has mainly come from general population studies. Barrett-Connor et al. reported a non-linear relationship between DBP and depression with a observed nadir of 75 mm Hg DBP for concurrent depressive symptoms in a general population sample [30]. In various cross-sectional studies involving mainly elderly population, depression has been observed to have a non-linear association with SBP [31-34] and DBP [30,35]. Similarly, increased prevalence of depressive symptoms has been observed with extreme values of total cholesterol [36,37] and HbA1c in general population samples [38], in a non-linear trend. There is as yet no published literature that we know of that examines the relationship between cardiovascular risk factors and depressive symptoms in those with cardiometabolic disease.
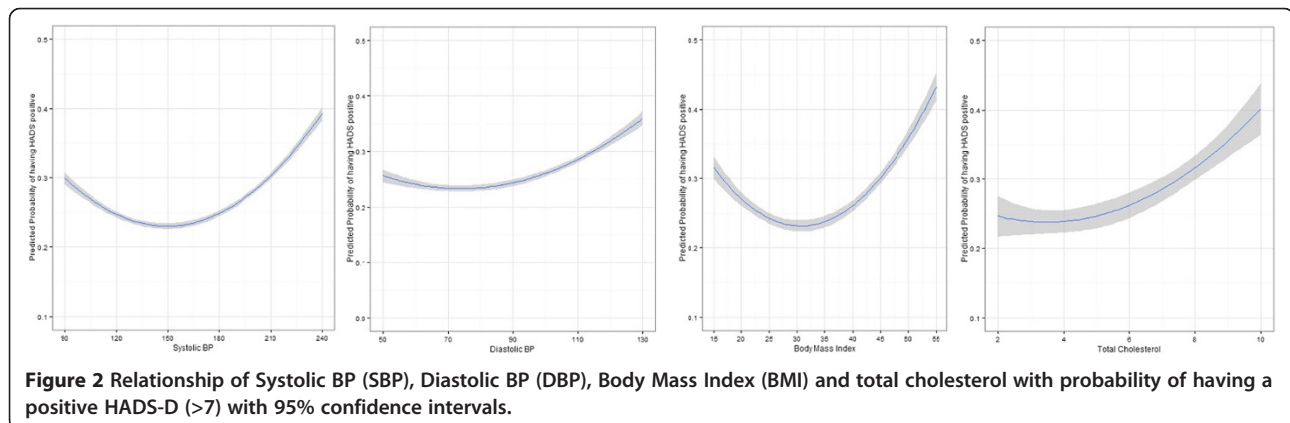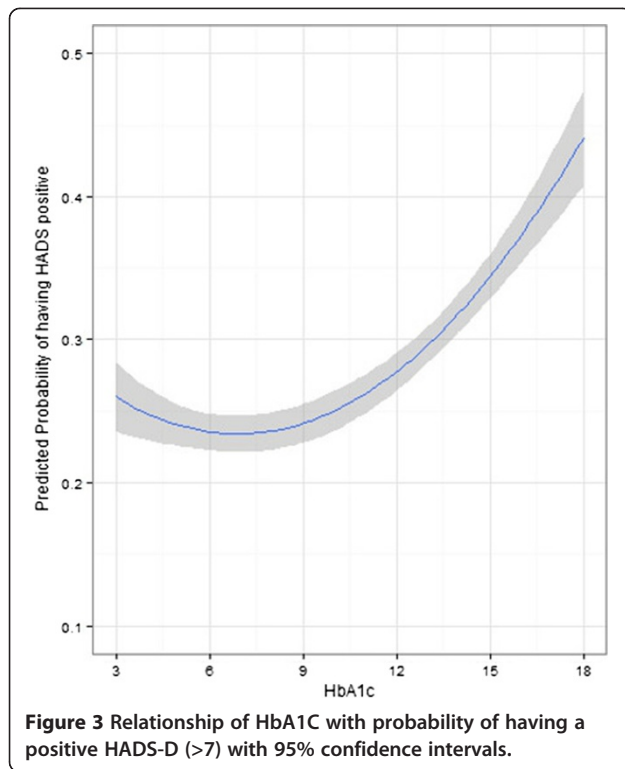

Figure 2 Relationship of Systolic BP (SBP), Diastolic BP (DBP), Body Mass Index (BMI) and total cholesterol with probability of having a positive HADS-D (>7) with 95% confidence intervals.

Figure 3 Relationship of HbA1C with probability of having a positive HADS-D (>7) with 95% confidence intervals.

Non-linear relationship between extreme values of SBP, DBP, BMI and HbA1c and adverse clinical outcomes such as increased incidence of vascular events and deaths in patients with cardiometabolic conditions has been reported extensively [39-43].

There are two potential implications of our findings. Firstly, if the association between extreme values of these risk factors with depressive symptoms in those with cardiometabolic disease is supported by prospective studies, then this relationship could be used to identify those at "high risk" of depression. This would then offer a mechanism for targeting of depression screening in those with cardiometabolic disease. Secondly, these results need to be replicated using other datasets and also prospectively to further explain the nature and direction of the observed association between depressive symptoms and cardiovascular risk factors values. Such further investigation is necessary in order to determine whether the lower cardiovascular risk factors are merely markers of other disease processes (for example, low total cholesterol levels associated with malnutrition, liver diseases and haematological diseases) [44-46] that may make patients more vulnerable to experiencing depressive symptoms or whether it could be attributed to a potential side-effect of aggressive cardiovascular risk factor management [47-50].

This study has a number of key strengths. The data came from a large, community based sample, and importantly reflecting real life clinical practice. There are several limitations. As the study was based on cross-sectional analysis, it is not possible to make causal inferences from the findings of this study. It is therefore unclear whether the observed non-linear association of cardiovascular risk factors with prevalent depressive symptoms is due to cause or effect.

Secondly, we did not have complete information on biobehavioural factors such as smoking status, alcohol intake and levels of physical activity which are likely to influence the values of cardiovascular risk factors considered and also the prevalence of depressive symptoms [51-54].

Since only a minority of the patients were actually screened, depression status was unknown for a large number of patients, which remains an important limitation. There may be important differences between patients with known depression status and those whose depression status was unknown, which are not clearly evident from their baseline demographic data. Practitioners may intuitively screen those patients where they are more likely to get a positive result, for instance patients with multimorbidity. Also, there is a possibility of reverse causality with GPs reviewing a patient whom they consider to have depression and offering screening subsequently. Previously reported barriers to discussing depression (or mental health) in patients with chronic disease in primary care, such as stigma associated around the 'label' and physicians' preconception of normalizing depression in patients with chronic disease, could be influencing factors behind low uptake of depression screening in our study [55,56].

Finally, the overall accuracy of depression screening in our study was reliant on HADS-D which is a self-reported measure and it is not a gold standard measure for assessing depressive symptoms in patients with cardiometabolic disease in a primary care setting [11,57,58]. We also did not have information on history of previous episodes of depression for patients in our study which may influence the prevalence levels for depressive symptoms.

Conclusion

In a general practice sample of patients with CHD, stroke, or diabetes, depressive symptoms were found to have a strong curvilinear association with SBP, BMI, and HbA1c; and a weaker curvilinear association with total cholesterol and DBP. Further investigation of these relationships is urgently needed to clarify the nature of these associations, in order to determine whether they have potentially important implications for clinical practice in relation to either risk stratification for depression or our approach to secondary prevention in individuals with cardiometabolic disease.

Additional files

Additional file 1: "Additional Analysis". Results of sensitivity and supplementary analysi.

Additional file 2: "Linear Regression with HADS-D as continuous measure". Results of statistical analysis with linear regression models using HADS-D as a continuous measure.

Author contributions
BDJ, JC, NS, SB and FSM designed the study and obtained the funding. BDJ, SB and GD analysed the data. All authors interpreted the findings and contributed to the drafting of this paper. FSM is the guarantor and the lead author for this manuscript. All authors read and approved the final manuscript.

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Appendix 3

Relationship Between Blood Pressure Values, Depressive Symptoms, and Cardiovascular Outcomes in Patients With Cardiometabolic Disease

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The authors studied the joint effect of blood pressure (BP) and depression on the risk of major adverse cardiovascular outcome in patients with existing cardiometabolic disease. A cohort of 35,537 patients with coronary heart disease, diabetes, or stroke underwent depression screening and BP measurement recorded concurrently. The authors used Cox's proportional hazards to calculate risk of major adverse cardiovascular event (MACE; myocardial infarction/heart failure/stroke or cardiovascular death) over 4 years associated with baseline BP and depression. A total of 11% (3939) had experienced a MACE within 4 years. Patients with very high systolic BP (160–240 mm Hg; hazard ratio, 1.28) and depression (hazard ratio, 1.22) at baseline had significantly higher adjusted risk. Depression had a significant interaction with systolic BP in risk prediction (*P*=.03). Patients with a combination of high systolic BP and depression at baseline had 83% higher adjusted risk of MACE, as compared with patients with reference systolic BP without depression. Patients with cardiometabolic disease and comorbid depression may benefit from closer monitoring of systolic BP. *J Clin Hypertens (Greenwich).* 2016;1–9. © 2016 The Authors. *The Journal of Clinical Hypertension* Published by Wiley Periodicals, Inc.

Blood pressure (BP) reduction is recommended for all patients diagnosed with hypertension by various guideline bodies, especially for patients with cardiometabolic disease (coronary heart disease [CHD], diabetes, and previous stroke), as it is associated with a reduction in the risk of future adverse cardiovascular (CV) outcomes.[1–4] However, a "J-shaped phenomenon" has also been reported in epidemiological and interventional studies for both systolic BP (SBP) and diastolic BP (DBP), whereby BP lower than 130/80 mm Hg has been associated with higher risk of adverse health outcomes including fatal and nonfatal myocardial infarction (MI) and stroke.[5–8] The optimal level of BP control in patients with existing cardiometabolic disease remains an area of ongoing debate.[9,10] Results from the recently published Systolic Blood Pressure Intervention Trial (SPRINT)[11] suggest that patients with CHD may have a lower risk of CV events with intensive SBP lowering (<120 mm Hg).[11]

Patients with existing cardiometabolic diseases such as CHD, diabetes, and stroke are two to three times more likely to experience depressive symptoms than the general population.[12–14] Moreover, comorbid depression in these patients with cardiometabolic disease is associated with higher risk of subsequent vascular events.[15–17] Depression screening, as a standalone intervention, in these patient groups has not shown any meaningful benefits in reducing CV events,[18,19] and it has been recommended that screening should be followed by further evaluation by a professional qualified in the diagnosis and management of depression.[20] Depression treatment with models such as collaborative care in cardiometabolic disease patient groups has been found to be beneficial in reducing depressive symptoms and improving treatment adherence but not useful in reducing CV events.[21,22]

The relationship between depressive symptoms and BP has been investigated in several cross-sectional epidemiological studies.[23–27] These studies have shown that depression has a nonlinear relationship to SBP and DBP, with greater depressive symptoms at both low and high BP values.[23–27] One longitudinal study concluded that persistent depression leads to lowering of both systolic and diastolic BP.[28]

The mediating mechanism for the observed higher risk of CV events in patients with cardiometabolic disease and comorbid depression remains unclear, with factors such as autonomic dysfunction and chronic inflammation proposed as contributors to a causal pathway.[29] We

hypothesized that patients with depression in cardiometabolic disease with poor BP control may represent a "high-risk" subtype, as the above mechanisms have also been associated with poor BP control.[30,31]

To date, the joint associations of depression and BP with the risk of CV disease has not been studied. In this study, we use data from a large cohort of primary care patients with cardiometabolic disease (CHD/diabetes/stroke), followed up for 4 years, to examine the associations of depression and BP with the risk of subsequent CV events. In doing so, we allow for both nonlinearity of their effects and of interactions between them.

METHODS

Study Design and Setting
The patient sample in this study was recruited from two health boards in the West of Scotland that serve a population of approximately 1.8 million. We received approvals from the National Research Ethics Service and National Services Scotland (NHS) Privacy Advisory Committee and NHS Greater Glasgow and Clyde Enhanced Services data group, which was the authorized "guardian" of this data set. We retrospectively analysed a large routinely collected data set, which was completely anonymous with no patient identifiers, therefore individual patient consent was not obtained.

The local health boards oversaw a program of incentivized depression screening in chronic disease as part of a wider chronic disease management program of Local Enhanced Services (LESs). Family practices in the health boards studied were paid under the LES scheme to carry out a comprehensive annual health assessment, which included depression screening, for all patients with one of the three common cardiometabolic conditions: CHD, diabetes, and stroke. However, there were no penalties for nonadherence. The nurse in the family practice usually carried out the annual health assessment and it lasted for approximately 1 hour. Patients recognized as being "under treatment" for depression at the time of their health assessment were exempt from depression screening. Patients with a positive result on depression screening were offered treatment as per routine care for management of depressive symptoms based on national guidelines.

Participants
The analysis described here was restricted to adults who had a health assessment recorded for at least one of the three conditions between January 4, 2008, to March 31, 2009, and were aged between 18 and 90 years, who underwent depression screening. The "DepChron" data set consisted of a total of 125,143 patients who were in a family practice disease register with a diagnosis of at least one of CHD, diabetes, or stroke in 2008–2009; all of these patients underwent a comprehensive health assessment as part of LES.[32,33] Patients were labeled as

under treatment for depression and exempt from depression screening if they were noted to be on antidepressants based on their prescription record at the time of depression screening.

Measurement of Clinical Variables
The depression subscale of the Hospital Anxiety and Depression Scale (HADS-D)[34] has a range of total score from 0 to 21. A threshold of >7 was used to define the presence of depressive symptoms, as endorsed by national guidelines.[35] The area-based Scottish Index of Multiple Deprivations (SIMD) was used as a measure of socioeconomic status, with patients categorized into deciles of deprivation relative to the Scottish population.[36] Patients who were identified to have depressive symptoms as a result of depression screening were offered "routine care," as recommended for management of depressive symptoms in national guidelines.[35] A new antidepressant prescription for a period up to 6 months after screening was labeled as "new treatment" for the screened patients. We also analyzed antidepressant prescriptions after excluding amitriptyline as it is often used in the management of chronic pain in primary care. No reliable information was available on the number of patients who were referred for psychological therapies following their depression screening.

SBP and DBP measurements and body mass index (BMI) were recorded determined from height and weight measurements. These BP measurements were performed by the primary care practice nurse during routine clinical assessments. As the data were collected during routine clinical practice, information on methods used for recording the BP (manual or digital; single reading or multiple readings) was not available. A blood sample was collected by the practice nurse at the time of assessment, and the result for total cholesterol was reported in mmol/L and glycated hemoglobin (only available for patients with diabetes) was reported in Diabetes Control and Complications Trial units. We restricted the values for CV risk factors to clinically plausible ranges based on both our clinical judgement and the findings of general population studies. SBP measurements were restricted to a range between 90 mm Hg and 240 mm Hg and DBP to a range between 50 mm Hg and 130 mm Hg.[37] Similarly, BMI was restricted to a range between 15 mg/dL and 55 mg/dL,[38] total cholesterol between 2 mg/dL and 10 mg/dL,[39] and glycated hemoglobin between 3% and 18%.[40] Observations in the data, which were outside these ranges, were excluded from the analysis.

Measurement of Outcome Variables
We used electronic data linkage methods to measure the outcome variables for the patient cohort recruited in our study for a follow-up duration of 4 years from April 2009 to March 2013. We electronically linked the health records for patients in primary care registers with

the records held by the Information Services Division Scotland for any occurrence of hospitalization or mortality during the follow-up period. We studied four different clinical outcomes for the patients in our study using the *International Classification of Diseases–10th Revision* (*ICD-10*).[41] The outcomes studied and their respective *ICD-10* codes are as follows:

1 Admission due to MI: I21
2 Admission due to stroke: I61-I64
3 Admission due to heart failure (HF): I50
4 Death due to CV causes: I00-I99.

Major adverse CV outcome (CV mortality or admission due to MI/stroke/HF) was used as the composite outcome variable. Patients were censored if they experienced a composite CV outcome as described above or if they died of reasons other than CV causes.

BP Measurement

There is no consensus among various guidelines published internationally for optimal BP targets in patients with existing cardiometabolic disease.[1–4] We classified BP into five different categories based on clinical judgement to improve interpretability of results. SBP was classified into five categories: very high (160–240 mm Hg), high (140–159 mm Hg), reference (130–139 mm Hg), tightly controlled (120–129 mm Hg), and low (80–119 mm Hg). DBP was also classified into: very high (100–130 mm Hg), high (90–99 mm Hg), reference (85–89 mm Hg), tightly controlled (80–84 mm Hg), and low (40–79 mm Hg). SBP and DBP were also added as continuous variables in the regression models as described below.

Statistical Analysis

We used time-to-event analysis to study the association between three predictors: (1) SBP categories, (2) DBP categories, and (3) presence of depressive symptoms, and the risk of major adverse CV outcome in the study population. Cox's proportional hazards regression analysis was performed, unadjusted and adjusted for potential confounding factors, and the results are presented in terms of hazard ratios (HRs) and 95% confidence intervals (CIs). We performed multivariable analysis adjusting for the following confounders: age (continuous), sex (male and female), socioeconomic status (deprived: SIMD deciles 1–5 vs affluent: SIMD deciles 6–10), initiation of antidepressants (yes/no), number of cardiometabolic comorbidities (range 1–3, representing a combination of one or more of the three cardiometabolic diseases under investigations: CHD, stroke, or diabetes), BMI (normal: 18.5–25 mg/dL, underweight 15–18.5 mg/dL, overweight 25–30 mg/dL, obese 30–50 mg/dL[42]), and total cholesterol levels (not raised vs raised: >5 mmol/L[4]).

To understand the relationship between BP and depressive symptoms in risk prediction of major adverse CV outcome, if any, we carried out an analysis of variance test to check for interaction between the BP categories and presence of depressive symptoms. In the event of a significant interaction, we also carried out a subgroup analysis to further study the nature of interaction. In the subgroup analysis, the study sample was divided on the basis of BP categories as described above. In each subgroup, a Cox's proportional hazards regression analysis was performed to study the risk of outcome with the presence of depressive symptoms at baseline, adjusting for potential confounders. Analysis was carried out using the R statistical software, version 3.0.2 (The R Project for Statistical Computing).

Sensitivity Analysis

We performed four different sensitivity analyses in the following patients: those with diabetes, those with affluent socioeconomic class, those with estimated glomerular filtration rate results available at baseline, and those with smoking and alcohol consumption results available.

RESULTS

Patient Population and Clinical Outcomes

A total of 125,143 patients with at least one of the following underwent a comprehensive health assessment in 2008–2009: diabetes (62,275 patients), CHD (62,990 patients), or previous stroke (26,060 patients). A total of 10,670 (8.5%) patients were exempt from depression screening as they were noted to be under treatment for depression and excluded from analysis, while the remaining 114,473 (91.5% of total sample size) were eligible for depression screening. The uptake of depression screening was low and HADS-D was recorded in 35,537 (31.1% of those eligible) of those undergoing the annual health assessment (Figure 1), and it is the data from this subset that we focus on in this paper. A total of 7080 of 35,537 patients had positive HADS-D results (>7) at baseline. Electronic data linkage between primary care disease registers and hospital discharge and mortality records, based on Community Health Index number was successful for 99.4% of patients.

Among the patients who were screened (n=35,537), 12,485 (35.1%) had diabetes only, 11,716 (32.9%) had CHD only, 3558 (10%) had previous stroke only, 7410 (20.8%) had two of these conditions, and 771 (2.1%) had all of the three conditions. Table I compares the demographic features, observed BP values, and the absolute number of adverse CV outcomes for the screened and unscreened population. In the study population of patients with depression screening results (n=35,537), 11% (3939) experienced at least one major adverse cardiovascular event (MACE) within 4 years. In the study population, the observed mean SBP at baseline was 133 mm Hg in the reference SBP group (130–139 mm Hg), 123 mm Hg in the tightly controlled SBP group (120–129 mm Hg), 109 mm Hg in the low SBP group (80–119 mm Hg), 145 mm Hg in the high SBP group (140–159 mm Hg), and 170 mm Hg in the very high SBP group (160–240 mm Hg).
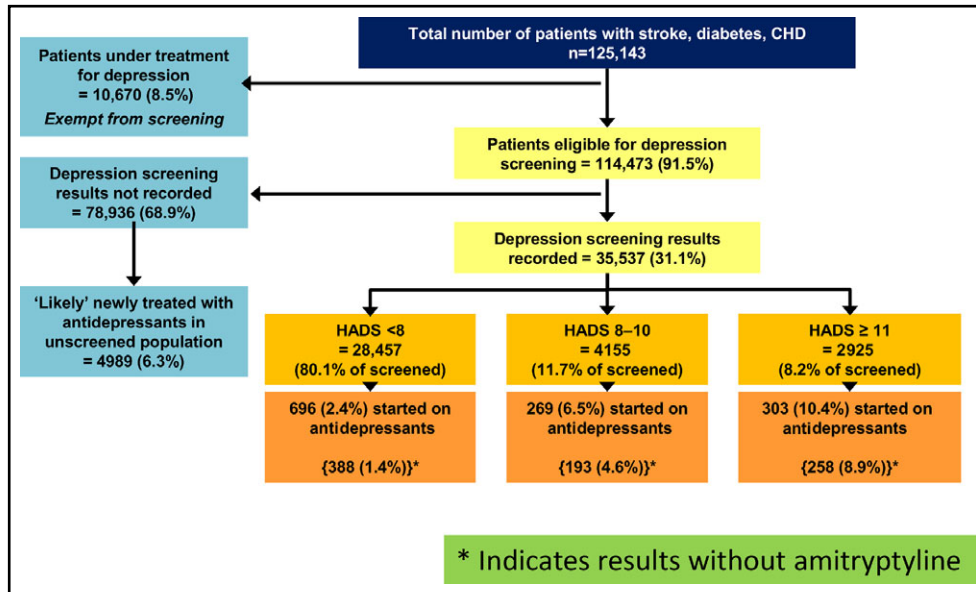
FIGURE 1. Study sample size and recruitment.[33] CHD indicates coronary heart disease; HADS, Hospital Anxiety and Depression Score.

BP, Depressive Symptoms, and Risk of a MACE at 4 Years

In the adjusted multivariable analyses for SBP categories, patients with very high SBP (160–240 mm Hg) and low SBP (80–119 mm Hg) at baseline had a significantly higher risk of a MACE at 4 years compared with patients with reference SBP (130–139 mm Hg) at baseline (Table II). There was no statistical difference in the risk between patients with reference SBP, tightly controlled SBP (120–129 mm Hg), and high SBP (140–159 mm Hg) at baseline. The adjusted risk was 15% higher for patients with low SBP and 28% higher for patients with very high SBP compared with patients with reference SBP at baseline. The presence of depressive symptoms (HADS-D >7) at baseline was associated with a 22% higher adjusted risk of a major CV event compared with those without depressive symptoms (Table II). Figure 2 shows that patients with high SBP had a significantly higher cumulative incidence rate compared with patients in the other SBP categories; similarly, patients with depressive symptoms had a higher cumulative incidence than those without depression.

In the adjusted analysis for DBP categories, none of the DBP categories at baseline had any statistically significant difference in the risk prediction of MACE, compared with the reference (Table II). The results were adjusted for age, sex, socioeconomic status, number of CV comorbidities, BMI, and total cholesterol values at baseline and initiation of antidepressants within 6 months of depression screening. Interestingly, initiation of antidepressants after depression screening did not have any significant impact on the risk of MACE (HR, 0.84; 95% CI, 0.68–1.04; *P*=.11). SBP as a continuous variable was found to have a significant nonlinear relationship with risk prediction of a MACE while DBP was not a significant predictor as a continuous variable in the multivariable analysis (results not shown). We also repeated the analysis by adjusting for different disease categories and combinations among the three cardiometabolic diseases, and there was no difference in the results (not shown).

Interaction Between SBP and Depressive Symptoms in Risk Prediction of Major CV Event at 4 Years

In the risk prediction of major CV event, the interaction between SBP categories and presence of depressive symptoms was statistically significant (*P*=.03). Patients with low SBP (80–119 mm Hg) only, with very high SBP (160–240 mm Hg) only, and depressive symptoms only at baseline had 10%, 19%, and 17% adjusted higher risks of a major CV event, respectively, compared with those without extremes of SBP and no depressive symptoms (Figure 3). In comparison, patients with both low SBP and depressive symptoms at baseline had a 36% (95% CI, 15%–62%) higher risk while patients with both very high SBP and depressive symptoms had the highest increased risk of 83% (95% CI, 46%–130%) compared with those in the reference SBP group without depressive symptoms.

In the multivariable subgroup analysis of five SBP categories, a nonlinear trend was observed in the association between presence of depressive symptoms and risk prediction of a major CV event at 4 years (Table III). There was no evidence of an association between the presence of depressive symptoms and adjusted risk of major adverse event for the subgroup of patients with reference SBP (130–139 mm Hg) at baseline. Presence of depressive symptoms was associated with significantly higher risk of a major CV adverse

TABLE I. Comparison of the Depression Screened and Unscreened Patient Groups in Existing Cardiometabolic Disease

	Depression Screened (n=35,537)	Depression Unscreened (n=78,936)	P Value
Age, mean (SD), y	69.0 (11.9)	67.0 (14.3)	<.001
White ethnicity, No. (%)	30,693 (92.4)	53,343 (90.3)	<.001
Male sex, No. (%)	20,658 (58.2)	42,727 (54.2)	<.001
Deprived socioeconomic status SIMD deciles ≤5, No. (%)	22,726 (65.3)	51,686 (67.4)	<.001
Number of cardiometabolic conditions, No. (%)			
One	27,356 (77.0)	65,417 (82.9)	<.001
Two	7410 (20.9)	12,265 (15.5)	
Three	771 (2.2)	1254 (1.6)	
Antidepressant initiation, No. (%)	1268 (3.5)	4989 (6.3)	<.001
Systolic blood pressure, No. (%)			
130–139 mm Hg (reference)	8389 (23.6)	13,315 (16.8)	<.001
120–129 mm Hg (tightly controlled)	6864 (19.3)	10,818 (13.7)	
80–119 mm Hg (low)	5711 (16.0)	9258 (11.7)	
140–159 mm Hg (high)	8624 (24.2)	15,969 (20.2)	
160–240 mm Hg (very high)	2514 (7.0)	5778 (7.3)	
Not available	3435 (9.6)	23,802 (30.1)	
Diastolic blood pressure, No. (%)			
85–89 mm Hg (reference)	1909 (5.3)	4219 (5.3)	<.001
80–84 mm Hg (tightly controlled)	7070 (19.8)	13,088 (16.5)	
40–79 mm Hg (low)	20,585 (57.9)	31,724 (40.1)	
90–99 mm Hg (high)	1981 (5.5)	4450 (5.6)	
100–130 mm Hg (very high)	562 (1.5)	1649 (2.0)	
Not available	3430 (9.6)	23,806 (30.1)	
Major adverse cardiovascular outcome (cardiovascular death or admission due to MI/stroke/heart failure), No. (%)	3939 (11)	10,990 (13.9)	<.001

Abbreviations: MI, myocardial infarction; SD, standard deviation; SIMD, Scottish Index of Multiple Deprivation.

TABLE II. SBP, DBP, and Presence of Depressive Symptoms (HADS-D >7) at Baseline and Risk Prediction of Major Adverse Cardiovascular Event (Cardiovascular Death or Admission Due to MI/Stroke/HF) in 35,537 Patients With Previous Stroke, Coronary Heart Disease, or Diabetes at 4 Years of Follow-Up

Predictors at Baseline	Major Adverse Cardiovascular Event	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[a]
SBP, mm Hg		
80–119	**1.15 (1.04–1.27)**	**1.15 (1.03–1.29)**
120–129	0.99 (0.90–1.09)	1.00 (0.90–1.12)
130–139	1	1
140–159	**1.10 (1.00–1.20)**	1.07 (0.96–1.18)
160–240	**1.43 (1.26–1.61)**	**1.28 (1.11–1.49)**
DBP, mm Hg		
40–79	**1.24 (1.07–1.44)**	0.94 (0.78–1.12)
80–84	1.05 (0.89–1.23)	0.93 (0.77–1.12)
85–89	1	1
90–99	0.83 (0.67–1.02)	0.90 (0.71–1.14)
100–130	1.03 (0.77–1.39)	1.15 (0.82–1.62)
HADS-D >7	**1.23 (1.14–1.32)**	**1.22 (1.11–1.33)**
Reference=HADS-D ≤7		

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; HADS-D, Hospital Anxiety and Depression Score-depression subscale; HF, heart failure; HR, hazard ratio; MI, myocardial infarction; SBP, systolic blood pressure.

[a]Adjusted for confounders including body mass index, total cholesterol, age, sex, socioeconomic status, number of comorbid conditions, and initiation of antidepressants. Significant results are indicated in bold.

event for patients in all other baseline SBP categories. Patients with very high SBP and concurrent depressive symptoms had the highest absolute event rate of 17.7%; moreover, the change in the adjusted risk with addition of depressive symptoms was highest for the subgroup of patients with very high SBP at 55% (Table III).

Sensitivity Analysis

In all four sensitivity analyses, the subgroup of patients with very high SBP and prevalent depressive symptoms were observed to have the highest absolute event rate of CV outcomes. In addition, in SBP subgroup analysis,

presence of depressive symptoms (against no depressive symptoms) was observed to have the highest adjusted effects size for MACE in the very high SBP subgroup for all four sensitivity analyses. These results are presented in detail in the supplementary information.

DISCUSSION

Summary of Findings

Patients with existing cardiometabolic disease and those with very high and low SBP at baseline were observed to have a significantly higher adjusted risk of a MACE than those with SBP in the reference range. Presence of depressive symptoms at baseline was also associated with a significantly higher risk of a MACE, while DBP was not a significant predictor in the multivariable analysis in either pooled or subgroup analysis. Presence of depressive symptoms compounded the risk of a MACE in patients with very high SBP.

Strengths and Limitations

This study has a number of key strengths, in that the data came from a large, community-based sample reflecting real-life clinical practice, and electronic data
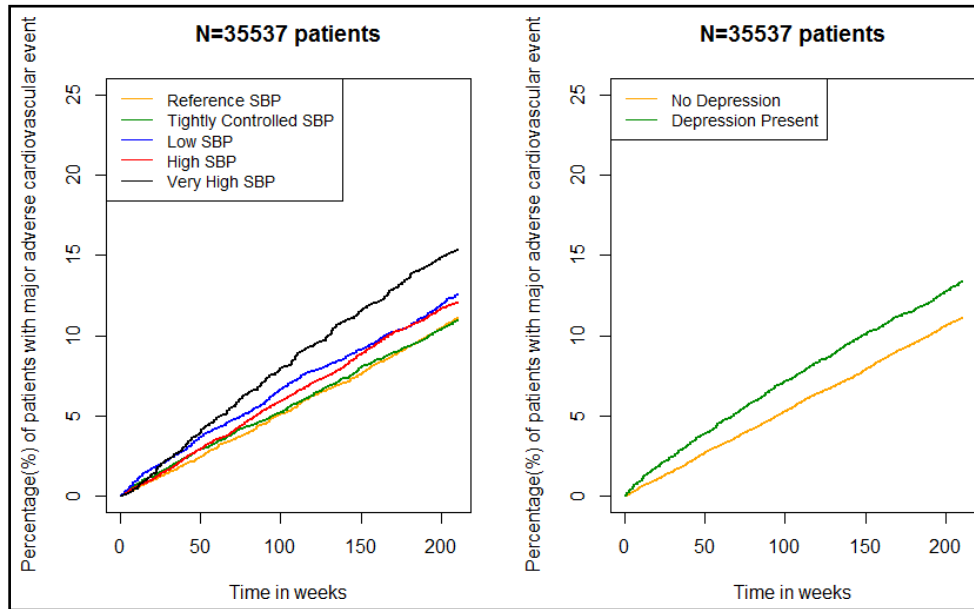
FIGURE 2. Kaplan-Meier plots comparing unadjusted cumulative event rates for major adverse cardiovascular outcome based on systolic blood pressure (SBP) values and presence of depressive symptoms at baseline in patients with existing cardiometabolic disease. A total of 35,537 patients with previous stroke, coronary heart disease, or diabetes. Major adverse cardiovascular event=cardiovascular death or admission due to myocardial infarction/stroke/heart failure. Reference SBP=130–139 mm Hg, tightly controlled SBP=120–129 mm Hg, low SBP=80–119 mm Hg, high SBP=140–159 mm Hg, very high SBP=160–240 mm Hg. Depressive symptoms (defined as Hospital Anxiety and Depression Scale-depression subscale >7) at baseline.
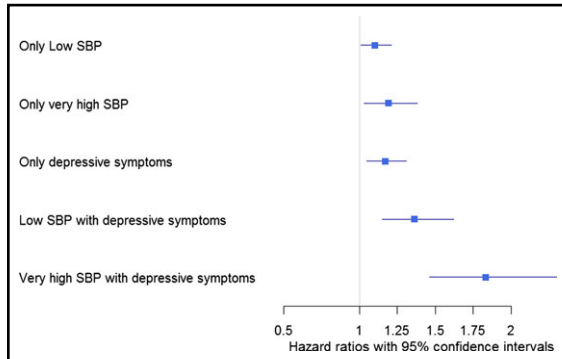
FIGURE 3. Forest plot showing interaction between depressive symptoms and extremes of systolic blood pressure (SBP) at baseline with the risk of major adverse cardiovascular event at 4 years in patients with existing cardiometabolic disease. A total of 35,537 patients with previous stroke, coronary heart disease, or diabetes. A forest plot for comparing cumulative hazard for major adverse cardiovascular event (cardiovascular death or admission due to myocardial infarction/stroke/heart failure) for patients with very high (160–240 mm Hg) and low (80–119 mm Hg) SBP and depressive symptoms (defined as Hospital Anxiety and Depression Scale-depression subscale >7) at baseline.

linkage enabled successful follow-up for the majority of patients in the cohort. There are several limitations. Only a minority of the patients in the sample had depression screening recorded despite incentivization. Consequently, there may be important differences between patients with known depression status and those whose depression status was unknown that were not recorded in our data. In addition, we did not have information on depression score or CV events for the cohort of patients noted to be "under treatment" for depression at the time of depression screening, which is an important limitation.

The observed association between low SBP at baseline and higher risk of major adverse CV outcomes could be caused by reverse causality, where patients with low SBP could be those with the most severe form of disease. There was no available information on disease severity for the study participants, which is an important limitation. Morevoer, we had insufficient information on biobehavioral factors such as smoking status, alcohol consumption, and levels of physical activity and hence we were unable to adjust the main results for these factors. Biobehavioral factors are likely to influence the prevalence of depressive symptoms in cardiometabolic disease patients and, in turn, may affect the ability to make positive health-related behavior changes and influence outcomes.[43–45] Information on cardiac-related medications was not available for these patients. However, these patients had existing cardiometabolic disease and were attending their primary care providers for annual health assessment. Hence, the majority of them were likely to be taking at least one medication that could lower BP. In addition, we had information only on initiation of antidepressants but did not have information either on duration of antidepressants or on the different class of antidepressants chosen.

TABLE III. Presence of Depressive Symptoms and the Risk of Major Adverse Cardiovascular Events (Cardiovascular Death or Admission for MI/Stroke/HF) for 35,537 Patients With Previous Stroke, Coronary Heart Disease, or Diabetes at 4 Years of Follow-Up Based on SBP at Baseline

	SBP Categories				
	Low, 80–119 mm Hg (n=5711)	Tightly Controlled, 120–129 mm Hg (n=6864)	Reference, 130–139 mm Hg (n=8389)	High, 140–159 mm Hg (n=8624)	Very High, 160–240 mm Hg (n=2514)
Not depressed event rate, No. (%)	492/4376 (11.2)	555/5464 (10.1)	730/6806 (10.7)	776/7028 (11.0)	278/2001 (13.8)
Depressed event rate, No. (%)	192/1335 (14.3)	171/1400 (12.2)	161/1583 (10.1)	226/1596 (14.1)	91/513 (17.7)
Depressed vs not depressed (*adjusted), HR (95% CI)	**1.23 (1.01–1.49)**	**1.34 (1.10–1.63)**	0.94 (0.77–1.14)	**1.29 (1.08–1.53)**	**1.55 (1.18–2.03)**
P value	<.001	<.001	.54	<.001	<.001

Abbreviations: CI, confidence interval; HADS-D, Hospital Anxiety and Depression Score-depressive subscale; HF, heart failure; HR, hazard ratio; MI, myocardial infarction; SBP, systolic blood pressure.

Adjusted for confounders including body mass index, total cholesterol, age, sex, socioeconomic status, number of comorbid conditions, and initiation of antidepressants. Significant results are indicated in bold.

Moreover, there are other adverse clinical outcomes of significance in this group of patients such as renal failure, angina pectoris, and retinopathy.[8,46] We did not have information available for these outcomes in our data. Finally, the overall accuracy of depression screening in our study was reliant on HADS-D, which is a self-reported measure and has accuracy-related drawbacks when used for assessing depressive symptoms in patients with cardiometabolic disease in a primary care setting.[18,47]

Comparison With Existing Literature
The association between presence of depressive symptoms and higher risk of adverse clinical outcomes in patients with preexisting cardiometabolic disease has been previously reported in the literature.[15–17] SBP at baseline was found to have a nonlinear relationship in our study with the risk prediction of a MACE, which has also been reported in various other studies.[5,6,8] Our study findings are contrary to the results observed in the SPRINT trial, but there are important differences such as the study design and setting. In addition, the SPRINT trial excluded patients with diabetes and previous stroke, who were included in our study.[11] With regards to DBP, our study found that DBP at baseline was not a significant predictor of adverse CV outcomes, and there are studies that have reported similar findings of better predictive power of SBP over DBP in predicting CV outcomes.[48,49]

This is the first study, to our knowledge, to investigate the interacting relationship between depressive symptoms and BP in risk prediction of adverse clinical outcomes in patients with preexisting cardiometabolic disease.

CLINICAL IMPLICATIONS
Firstly, there may be potential benefits of depression screening for patients with cardiometabolic disease who have extremes of SBP. Secondly, cardiometabolic disease patients who are diagnosed with comorbid depressive symptoms may benefit from closer monitoring of their SBP for secondary prevention of CV events. Further research is needed in this area before making any clinical recommendations.

CONCLUSIONS
SBP and depressive symptoms at baseline were independent predictors of a MACE at 4 years in patients with existing cardiometabolic disease, while DBP at baseline did not have a significant effect. Presence of depressive symptoms compounded the risk of a MACE in SBP categories both higher and lower than the reference SBP, especially in patients with very high SBP. There may be potential benefits from closer monitoring (over and above routine care) of BP in patients with cardiometabolic disease and comorbid depression. Further research is needed to understand the relationship between extremes of BP and depressive symptoms in patients with existing cardiometabolic disease and the underpinning biological mechanisms.

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Supporting Information

Additional supporting information may be found online in the supporting information tab for this article.

Data S1. Sensitivity Analysis for Patients With Diabetes, Affluent Socio-economic Class, for Patients With Results of Estimated Glomerular Filtration Rate Available and for Patients With Results of Smoking and Alcohol Consumption Available at Baseline

Table S1. Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Presence of Depressive Symptoms (HADS-D >7) at Baseline and Risk Prediction of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/New HF

Diagnosis or HF Admission) in a Subset of Patients (n=18,453) With Diabetes at 4 Years of Follow-Up

Table S2. Presence of Depressive Symptoms and the Risk of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/Heart Failure) in a Subset of Patients (n=18,453) With Diabetes at 4 Years of Follow-Up Based on Systolic Blood Pressure at Baseline

Table S3. Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Presence of Depressive Symptoms (HADS-D >7) at Baseline and Risk Prediction of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/New HF Diagnosis or HF Admission) in a Subset of Patients (n=12,079) With Affluent Socioeconomic Status at 4 Years of Follow-Up

Table S4. Presence of Depressive Symptoms and the Risk of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/Heart Failure) in a Subset of Patients (n=12,079) With Affluent Socioeconomic Status at 4 Years of Follow-Up Based on Systolic Blood Pressure at Baseline

Table S5. Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Presence of Depressive Symptoms (HADS-D >7) at Baseline and Risk Predic-

tion of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/New HF Diagnosis or HF Admission) in a Subset of Patients With Available eGFR Results (n=15,982) at 4 Years of Follow-Up

Table S6. Presence of Depressive Symptoms and the Risk of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/Heart Failure) in a Subset of Patients With Available eGFR (n=15,982) at 4 Years of Follow-Up Based on Systolic Blood Pressure at Baseline

Table S7. Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and Presence of Depressive Symptoms (HADS-D >7) at Baseline and Risk Prediction of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/New HF Diagnosis or HF Admission) in Subset of Patients With Smoking and Alcohol Consumption Available (n=13,676) at 4 Years of Follow-Up

Table S8. Presence of Depressive Symptoms and the Risk of Major Adverse Cardiovascular Event (Cardiovascular Death/Stroke/Myocardial Infarction/Heart Failure) in a Subset of Patients With Smoking and Alcohol Consumption Available (n=13,676) at 4 Years of Follow-Up Based on Systolic Blood Pressure at Baseline

Appendix 4

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NHS
Greater Glasgow
and Clyde

Date   21st March 2013
Your Ref
Our Ref

Enquiries to
Direct line   0141 201 4541
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Dr Bhautesh Jani
CSO Clinical Academic Fellow
University of Glasgow
1 Horselethill Road
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G12 9LX

Dear Dr Jani,

Record linkage proposal using NHS Greater Glasgow & Clyde Local Enhanced Services data

I am pleased to confirm that your proposal to request NHS ISD Scotland to undertake individual patient level record linkage between our Local Enhanced Services chronic disease management dataset and SMR 01 data, has received prior approval by our local Keep Well/GMS Enhanced Services Data Group. As you are aware, this group oversees information governance with regard to safe use of GMS data within the terms of a data sharing agreement with practices and the LMC.

I note that you also wish to undertake record linkage with mortality data, however, I should make you aware that mortality data are actually held by National Records of Scotland (NRS), although it is possible that ISD Scotland may receive a regular download from NRS (NHSGGC does, so I imagine they will do likewise). If this is not the case, we can help with that aspect locally.

I trust that this information contains all that you require. Please do not hesitate to contact me if I can provide any further detail or clarification.

Yours sincerely,

Anne Scoular

Anne Scoular
Consultant in Public Health Medicine

cc. Professor Frances Mair

NRES Committee London - Hampstead

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24 October 2012

Dr Bhautesh Jani
SCREDS Clinical Lecturer in General Practice
University of Glasgow
General Practice and Primary Care
1 Horsetlethill Road
Glasgow   G12 9LX

Dear Dr Jani

Study title:	**Exploring the potential role of allostatic load bio-markers in risk assessment of patients presenting with depressive symptoms. Short Title: Allostatic LOad Use in Depression-ALOUD study**
REC reference:	**12/LO/1622**

The Proportionate Review Sub-committee of the NRES Committee London - Hampstead reviewed the above application in correspondence.

Ethical opinion

- **It was asked whether the researchers had contacted the NHS Scotland Privacy Advisory Committee.**

It was confirmed that the NHS Scotland Privacy Advisory Committee (PAC) was contacted and their advise was to get REC approval prior to making an application to PAC committee. It was also confirmed that they would be seeking PAC approval prior to carrying out data linkage once we have had the REC approval.

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The documents reviewed and approved were:

Document	Version	Date
Covering Letter	from Dr Bhautesh Jani	
Investigator CV	for Dr Bhautesh Jani	
Other: Summary CV of Dr Jonathan Cavanagh - Supervisor		
Other: Summary CV for Professor Frances Mair - Supervisor		
Other: Summary CV for Professor Naveed Sattar - Supervisor		
Other: CSO Award letter from Dr Elaine Moir		31 July 2012
Other: Peer review from Karen Ford		19 June 2012
Other: Letter from Funder - BUPAFoundation		07 July 2011
Other: Summary CV for Dr Sarah Barry - Supervisor		
Protocol	1	29 June 2012
REC application		10 September 2012
Referees or other scientific critique report	from Professor Frances Mair	03 April 2012

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.
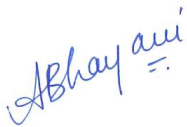
Further information is available at National Research Ethics Service website > After Review

| 12/LO/1622 | Please quote this number on all correspondence |

With the Committee's best wishes for the success of this project

Yours sincerely
PP

[signature]

Miss Stephanie Ellis
Chair

Email: NRESCommittee.London-Hampstead@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

                        "After ethical review – guidance for researchers" [SL-AR2]

Copy to:

Debra Stuart
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The Tennent Institute
38 Church Street
Glasgow G11 6NT

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England

NRES Committee London - Hampstead

Attendance at PRS Sub-Committee of the REC meeting in correspondence

Committee Members:

Name	Profession	Present	Notes
Miss Stephanie Ellis **– Chair**	Former Civil Servant	Yes	
Dr Andrew Hilson **– Alternate Vice Chair**	Consultant in Nuclear Medicine	Yes	
Mrs Wendy Spicer	Pharmacist	Yes	

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University of Glasgow
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GLASGOW
G12 9LX

Date	24 May 2013
Your Ref	
Our Ref	PAC 85/12

Enquiries to	Janet Murray	
Direct Line	0131 275 6954	
Email	janet.murray1@nhs.net	

Dear Dr Jani

Re: Exploring the Potential Role of Allostatic Load Biomakers in Risk Assessment of Patients presenting with Depressive Symptoms "Aloud" Study - DepChron dataset

The Privacy Advisory Committee has considered and approved your application for a data linkage in support of the above study.

Conditions applied: None

Time period: As specified

Points highlighted: None

The approval of the Committee is for a period of 5 years from the date of this letter.   Any change to the terms of your application, including changes in data user(s), additional data fields or extension of the time period approved must be requested through Susan Kerr, PAC Administrator on 0131 275 6445 or nss.pac@nhs.net

In order to progress your request please contact the eDRIS team on telephone 0131 275 7333 or email nss.eDRIS@nhs.net.

Please note that the following details about your application will be published under the following headings on the PAC website at http://www.nhsnss.org/pages/corporate/pac_meetings_and_decision_making.php later this year:

No	Title	Type	Summary	Date sent to PAC	PAC Responses	NSS Decision	Date Completed

If you have any queries about this please contact Patricia Ruddy patricia.ruddy@nhs.net.

Kind regards.

Yours sincerely

Dr Janet Murray
Consultant in Public Health Medicine

cc eDRIS

Appendix 5

Area 151A
Gyle Square
1 South Gyle Crescent
Edinburgh, EH12 9EB
Telephone 0131 275 6000
Fax 0131 275 7606
www.isdscotland.org

NHS

**National
Services
Scotland**

Dr B Jani
Clinical Academic Fellow
College of Medical, Veterinary and Life Sciences
University of Glasgow
1 Horselethill
GLASGOW
G12 9LX

Date	24 May 2013
Your Ref	
Our Ref	PAC 86/12

Enquiries to	Janet Murray
Direct Line	0131 275 6954
Email	janet.murray1@nhs.net

Dear Dr Jani

Re: Exploring the Potential Role of Allostatic Load Biomakers in Risk Assessment of Patients presenting with Depressive Symptoms "Aloud" Study - Psobid Dataset

The Privacy Advisory Committee has considered and approved your application for a data linkage in support of the above study.

Conditions applied: None

Time period: As specified

Points highlighted: None

The approval of the Committee is for a period of 5 years from the date of this letter.   Any change to the terms of your application, including changes in data user(s), additional data fields or extension of the time period approved must be requested through Susan Kerr, PAC Administrator on 0131 275 6445 or nss.pac@nhs.net

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Kind regards.

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Dr Janet Murray
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cc eDRIS

Interim Chair   Professor Elizabeth Ireland
Chief Executive   Ian Crichton
Director   Susan Burney
NHS National Services Scotland is the common name of the Common Services Agency for the Scottish Health Service.

9 June 2005

Glasgow Royal Infirmary LREC (2)
4th floor, Walton Building
Glasgow Royal Infirmary
84 Castle Street
GLASGOW
G4 0SF

Telephone: 0141 211 4020
Facsimile: 0141 232 0752

Professor Chris Packard
Consultant Clinical Scientist
Professor of Vascular Biochemistry
Dept. Vascular Biochemistry
4th Floor University Block
Glasgow Royal Infirmary
Glasgow G31 2ER

Dear Professor Packard

Full title of study: **Factors determining physical and mental well being in affluent and deprived communities in Glasgow (Psychological and Biological Determinants of Disease, pSoBid1).**
REC reference: **05/S0705/40**

Thank you for your letter of 7 June 2005, responding to the Committee's request for further information on the above research and submitting revised documentation. The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

The Committee has designated this study as having "no local investigators". There is no requirement for other Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application	2	07 June 2005
Investigator CV	Dec 2004	(None Specified)
Protocol	1	05 May 2005
Covering Letter		04 May 2005
Summary/Synopsis - flow chart, sampling, demographic data questionnaire	1	05 May 2005

Peer Review	Various	(None Specified)
Personal demographics and lifestyle Questionnaire	1	05 May 2005
Questionnaire at the end of each visit	1	05 May 2005
First Letter of Invitation to Participants	2	06 June 2005
Second Letter of Invitation to Participants	2	05 June 2005
First letter to GP	2	06 June 2005
Letter to GP at the end of the study	2	06 June 2005
Participant Information Sheet	2	06 June 2005
Participant Consent Form	2	06 June 2005
Participant Consent Form - personal copy	2	(None Specified)
Response to Request for Further Information		07 June 2005
Reply form to letters of invitation	2	05 June 2005

Management approval

You should arrange for all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter.

All researchers and research collaborators who will be participating in the research must obtain management approval from the relevant care organisation before commencing any research procedures. Where a substantive contract is not held with the care organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/S0705/40	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project,

Yours sincerely

Dr Malcolm Booth
Chair

Email: emma.stoica@northglasgow.scot.nhs.uk

Enclosures: Standard approval conditions

Glasgow Royal Infirmary LREC (2)

Attendance at Committee meeting on 20 May 2005

Dr Malcolm Booth *(in the Chair)*
Consultant

Miss Fiona Mackelvie
Lay member

Mr Julian May
Lay member

Mr Angus McFadyen
Statistician

Mr Colin McKay
Consultant

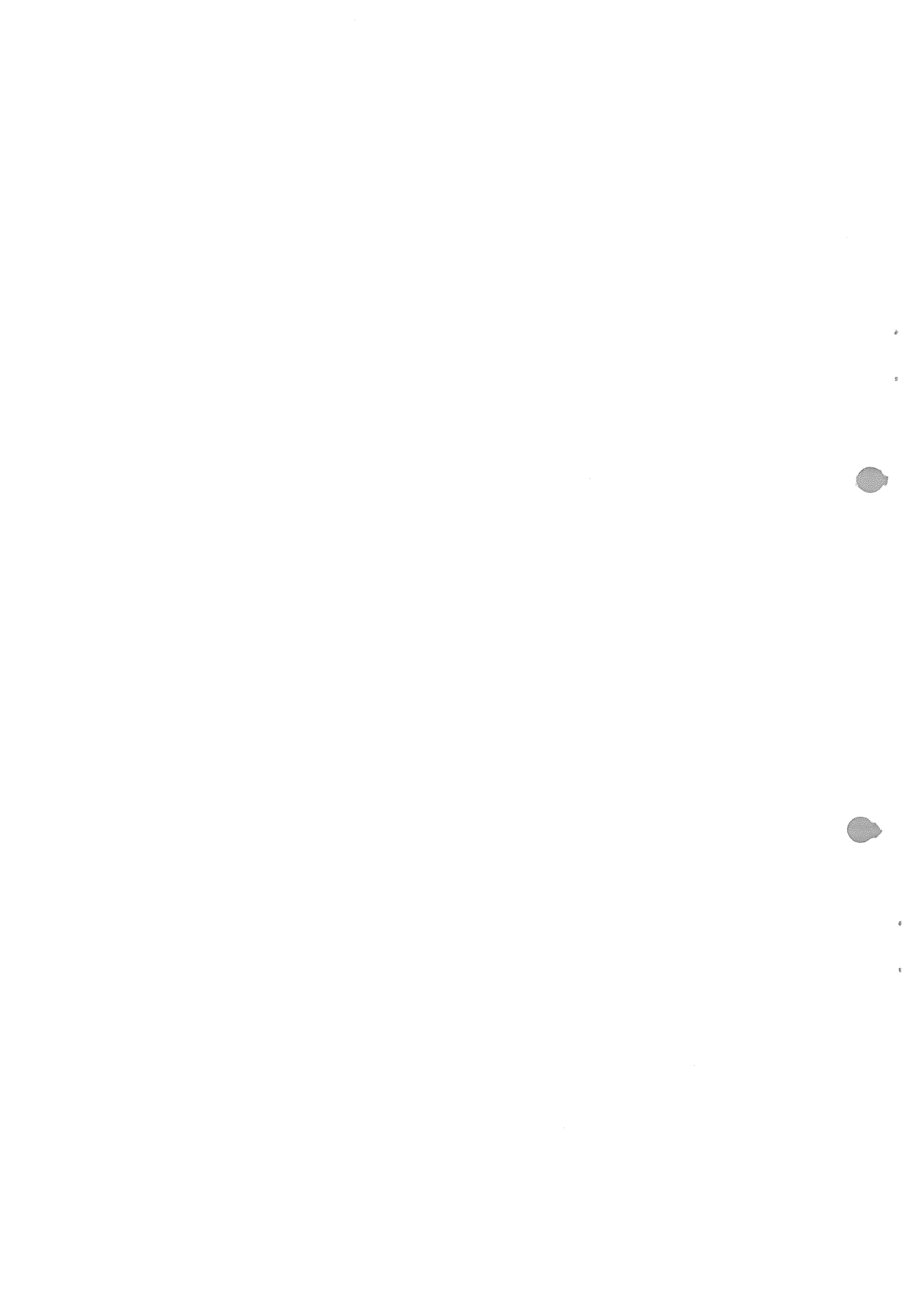**Mrs Fiona McMillan**
Pharmacist

Dr Anne Parker
Consultant

Mr A S Weatherhead
Lay member

Mr Michael Bromby
Lay member

Appendix 6

AMSTAR Score for quality appraisal of systematic reviews included in Chapter 3

In Chapter 3, the background chapter on depression in cardiometabolic disease, there were 3 tables (table 3.1, table 3.2 & table 3.3) describing the key characteristics and findings of 15 systematic review/meta-analysis on various topics. The performance of these studies against the individual items of the AMSTAR quality assessment tool is presented in a table below. The list of these 15 studies is as follows:

1. Thombs BD, Bass EB, Ford DE, Stewart KJ, Tsilidis KK, Patel U, et al. Prevalence of depression in survivors of acute myocardial infarction. J Gen Intern Med. 2006;21(1):30–8.

2. Roy T, Lloyd CE. Epidemiology of depression and diabetes: a systematic review. J Affect Disord. 2012 Oct;142 Suppl:S8–21.

3. Robinson RG, Spalletta G. Poststroke depression: a review. Can J Psychiatry. 2010 Jun;55(6):341–9.

4. Meijer A, Conradi HJ, Bos EH, Anselmino M, Carney RM, Denollet J, et al. Adjusted prognostic association of depression following myocardial infarction with mortality and cardiovascular events: individual patient data meta-analysis. Br J Psychiatry. 2013 Aug 1;203(2):90–102.

5. Park M, Katon WJ, Wolf FM. Depression and risk of mortality in individuals with diabetes: a meta-analysis and systematic review. Gen Hosp Psychiatry. Jan;35(3):217–25.

6. Bartoli F, Lillia N, Lax A, Crocamo C, Mantero V, Carrà G, et al. Depression after stroke and risk of mortality: a systematic review and meta-analysis. Stroke Res Treat. 2013 Jan;2013:862978.

7. Thombs BD, Roseman M, Coyne JC, de Jonge P, Delisle VC, Arthurs E, et al. Does Evidence Support the American Heart Association's Recommendation to Screen Patients for Depression in Cardiovascular Care? An Updated Systematic Review. PLoS One. 2013;8(1):e52654.

8. Roy T, Lloyd CE, Pouwer F, Holt RIG, Sartorius N. Screening tools used for measuring depression among people with Type 1 and Type 2 diabetes: a systematic review. Diabet Med. 2012 Feb;29(2):164–75.

9. Meader N, Moe-Byrne T, Llewellyn A, Mitchell AJ. Screening for poststroke major depression: a meta-analysis of diagnostic validity studies. J Neurol Neurosurg Psychiatry. 2014 Feb;85(2):198–206.

10. Baumeister H, Hutter N, Bengel J. Psychological and pharmacological interventions for depression in patients with coronary artery disease. Cochrane Database Syst Rev. 2011;9:CD008012.

11. Tully PJ, Baumeister H. Collaborative care for comorbid depression and coronary heart disease: a systematic review and meta-analysis of randomised controlled trials. BMJ Open. 2015 Jan 21;5(12):e009128.

12. Baumeister H, Hutter N, Bengel J. Psychological and pharmacological interventions for depression in patients with diabetes mellitus and depression. Cochrane Database Syst Rev. John Wiley & Sons, Ltd; 2012;12:CD008381.

13. Atlantis E, Fahey P, Foster J. Collaborative care for comorbid depression and diabetes: a systematic review and meta-analysis. BMJ Open. 2014 Jan;4(4):e004706.

14. Mead GE, Hsieh C-F, Lee R, Kutlubaev MA, Claxton A, Hankey GJ, et al. Selective serotonin reuptake inhibitors (SSRIs) for stroke recovery. Cochrane database Syst Rev. 2012 Jan;11:CD009286.

15. Eng JJ, Reime B. Exercise for depressive symptoms in stroke patients: a systematic review and meta-analysis. Clin Rehabil. 2014 Feb 17;28(8):731–9.

Table: AMSTAR scores of the 15 key studies included in Chapter 3

Name of the lead author (publication year)	AMSTAR Total Score (out of 11)	'Priori' design provided- yes/no	Study selection and Data Extraction by two reviewers yes/no	Comprehensive Search Strategy (at least two sources) yes/no	Status of study used as inclusion criteria- yes/no	List of included and excluded studies provided yes/no	Characteristics of included study provided yes/no	Scientific quality of included studies assessed and documented yes/no	Scientific quality of include studies used in forming conclusion yes/no	Methods used to combine studies appropriate yes/no	Possibility of publication bias assessed yes/no	Conflict of interest included yes/no
Thombs (2006)(1)	8	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Roy (2012)(2)	7	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Robinson (2010)(3)	5	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes
Meijer (2013)(4)	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Park (2013)(5)	10	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bartoli (2013)(6)	9	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Thombs (2013)(7)	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Roy (2012)(8)	9	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Meader (2014)(9)	9	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Baumeister (2011)(10)	11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tully (2015)(11)	11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Baumeister (2012)(12)	11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Atlantis (2014)(13)	8	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
Mead (2012)(14)	10	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eng (2014)(15)	10	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes