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**Some systemic markers of inflammation
in older adults with psychiatric disorders**

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A dissertation for the degree of PhD
June 2022

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Contents

1 Acknowledgement

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2 Abbreviations

AAGP: American Association for Geriatric Psychiatry
BDI: Becks Depression Inventory
BDNF: Brain-Derived Neurotrophic Factor
BMI: Body Mass Index
CDT: The Clock-Drawing Test
CES-D: Centre for Epidemiological Studies-Depression Scale
CIDI: Composite International Diagnostic Interview
CIS-R: Clinical Interview Schedule-Revised
CNS: Central Nervous System
CRP: C-Reactive Protein
CSDD: Cornell Scale for Depression in Dementia
DALY: Disability-Adjusted Life Years
DAMPS: Damage-Associated Molecular Patterns
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
ELISA: Enzyme-Linked Immunosorbent Assay
ELSA: English Longitudinal study of Ageing
EOS: Early-Onset Schizophrenia
FDR-P: False Detection Rate P-Value
GC: Glucocorticoids
GC-Receptor: Glucocorticoid Receptor
GDP: Gross Domestic Product
GDS: Geriatric Depression Scale
GHQ-30: General Health Questionnaire
GP: General Practitioner
HADS: Hospital Anxiety and Depression Scale
HADS-A: Hospital Anxiety and Depression Scale – Anxiety Subscale
HADS-D: Hospital Anxiety and Depression Scale – Depression Subscale
HALE: Healthy Life Expectancy
HDRS: Hamilton Depression Rating Scale
HPA-Axis: Hypothalamic–Pituitary–Adrenal Axis
Hs-CRP: High-Sensitivity C-Reactive Protein
IDO: Indoleamine Oxygenase
IL: Interleukin
INF-Γ: Interferon Gamma
LOS: Late-Onset Schizophrenia
MADRS: Montgomery and Åsberg Depression Rating Scale
MINI: MINI International Neuropsychiatric Interview
MMSE: Mini Mental State Examination
MOD: Major Depressive Disorder
N: Number of Individuals
NF-kb: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B-cells
NKC: Natural Killer Cells
NMDA-Receptors: N-Methyl-D-Aspartate Receptors

NSAID: Non-Steroidal Anti-Inflammatory Drugs
OECD: Organisation for Economic Co-Operation and Development
PAMPS: Pathogen-Associated Molecular Patterns
RCT: Randomized Controlled Trials
ROS: Reactive Oxygen Species
SCID-IV: Structured Clinical Interview For DSM-IV
SNS: Sympathetic Nervous System
SPSS: Statistical Package for the Social Sciences
TCR: T-Cell Receptor
TNF: Tumour Necrosis Factor
VC: Variance Coefficient
VIF: Variance inflation

3   List of papers

3.1   Paper I

Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole
Kristian Grønli: *Cytokine profiles and diagnoses in elderly, hospitalized psychiatric patients*.
BMC Psychiatry, Volume 18, September 2018.
Link: https://bmcpsychiatry.biomedcentral.com/articles/10.1186/s12888-018-1900-y

3.2   Paper II

Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole
Kristian Grønli: *Changes in cytokines during treatment of elderly, hospitalized psychiatric
patients – a naturalistic study*. Psychoneuroendocrinology, volume 108, October 2019.
Link: https://www.sciencedirect.com/science/article/pii/S0306453019304019

3.3   Paper III

Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole
Kristian Grønli: *C-reactive protein levels and depression in younger and older adults - a study
of 19,947 individuals. The Tromsø Study*. Submitted.

4 Summary

Research has demonstrated that inflammation is a central part of several psychiatric disorders, of which depression in the most researched condition. It has been suggested that patterns and levels of systemic inflammatory markers could be used as gauging tools in the diagnosis and assessment of psychiatric disorders. Most studies have, however, been conducted on younger adults, and studies on elderly are far less frequent. This thesis explores the possible association between systemic biomarkers of inflammation, i.e. a panel of 27 cytokines and C-reactive protein (CRP), and characteristics - depression in particular - of older psychiatric patients.

In study I, we investigated possible correlations between the levels of 27 cytokines, and diagnoses, clinical and demographical variables, in diagnostically unselected in-patients that were 60 years and older (N = 98). We found no significant associations between cytokine levels and diagnoses, nor any other variables. However, we did find higher levels of 10 cytokines in the non-depressed patients, possibly as a result of the higher prevalence of cardiovascular disease and dementia in the non-depressed group.

In study II, we explored whether changes in the level of 27 cytokines during the treatment of diagnostically unselected psychiatric in-patients aged 60 years or more (N = 49) could be related to diagnoses, clinical and demographical variables, as well as self-reported clinical improvement. We found a positive correlation between clinical improvement and falling cytokine levels (p < 0.033), irrespective of psychiatric diagnoses or other variables.

In study III, we investigated possible correlations between two levels of depression - moderate and moderate-severe depression - and CRP, in younger (40 - 59 years) versus older adults (≥ 60 years), participating in 7th Tromsø survey (N = 19,947). We found a multi-adjusted association between depression and elevated CRP in younger adults, but not in older adults.

The studies of this thesis could not confirm an association between markers of systemic inflammation, and diagnoses and other characteristics of older adults with psychiatric disorders. In particular, this research could not, unlike most studies on younger adults, confirm a link between markers of systemic inflammation and depression in older adults.

5 Introduction

5.1 Elderly or older?

Before getting into the issue at hand, a short discussion regarding a potentially contentious term - *elderly* - is needed. Some recommend not using this term, and some have even called its use ageist - fostering discrimination against older people - comparable to the use of derogatory labels applied by racists and sexists (1). I disagree. Consequently, I have chosen to use the term in this thesis, simply because *elderly* translated to Norwegian - *eldre* - does not have specific negative connotations. It simply means being older, i.e. that a person or a group is older than another person or group, though in this context, it is commonly denoting people of about 60-65 years of age and older. In Norwegian, *elderly* does not equal to disparaging stereotypes, and in certain cultures it is even a honorary title, signifying high status, wisdom etc. For instance, in Malaysia and Brunei, age is often used as an authority-ranking device, partially reflected by the use of age-referring captions that Europeans and Americans would instantly find "geriatric". One example is the use of the honorary title *Dato* (or Datin for a female) in Malaysia and Brunei, which literally means *grandfather* (or *grandmother*), but is used to indicate that a person is wise and honourable, irrespective of his/her age.

The sometimes-recommended substitute for *elderly* in scientific papers, *older adults*, is, in my opinion, less precise than *elderly*, at least in its translated Norwegian meaning. Using the phrase *older adults* in the Norwegian language would immediately spawn the question "What does that mean?" from a Norwegian. I appreciate that there might be cultural and historical reasons for a translatable term being perceived very different by two lingual counterparts, but I believe that my argument is supported by the fact that neither BMC Psychiatry (article 1), nor Psychoneuroendocrinology (article 2) have objected to use of *elderly* in the title or in the text.

In any case, I have used the two terms interchangeably, mixed with idioms such as *geriatric, old, older individuals, older adults* etc., throughout this thesis, simply to denote a group of people of a certain age, i.e. the elderly.

5.2   Aging and geropsychiatry

5.2.1   Global aging

In 2017, the world population aged 60 years and older numbered 962 million, more than twice as many as in 1980 (2). By 2050, the number of persons older than 60 and 65 years are projected to reach 2.1 billion and 1.5 billion, respectively. Aging populations are a global phenomenon, as the quantity of older people is increasing in virtually every country in the world. Worldwide, the share of people aged 65 years and older was 9 % in 2019, projected to increase to 16 % by 2050 (3). During the same period, the global number of persons aged 80 years and above is estimated to triple, from 143 million to 426 million (4). Norway is not exempted from these projections; the number of people ≥ 65 years will almost be doubled by 2060, whilst the number of those 80 years and older will be tripled. Just within ten years there will be, for the first time, more people over the age of 65 years than here are children and adolescents (0-19 years) in Norway (5).

5.2.2   People live longer and need more support

One reason for the relative and absolute increase in the number of older people, is that the survival beyond 65 years is improving. Globally, life expectancy has increased from 66.8 years in 2000 to 73.4 years in 2019. Despite increased life expectancy, the healthy life expectancy (HALE), i.e. numbers of years living in good health, has not increased proportionally (6). Consequently, the aging populations of the world represent significant challenges for the various support systems and health care for the elderly, psychiatry being no exception. Most psychiatric disorders are probably not more common in older adults compared to younger adults. However, the need for additional support when being afflicted by a psychiatric disorder, i.e. support beyond the mere treatment of the psychiatric disorder, arises far more frequently in older patients than in younger ones (7). The reason is that the geropsychiatric population is significantly more vulnerable to loss of function, complications and aberrant reactions to treatment. First, there is the increase in biological vulnerability due to age itself. As we grow older, the working of our biological machinery deteriorates, resulting in slower and weaker responses to stress. Second, psychiatric patients tend to have poorer physical health than the general population - they tend to smoke more often, have higher blood pressure, have a higher BMI etc. (8, 9). Third, multimorbidity, defined as the

coexistence of more than two chronic somatic diseases, is common in the elderly, with a prevalence of more than 60% for those aged 65–74 years, and more than 80% for those aged ≥ 85 years (10). Fourth, treatment with psychotropic drugs in older adults, indeed with any class of drugs, involves an elevated risk of poor effect, adverse effect and/or interactions (11), particularly when several drugs are combined. The combined result of all these factors entails a high risk of taxing the functional capacity of an older psychiatric patient, to the extent that he/she will need additional support.

Dementia is the one psychiatric disorder that disproportionally affects older people more frequently than younger adults. In fact, ageing is the primary driver of the frequency of dementia, as the incidence of dementia doubles with every 5.9 years increase in age, from 3.1/1000 person years at age 60-64, to 175.0/1000 person years at age 95 (12).

5.2.3 The cost of ageing populations

Given the fact that people over 65 years are shouldering a disproportionate burden of disease, accounting for more than 50 % of disability-adjusted life years (i.e. DALY: Number of years lost due to ill-health, disability or early death) (13), age is a major determinant of health care costs. For instance, the health care spending curve in OECD remains relatively flat until the age of 50-54 years, but raises steeply form the age of 65 and onwards, accounting for approx. 60 % of health care spending (14). Parallel to the aging of the populations, the health care expenditure has outpaced gross domestic product (GDP) for most of the past half century across the OECD countries, and there is little sign of deceleration. As the populations of the world become older the expenses are expected to rise, not only in terms of increased health care and support expenditures, but also because of an increase in pension payments and a reduction in labour force (15).

Dementia, of which Alzheimer is the most common form, has significant economic and social consequences in terms of direct medical and social care costs, as well as the costs of informal care. In 2015, the total global societal cost of dementia was estimated to be 818 billion USD[1], equivalent to 1.1% of global gross domestic product (GDP)(16). In Norway the cost of dementia was calculated at 95 billion Nkr in 2020, including both direct and indirect

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[1] Ca. 7,1 billioner norske kroner

costs (17).

5.2.4 Research on older adults

Despite the aging demographics and the related increase in health care expenses, older adults are vastly underrepresented in research in both somatic and psychiatric medicine (18-20). Research on dementia is of course the one exception, but still, the research funding does not reflect the size of health care spending related to dementia. In the UK, for instance, research has revealed that for every £10 of health and social care costs attributable to each disease, cancer received £1.08 in research funding, chronic heart disease £0.65, stroke £0.19 and dementia £0.08. In fact, in the UK, dementia has higher health and social care costs than cancer and chronic heart disease combined (21).

"More research is needed" is perhaps an overly used phrase, but when it comes to research on the older population it is still a highly relevant statement. For one thing, the aging populations of the world makes this unescapable; we simply must know more about aging itself, healthy and unhealthy aging, how disease and aging is connected, etc. to handle the impending challenges. To achieve this, the National Institute of Aging has pinpointed seven areas of research to uncover the underpinnings of aging; adaption to stress, epigenetics, metabolism, macromolecular damage, proteostatis, and last, but not least, *inflammation* (22). The latter is the focal point of this thesis. Research on younger adults have established inflammation as a part of several psychiatric disorders, particularly depression. Whether this is true for elderly psychiatric patient, is less clear. One complicating factor is the natural inflammatory process of aging, i.e. if and how the natural age-related inflammation influences the psychoneuroimmunology of psychiatric disorders in the elderly.

5.2.5 What is geropsychiatry?

Geropsychiatry - also known as geriatric psychiatry, psychiatry of old age, psychogeriatrics or gerontopsychiatry - is a discipline in psychiatry dealing with mental disorders in older people. While the United Nations refers to those aged 60 years and over as "older people", most developed countries have accepted the age of 65 years as the threshold of an "older person". Though there is no scientific foundation for this chronological definition, there are good reasons why geriatric psychiatry is a speciality in its own right. First, most psychiatric

disorders in the elderly are not identical to the corresponding disorders in younger people in terms of development, presentation, consequences etc (23, 24). Then, there are the dementias, a group of disorders predominantly of geriatric origin, commonly presenting as other psychiatric conditions, such as depression or anxiety. Second, the treatment of elderly psychiatric patients differs from the treatment of younger adults in several important aspects. For instance, in psychotherapy, older and younger patients have different preferences with regard to approach and methodology (25, 26). Pharmacological interventions in the elderly often necessitate careful and stepwise applications due to the increased risk of side effects, even serious adverse effects, owing to the vulnerability of the aging homeostatic system. Third, the high frequency of somatic comorbidity in the elderly requires an integrated approach, demanding the old age psychiatrist to be competent in somatic disciplines as well. In other words, geropsychiatry is not just "psychiatry for older people", but a psychiatric sub-specialty.

5.2.6 Historical origin

The actual genesis of modern geropsychiatry is difficult to pinpoint, but certainly the publication by Alois Alzheimer in 1907 of a paper describing a female patient with "presenile dementia" is one of the early milestones. Alzheimer stained samples of brain tissue of a female patient, Frau Auguste Deter, and demonstrated the typical amyloid plaques and neurofibrillary tangles of what later became known as Alzheimer's disease. However, Alzheimer's discovery did not change the perception of old age psychiatry as just an extension of "ordinary" psychiatry, albeit that the prevalence of most conditions was considered to be very low in old people. To the extent that old age psychiatry was a thing of its own, it was confined to the neurodegenerative disorders, mostly dubbed some form of "dementia" or "psychosis". In addition, there was a tendency to consider psychiatric symptoms debuting in old age to be an inflation of already existing character traits, either caused by old age itself or by age-related brain-degeneration leading to disinhibition of previously controlled personality traits. Thus, melancholic traits[2] in adulthood would become symptoms of depression or anxiety in old age, i.e. excessive manifestations of a pre-existing

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[2] At that time, the meaning of *melancholia* was not limited to depression, but also encompassed anxiety.

constitution, but not as psychiatric disorders per se (27, 28).

In any case, there was a growing interest in the psychiatric disorders of elderly in Europe in the first part of the 20th century. In the UK in the 1940s, two of the most influential psychiatrists of their day, David K. Henderson and Aubrey Lewis, took an interest in the mental disorders of older people - in those days "old" meant people above 60 years of age. At instigation of professor Aubrey Lewis, the first dedicated psychiatric "geriatric unit" in Europe was opened at the Bethlehem Hospital in South London after WW2 (29). Further development in the field was slow, however, and it was not until the 60s and 70s that there was a growing realization that old age psychiatry was more than just neurogenerative disorders, and that geropsychiatry included many of the same disorders as psychiatry in general, but with its own set of phenomenology and approaches. Eventually, geropsychiatry was recognized as a clinical speciality in the 1970s and 1980s. For instance, the European Association of Geriatric Psychiatry was founded in 1973 (though formally not registered until 1987), the American Association for Geriatric Psychiatry (AAGP) in 1978, and the International Psychogeriatric Association in 1982. Still, it took another decade or two until countries started to formalize the certification and education of physicians in geropsychiatry. For instance, it was not until 1991 that the AAGP first introduced mandatory exams for the certification as a geropsychiatrist (in the US, *a geriatric psychiatrist*) – a subspecialty within psychiatry - with a required re-certification every 10 years (30). Even today, many countries, Norway included, do not have any certifications or specific requirements for psychiatrists working in geropsychiatric units.

5.2.7   Geropsychiatry in Norway

Local psychiatric out-patient clinics in Norway treat all adults, including geropsychiatric patients. More specifically, they treat geropsychiatric patients with "uncomplicated" psychiatric disorders (for instance, depression) or chronic psychiatric disorders (such as schizophrenia and bipolar disorder). It is noteworthy, that the assessment and treatment of patients with uncomplicated dementia are for the most part undertaken by the primary health care services, incl. GPs.

In addition to the regular out-patient services, there are 22 geropsychiatric departments,

typically consisting of an out-patient clinic and an inpatient ward (31). The geropsychiatric out-patient clinics provide counselling to the regular psychiatric out-patient services and community health care services, as well as assess and treat complicated geropsychiatric cases. The geropsychiatric wards operate analogous to this; they accept cases too complex to handle in an out-patient setting. The majority of the wards have a general profile, i.e. they accept, in principle, all diagnoses, but some of the larger wards are organized with two subunits; one ward for the complicated dementias and one for other (complicated) geropsychiatric disorders. In the geropsychiatric wards, the most prevalent diagnostic categories are depression, dementia-related problems, bipolar disorders and psychosis (31).

5.3 Prevalence of psychiatric disorders in older adults

5.3.1 Anxiety and depression

In principle, older people suffer from the same psychiatric disorders as other adults, at least in nosological terms. However, the frequency of the various psychiatric disorders may differ between younger and older adults, and more often than not, the clinical presentations in older adults are somewhat different than the "classical" clinical syndromes encased in the diagnostic systems (32, 33). The likely reason is that the diagnostic criteria are for the most part based on studies on younger adults. This illustrates a longstanding issue in psychiatric research; there is a paucity on studies on the geropsychiatric populations. Possibly as a result of this paucity, in particular the scarcity of large-scale population studies on older people, there is a substantial variation in the reported prevalence of most psychiatric disorders in the elderly. Some of the variation might also be the result of differences in population samples (e.g. elderly in nursing homes versus community-living elderly), including the definition of "older", or in methodology, such as different questionnaires or diagnostic methods. For instance, a review based on 55 studies reported a prevalence of depression in elderly, in different settings, ranging from 2 % to 64 % (34).

A short interjection; I will not elaborate on the nomenclatural differences between subgroups of major depressive disorders and *late-life depression* or *geriatric depression*, and I will use these terms interchangeably, in conjunction with the generic term *depression,*

throughout the text. In chapter 9.3, I will debate, albeit briefly, if depression in older individuals is a closely related, but still different disorder than depression in younger persons.

The prevalence of anxiety disorders displays a similar pattern of variation, with estimates ranging from 1.2 % to 15 % (35). In any case, with the exception of dementia, most reviews and meta-analysis report decreased rates of psychiatric disorders in older adults compared to younger adults (36-38). Within the group of elderly, however, several studies have demonstrated an age-related trend of increasing prevalence of depression (39-41). One explanatory factor could be declining health with advancing age, as the increasing rates of depression as people grow older are associated with a higher occurrence of chronic somatic diseases (42-44). Besides problems with physical health, there is the trinity of social risk factors for all psychiatric disorders in the elderly (indeed for all psychiatric disorders irrespective of age); lack of social/emotional support, stressful life events and low socio-economic status (45).

An overview of the prevalence of psychiatric disorders commonly found in older adults is presented in table 1 (*Table 1: Prevalence of common psychiatric disorders in older adults*)

5.3.2   Dementia

Dementia is the one age-related disorder in psychiatry that has been most consistently researched, with a prevalence rate in people aged 60 and over ranging from 5-8 % (46, 47). The most common form of dementia is Alzheimer's disease, representing approx. 60 - 70 % of cases. Vascular dementia constitutes 20 % of the dementias, whilst Lewy body dementia, a disorder associated with Parkinson's disease, accounts for about 10 %. Next, 5 – 10 % of the dementias are of the frontotemporal type, and finally there is a small, heterogenous group of rare dementias, such as atypical Alzheimer's disease, hereditary vascular dementia (CADASIL-dementia), dementia in Creutzfeldt-Jakob's disease, dementia in Huntington's chorea, corticobasal syndrome, hippocampal sclerosis etc. (48, 49).

In Norway, there is estimated to be approx. 101 000 persons with dementia (49). As in the rest of the world, the proportion of dementia in the Norwegian population, particularly Alzheimer's disease and vascular dementia, increases with age [3]. Dementia also affects more

older women than men. Especially, there is a disproportionate share of women from the age of 85 years and upwards being affected by dementia (50).

Despite the absolute increase in dementia worldwide as the population of the planet gets older, the incidence rates of dementia seem to be falling (51-53). Different theories about the causes circulate - reduced burden of Alzheimer (54), lower rates of cardiovascular disease, fewer strokes (55) - but the truth is that we do not know why such a favourable, but unexpected, trend has developed.

Age and sex estimates of prevalence of dementia in Norway are presented in table 2 (*Table 2: Age and sex estimates of prevalence of dementia in Norway in 2020*).

5.3.3   Psychosis

In general, psychosis and psychotic symptoms are uncommon in the elderly. In a community-based study from Sweden (N = 894) of non-demented elderly, the one-year prevalence of any psychotic symptoms was 0.9 % among those aged 70 years, and 1.2 % among those aged 78 and 82 years (56). Psychotic symptoms are, however, quite frequent in the dementias, and psychotic disorders such as schizophrenia do subsist in older adults (57).

An overview of epidemiology and clinical features of geriatric psychosis is presented in table 3 (*Table 3: Epidemiology and clinical features of psychosis in later life*).

Table 1: Prevalence of common psychiatric disorders in older adults

Author, year	Number of participants	Country/region, study type	Age limit/ age range	Proportion of females	Diagnostic procedure	Prevalence range/type	Prevalence of disorder	
DEPRESSION, ANXIETY, SUBSTANCE/ALCOHOL DISORDER								
Jimenez et al, 2010[54]	2375	USA, community, ethnic minorities	≥ 60 years	Not stated*	WMH-CIDI (interview)	12 months	Depression Anxiety Substance/alcohol	5.4 – 8.1 % 10.9 – 15.3 % 1.3 – 9.0 % (total)
Seitz et al, 2010[55]	1.317.292	USA, nursing homes	≥ 65 years	71.2 %	Review of medical records	Point	Depression Anxiety Substance/alcohol	36.9 % 11.7 % 1.0 %
Reynolds et al, 2015[56]	12.312	USA, community	≥ 55 years	55.0 %	AUDADIS-IV (interview)	12 months	Depression Anxiety Substance/alcohol	5.6 % 11.4 % 3.8 % (total)
Andreas et al, 2017[57]	3142	Europe, community	≥ 65 – 84 years	50.7 %	CIDI65+ (interview)	12 months	Depression Anxiety Substance/alcohol	11.6 % 17.2 % 8.9 % (total)
McCombe et al, 2018[39]	74.261	Europe, general practice	≥ 55 years	64.1 %	Electronic records (GP consultation)	9 months	Depression Anxiety Substance/alcohol	15.1 % 7.6 % 3.2 %/4.6 %
DEMENTIA								
Lobo et al, 2000[48]	71 – 482 (review, 8 studies)	Europe, community	≥ 65 years	Not stated	Miscellaneous	Point	Dementia (overall) Alzheimer's disease Vascular dementia	6.4 % 4.4 % 1.6 %
Berr et al, 2005[58]	1016 – 31,035 (review, 10 studies)	Europe, community	≥ 65 years	Not stated	Miscellaneous	Point	Dementia overall	5.9 - 9.4 %
Plassman et al, 2007[59]	856	USA, community	≥ 71 years	60.7 %	Miscellaneous	Point	Dementia (overall) Alzheimer's disease Vascular dementia	36 % 27 % 5.6 %
Prince et al, 2013[44]	Total not stated (review, 135 studies)	Worldwide, community	≥ 60 years	Not stated	Miscellaneous	Point	Dementia overall	5.0 – 7.0 %
Bacigalupo et al, 2018[60]	18,267 (review, 15 studies)	Europe, community	≥ 55 years	Not stated	Miscellaneous	Point	Dementia overall (prevalence correlating with age)	2.7 – 35.7 %

Table 2: Age and sex estimates of prevalence of dementia in Norway in 2020 (percent)

Age, years	Total	Males	Females
30-64	0,1	0,1	0,1
60-64	0,3	0,3	0,3
65-69	0,7	0,6	0,9
70-74	5,6	6,4	4,8
75-79	9,5	10,0	9,0
80-84	17,9	17,8	18,0
85-89	33,0	30,4	34,6
90+	48,1	41,5	50,9
70+	14,6	13,0	15,9
60+	8,0	6,8	9,1

Reference: Folkehelseinstituttet: Demens. https://www.fhi.no/nettpub/hin/ikke-smittsomme/demens/

Table 3: Epidemiology and clinical features of psychosis in later life

Disorder	Epidemiology	Clinical features
Late onset schizophrenia (> 45 years)	1.0% lifetime prevalence	Compared to EOS, patients with LOS more commonly have visual, olfactory, and tactile hallucinations, as well as persecutory and partition delusions.
Delusional disorder	0.18% lifetime prevalence	Delusions of theft, surveillance and persecution are common, and tend to be of a systematized nature. No cognitive impairment, functional decline or bizarre behaviour.
Psychotic depression	0.35% lifetime prevalence	MOD, usually severe, combined with psychotic features, often mood congruent delusions, i.e. delusions a dysthymic nature (somatic delusions are common).
Schizoaffective disorder	0.32% lifetime prevalence	Symptoms of both psychosis and mood disorder. Differential diagnosis can be challenging, as psychotic and mood symptoms appears in several other disorders.
Dementias	43 % – 89 %	Visual hallucinations are common in dementias. Delusions tend to be simple and non-complex; the most common delusion is one of theft.

Adapted from Colijn et al[55]
EOS: early-onset schizophrenia. LOS: late-onset schizophrenia. MOD: major depressive disorder

5.4   Inflammation and psychiatric disorders

5.4.1   Psychoneuroimmunology – a young discipline

Mens sana in corpore sano (58) - a healthy mind in a healthy body. Though the ancient phrase is aimed mainly at the positive effects of a healthy body on the mind[c], it demonstrates that an integrated perspective on body and mind is not a recent concept. Still, even in the 1960s, the idea of an integration of emotions, stress, immunological functioning, and diseases was considered speculative. When George F. Solomon published his landmark article *Emotions, immunity, and disease: a speculative theoretical integration* in 1964 (59), and coined the term *psychoimmunology* (later re-dubbed *psychoneuroimmunology*)*,* it was reservedly considered interesting, but without any scientific merit (60). Besides anecdotes alike contracting a cold when stressed, and how *The power of positive thinking* could provide success and a disease-free body (61), it was not until the 1980s that the scientific basis of the interactions between the brain and the immune system were revealed. One momentous discovery came in 1981, when David L. Felten and colleagues found nerves in the thymus and spleen, terminating near clusters of lymphocytes, macrophages, and mast cells, thus pinpointing how the nervous system could trigger and regulate the immune system (62). Similar reports followed, and in the late 1980s and early 1990s, a connection between depression and immunological changes emerged (63, 64). Initially, the focus was on the link between autoimmune diseases, particularly rheumatoid arthritis and systemic lupus erythematosus, and depression. However, it soon became evident that the immune system was independently involved in all forms of clinical depression, and in 1991, Robert S. Smith presented one of the first well-founded hypotheses of the inflammation-depression link (65). Smith wrote: "*Excessive secretion of macrophage monokines[d] is proposed as the cause of depression. … Interleukin-1 (IL-I) can provoke the hormone abnormalities linked with depression*". In the following decades, numerous reports about inflammatory changes in depression and other psychiatric disorders were published, and it is now broadly accepted that inflammation is an integral part of most major psychiatric syndromes. Still, psychoneuroimmunology is still in its infancy, as exemplified by the fact that no principal

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[c] A phrase taken from one of the satirical poems of the roman writer Decimus Junius Juvenalis, living in the late first and early second century AD.
[d] A monokine is a type of cytokine released primarily by monocytes and macrophages.

immunomodulatory treatment is yet available in psychiatry[e].

5.4.2   Systemic inflammation in psychiatric disorders

Systemic inflammation has been demonstrated in several psychiatric disorders, ranging from schizophrenia (66) and autism (67), to anxiety disorders (68) and depression (69). The level of inflammatory markers in blood, such as certain cytokines and C-reactive protein, seem to correspond to the severity of the psychiatric disorder, i.e. the more severe the disorder, the higher the level of the inflammatory markers (70-73). Furthermore, it has been suggested that patterns of cytokines may aid the diagnostic process, i.e. in determining the severity, the subtype and the stage of certain psychiatric illnesses, particularly depressive syndromes (74-76). Moreover, a range of studies have demonstrated aberrant levels and activity in various groups of blood immune cells in different disorders, for instance increased levels of CD4^{+} T-lymphocytes in depression (77) and natural killer cells dysfunction in psychosis (78, 79).

5.4.3   Brain inflammation in psychiatric disorders

Besides systemic inflammation, mental illness is paralleled by inflammatory changes in the brain, mainly as an activation of the microglial cells (80). For the most part, this activation of the microglia is an adaptive and functional process, at least in the shorter term (81). However, sustained activation of cytotoxic microglial cells (M1) will eventually lead to a neuroinflammatory process causing neuronal death (82-85). An important contribution to neuronal death is the accumulation of neurotoxic substances due to downregulation of the kynurenine pathway[f], (86) (see 5.3.5). Prolonged microglial activation and neuronal death have been found in chronic stress, post-traumatic stress disorder, long term depression, bipolar disorder and schizophrenia (86-91).

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[e] Some antidepressants and antipsychotics may hold anti-inflammatory properties, but the significance of this is uncertain (in terms of therapeutic effect).
[f] The kynurenine pathway is a metabolic pathway leading to the production of nicotinamide adenine dinucleotide (NAD+). Nicotinamide adenine dinucleotide (NAD) is a coenzyme central to metabolism.

5.4.4 From the brain to the body

The psychoneuroimmunological mechanisms of psychiatric disorders are immensely complex and an elaborated presentation of these mechanisms is beyond the scope of this thesis. Still, it is useful to provide a rudimentary background on some of the principles of the psychoneuroimmunological crosstalk, incl. some neuroendocrine pathways.

An initial caveat; for didactic reasons, I have presented the interplay between the nervous and the immune system as directional and sequential, starting in the body or in the brain. Though this might be true in some cases, it is important to keep in mind that the psychoneuroimmunological system is *one* highly integrated system, with continuous bidirectional communication between immune, endocrine end nervous tissues. This is illustrated by the facts that neurons, microglias and endocrine cells all have receptors for cytokines, neurotransmitters and hormones (92). Consequently, elevated levels of certain cytokines and CRP in peripheral blood are reliable indicators of both peripheral and central (brain) inflammation (93).

Prolonged psychological stress, herein psychiatric disorders, can cause systemic inflammation through the activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (94). In short, the SNS triggers the HPA axis, resulting in release of glucocorticoids (GC), most importantly cortisol, from the cortex of the adrenal glands. Short bursts of GCs are adaptive and inhibit inflammation, but a protracted elevation of GCs increases inflammation, including a longstanding activation of pro-inflammatory cytokines, such as IL-1β, IL-6, TNF and INF-γ, *and* creates a blunted anti-inflammatory response when superimposed stress or infection occurs. A critical factor in the self-perpetuating inflammation is the gradual weakening of the negative feedback mechanism caused by a reduction in GC-receptor activity and sensitivity, both in the immune system and the CNS (95).

Neurotransmitters of the SNS, primarily norepinephrine and neuropeptide Y, also promote inflammation (96). First, by directly stimulating immune tissues for activation of immune cells and release of pro-inflammatory factors, and second, by the release of catecholamines from the adrenal medulla into the blood stream, thus fuelling the inflammation further.

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5.4.5 From the body to the brain

Upon activation of systemic T- and B-cells (by endogenous and exogenous antigens, DAMPS and PAMPS, respectively), pro-inflammatory cytokines are released by the transcription of NF-κB[g]. Both the activated lymphocytes[h] and their cytokines are able to pass the blood-brain barrier, consequently triggering the immune system of the brain (stress and inflammation can alter the permeability of the blood-brain-barrier) (97). The primary agents of the neuroimmune system, the microglias, react by discharging cytokines, reactive oxygen species (ROS) and other inflammatory mediators. These mediators affect monoamine neurotransmission through a variety of mechanisms, thereby contributing to psychiatric symptoms and disorders (98). The inflammatory mediators also induce aberrant activity in indolamine oxygenase (IDO), a central enzyme in the formation of kynurenine, leading to increased amounts of kynurenine, quinolinic acid and other neurotoxic metabolites in the brain (86). In addition, IDO fosters an increase in glutamate, the main excitatory transmitter of the CNS, as well as hypoactivation of NMDA-receptors and a decrease in brain derived neurotrophic factor (BDNF) (99). High levels of glutamate are involved in stress and several psychiatric disorders (100) , and the combination of excess glutamate and reduced BDNF, a neuroprotective factor, is potentially neurotoxic (101, 102). The aberrant kynurenine pathway also depletes tryptophan, the primary precursor of serotonin (103).

Another pathway to psychiatric symptomatology and syndromes, is the production of brain reactive antibodies (BRA) by B-lymphocytes. BRA can be produced in numerous autoimmune disorders and infections. The BRA can block neuroreceptor binding (e.g. in NMDA receptors), enhance neuroreceptor binding (e.g. glutamate receptors), or block ion-channels in the brain, all of which can cause mental illness, even psychosis (104).

Finally, it is worth mentioning that inflammatory triggered microglias and astrocytes release tumour necrosis factor (TNF). TNF can kill oligodendrocytes, the myelinating glias of the central nervous system (CNS), vital for transmittance in neural tissue. High levels of TNF in brain tissue/CSF have been found in schizophrenia and neurodegenerative disorders (105).

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[g] NF-κB, or *nuclear factor kappa-light-chain-enhancer of activated B cells*, is a transcription factor that controls cytokine production and is involved in cellular responses to stimuli such as infection, cellular tress and damage. NF-κB has also been implicated in processes of synaptic plasticity and memory.
[h] For the most part, in this thesis, I use the terms *T-cells* and *B-cells*, which is synonymous for *T-lymphocytes* and *B-lymphocytes*.

5.5 Age-related changes to the immune system

5.5.1 The immune system

The immune system is commonly divided into the innate and the adaptive immune system, though this division is slightly artificial as innate and adaptive responses are found in both systems.

A basic overview of the immune system is presented in table 4 (*Table 4: Overview innate and adaptive immune system*).

5.5.2 Cytokines and C-reactive protein

The measurement of a set of cytokines (study I & II) and C-reactive protein/CRP (study III) were used to estimate levels of inflammation in the studies of this thesis.

Cytokines: An initial clarification; some differentiate between *cytokines* and *chemokines*, but here, the term *cytokines* are used as a term for both. It is also worth noting that the broader term *cytokines* traditionally include a range of molecules with mainly trophic and reparative qualities.

Cytokines are a large and heterogenous group of signalling molecules (proteins, peptides or glycoproteins), primarily working in the innate and the adaptive immune systems, as they regulate immunity, inflammation and haematopoiesis. Cytokines are secreted mainly by lymphocytes, macrophages and neutrophils, but also by several other cell categories, including the microglias of the central nervous system. In relation to inflammation, some cytokines are pro-inflammatory, others are anti-inflammatory, and then there are those who are both pro- and anti-inflammatory, depending on the stage of the inflammatory process.

The blood level of cytokines is highly individual, contingent on the health status and age of the person in question. In young, healthy, non-obese adults, however, most inflammatory cytokines are not detectable or at a very low level (106-108).

C-reactive protein also: CRP is an acute-phase protein, produced predominantly by hepatocytes, but in smaller amounts also in neurons, lymphocytes, adipocytes and atherosclerotic lesions, in response to pro-inflammatory cytokines (109). Specifically, CRP synthesis is induced by IL-1, IL-6, and IL-17 (110).

For a long time, CRP was considered a passive consequence of inflammation, exerting no significant role in immunological regulation or pathogenesis. Recent studies have demonstrated that CRP exerts a role in complement pathways, apoptosis, phagocytosis, nitric oxide release, and the production of cytokines (111).

5.5.3 Immunosenescence

As we grow older the immune system becomes less effective, rendering us susceptible to infections, vaccine failure and cancer. The aging of the immune system is often referred to as *immunosenescence (112)*. Immunosenescence affects both branches of the immune system, but to a lesser extent the innate immune system than the adaptive immune system *(Figure 1: Immunosenescence)*. Immunosenescence includes changes such as

- Decline in the self-renewal of hematopoietic stem cells (in the bone marrow), which in turn leads to a lower number of immune cells being produced.
- Involution of the thymus, resulting in lower maturation rate of T- and B-cells, and an increased number of dysfunctional T- and B-cells.
- Slower development of new monoclonal B-cells, and slower and lower production of specific antibodies from monoclonal B-cells.
- The repertoire of T-cell receptors (TCR), is diminished, as is the TCR response when triggered.
- Increased number of natural killer cells (NKC), but their ability to kill infected and transformed cells is diminished.
- The phagocytotic capacity of the macrophages declines, and so does their capability as antigen presenters.
- A gradual shift from M2 (anti-inflammatory, associated with healing and repair) to M1 macrophages (pro-inflammatory), providing a relative increase in M1 macrophages.
- Higher levels of autoantibodies, i.e. antigens against own tissue.
- Increased systemic levels of CRP and certain cytokines, most notably pro-inflammatory cytokines such as TNF, IL1β and IL-6.

Table 4: Overview innate and adaptive immune system

	Innate immune system	Adaptive immune system
Response	Rapid, with immediate effector activation	Slow, with delayed effector activation
Effector mechanisms	Opsonization, phagocytosis, granuloma formation, complement lysis, leukocyte recruitment, inflammation, healing response	Clonal expansion of antigen-specific B and T-cells, antigen specific immunoglobulins
Cells	Natural killer cells, macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils	T-lymphocytes, B-lymphocytes.
Recognition	Molecular patterns selected over evolutionary time	Molecular structure selected over lifetime of individual (antigenic epitope)
Recognition receptors [R]	Complement-R, Mannose-R, Toll-like-R, inflammasomes, low affinity immunoglobulins	B-cell-receptors, T-cell-receptors, MHC-I, MHC-II, High affinity IgG
Receptor genes	Single gene, no rearrangement required	Encoded in gene segments, rearrangement required
Receptor clonality	Non-clonal	Clonal

Other components of the immune system include acute phase proteins (e.g. C-reactive protein), complement, non-specific antibodies, and cytokines

5.5.4 Inflammaging

As mentioned in the introduction, immunosenescence affects the adaptive immune system more than the innate immune system. Possibly as a compensatory mechanism, but also because of age-related tissue damage of various origins, the innate immune mechanisms become more active. Accordingly, the number of NKCs and M1-macrophages increases, and the level of pro-inflammatory cytokines and inflammatory markers increase. In sum, this creates a sterile, low-grade, chronic inflammation in the elderly, also referred to as *inflammaging* (113). Inflammaging contributes to biological aging and represents a risk factor for age-related diseases, such as Alzheimer's disease (114), cancer (115) and cardiovascular disease (116).

5.5.5 Immunosenescence, inflammaging and inflammation in geropsychiatric diseases

To summarize, there is a link between systemic inflammation and psychiatric disorders in younger adults. Whether this link is present in older adults is yet not established, largely due to the fact that studies on older psychiatric patients are scant (dementia excluded). Provided that inflammation is the generic biological response to stress, infection and trauma, it seems likely that psychiatric disorders in elderly also should be accompanied by inflammatory changes. On the other hand, immunosenescence and inflammaging could challenge such a presupposition, making the psycho-inflammatory connection invalid or significantly different from that of younger adults.

The changes of immunosenescence is presented in figure 1 (*Figure 1: Immunosenescence)*.

Figure 1: Immunosenescence

OVERALL CHANGES

- Increased risk of infection
- Increased risk of cancer
- Increased autoimmune and inflammatory reactions
- Reduced vaccine response
- Reduced wound healing
- Inflammaging ($\uparrow$ CRP, IL-6 etc)

T-CELL CHANGES

- Reduced number of T-cells
- Increased number of dysfunctional T-cells
- Reduced T-cell receptor function and diversity
- Accumulation of non-functional memory T-cells
- Increased proinflammatory cytokines

B-CELL CHANGES

- Reduced number of B-cells
- Reduced B-cell receptor function and diversity
- Reduced proliferation of antibodies
- Increase in autoantibody titers
- Increased levels of IgG and IgA

OTHER CELL CHANGES

- Increased number of natural killer cells
- Reduced function of natural killer cells, incl. impaired cytotoxic capacity
- Reduced autophagy and phagocytosis by macrophages
- Dysregulated secretion of pro-inflammatory mediators (e.g. cytokines) by macrophages
- Inhibited response to growth factors in macrophages
- Macrophages shift from anti-inflammatory phenotype (M2), towards pro-inflammatory phenotype (M1)

6 Aim and objectives of the thesis

6.1 Aim

The aim of this thesis is to explore possible correlations between characteristics of older adults with psychiatric disorders, in particular depression, and biomarkers of systemic inflammation, as measured by cytokines and c-reactive protein.

6.2 Objectives

- Investigate possible correlations between cytokine levels, and diagnoses, clinical variables and demographical data, in diagnostically unselected elderly patients admitted to a psychiatric hospital.

- Investigate changes in cytokine levels during the treatment of diagnostically unselected elderly psychiatric in-patients, and explore whether these changes could be related to diagnoses, clinical variables and demographical data, as well as outcome of treatment (self-reported clinical improvement).

- Investigate possible correlation between two levels of depression (moderate and moderate-severe depression) and CRP, in younger adults (40 – 59 years) compared to older adults (≥ 60 years), in a large community sample.

7   Materials and methods

7.1   Subjects in study I

The subjects were 98 patients, 60 years and older, consecutively admitted to a geropsychiatric ward at the University Hospital of North Norway in Tromsø, Norway (69°N), during March 2010 - December 2011 (18 months). The catchment area of the hospital included approximately 255,000 citizens. The ward is the only intramural geropsychiatric unit in the northernmost part of Norway, i.e. the regions of Finnmark, Troms and Ofoten, covering about 83 000 km^2 (approx. 25 % of mainland Norway).

As most geropsychiatric patients in the catchment area are treated locally, the patients in this study were acutely and severely ill, or had some sort of treatment-resistant psychiatric disorder.

Patients unable to communicate and cooperate, or suffering from a medical condition likely to significantly affect the blood/plasma analysis, were excluded from the study.

A total of 107 patients were asked to participate in the study. Five patients declined, two patients were excluded due to an ongoing infection, and two patients were omitted because of erroneous data.

Figure 2 presents a flow-chart of the inclusion and exclusion of participants of study I (*Figure 2: Flow-chart inclusion/exclusion Study I & II*).

Besides registering demographic and life-style data, a thorough clinical assessment of all patients was undertaken by experienced clinicians, including clinical interviewing and examination, application of various psychometric tools, and reviews of medical records. Next of kin were also interviewed when appropriate.

During the first 3 days of admittance, morning blood samples were drawn for a range of analyses, including 27 cytokines, representing a broad spectrum of inflammatory markers.

There has been two previous publications on this population; one about Vitamin D deficiency (117), and one about zinc deficiency (118).

Trial registration: Retrospectively registered in the ISRCTN registry study, with study ID ISRCTN71047363.

7.2 Subjects in study II

The subjects in study II included 81 patients, and were drawn from the subjects in study I, i.e. 81 of the 98 patients from study I had their plasma analysed for cytokines both at admission and discharge (the 98 patients included in study I, undertook a plasma analysis of cytokines at admission). 17 patients did not have a cytokine analysis at discharge, as some declined, while others were lost due to insufficient procedures. 49 of the 81 patients included in study II also completed a self-reported clinical, psychiatric status form at discharge. The status form had 5 categories: Complete recovery, Almost complete recovery, Partial recovery, No recovery and Worsening.

Figure 2 presents a flow-chart of the inclusion and exclusion of participants of study II (*Figure 2 Flow-chart inclusion/exclusion Study I & II*).

Otherwise there was no procedural difference between the participants in study I and study II.

Trial registration: Retrospectively registered in the ISRCTN registry study, with study ID ISRCTN71047363.

7.3 Subjects in study III

The subjects in study III consisted of participants of the 7th and latest survey of the Tromsø Study, undertaken in 2015 - 2016. The Tromsø Study is a community-based, prospective study of inhabitants of the municipality of Tromsø, Norway. The study design includes repeated population surveys to which total birth cohorts and random samples are invited. The study was initiated in 1974 (Tromsø 1), with repeated health surveys in 1979 - 80 (Tromsø 2), 1986 - 87 (Tromsø 3), 1994 - 95 (Tromsø 4), 2001 (Tromsø 5), 2007 - 08 (Tromsø 6) and 2015 - 16 (Tromsø 7). The survey included clinical interviewing and examination, biological sampling (incl. blood samples), and self-administered questionnaires about life style, mental health, smoking, alcohol, etc.

21,083 individuals ≥ 40 years were originally included in the study, i.e. 64.7 % of the eligible inhabitants at the time (all inhabitants ≥ 40 years in Tromsø were invited to participate). However, individuals with an invalid or missing score on the subscale for depression of the

Hospital Anxiety and Depression Scale (HADS-D) were excluded (N = 499), as were individuals with a serum C-reactive protein (CRP) higher than 10 mg/L (N = 484) and individuals with BMI < 18.5 (N = 153), leaving a total of 19,947, i.e. providing a participation rate of 61.2 %.

Figure 3 presents a flow-chart of the inclusion and exclusion of participants of study III (*Figure 3: Flow-chart inclusion/exclusion Study I & II*).

7.4 Ethics

All patients in study I and II were provided with oral and written information about the study, herein that data would be treated in strict confidence. Competency to provide consent was evaluated according to established guidelines (119). For those patients who were considered unable to give individual consent due to their medical condition, information about the study was provided to their next of kin. All patients, and their next of kin when relevant, had to sign a written consent form in order to participate in the study. Approval of study I and II was obtained from the Regional Committee for Medical and Health Research Ethics of Northern Norway (REC North, reg. nr. 2009/1388).

As for study III, all participants in the Tromsø Study were provided with oral and written information about the study, herein that data would be treated in strict confidence. All participants had to sign a written consent form in order to participate in the study. The Tromsø study has been approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics of Northern Norway (REC North, reference no. 2014/940), as has this particular study (REC North, reference no. 2020/88232).

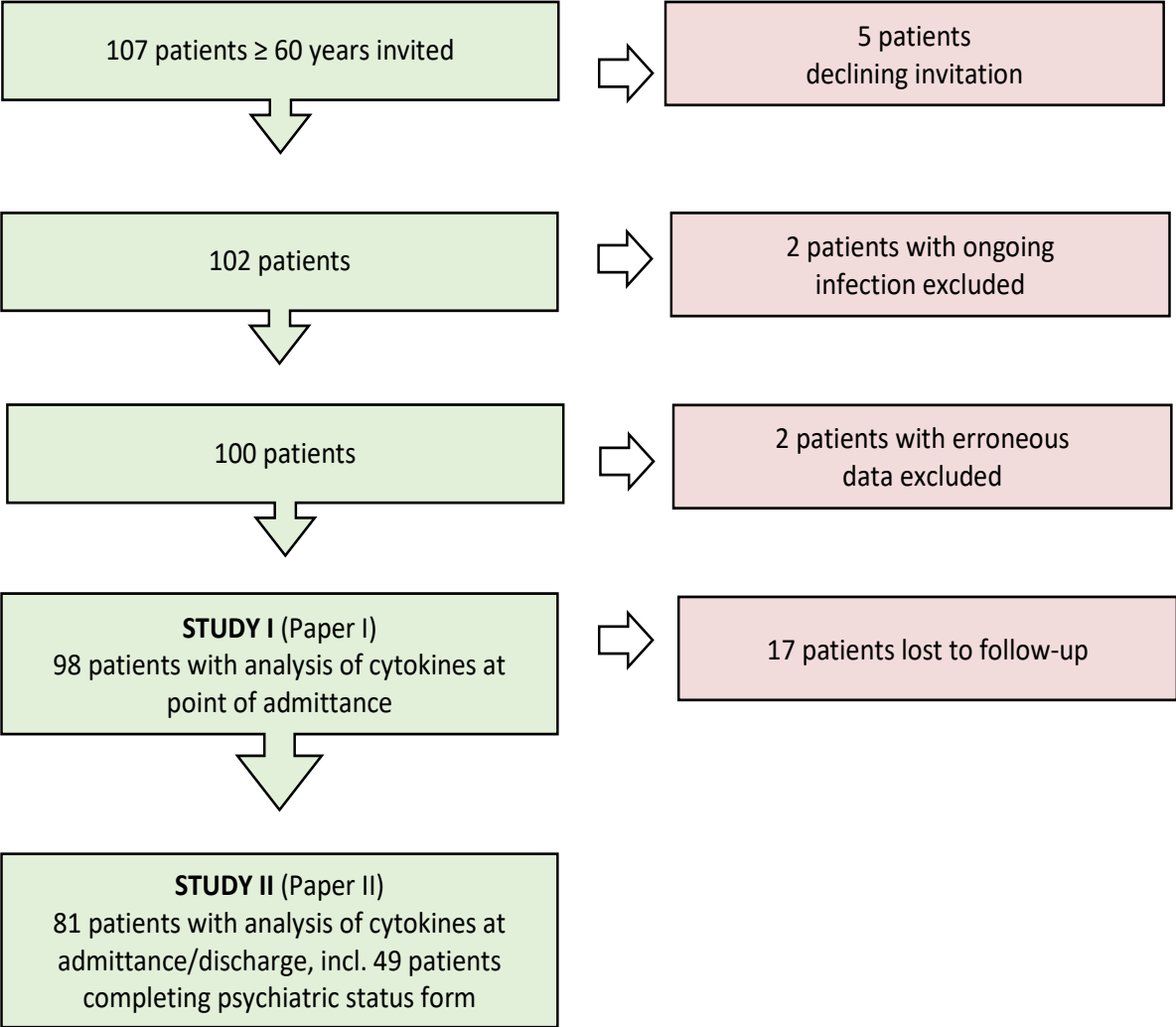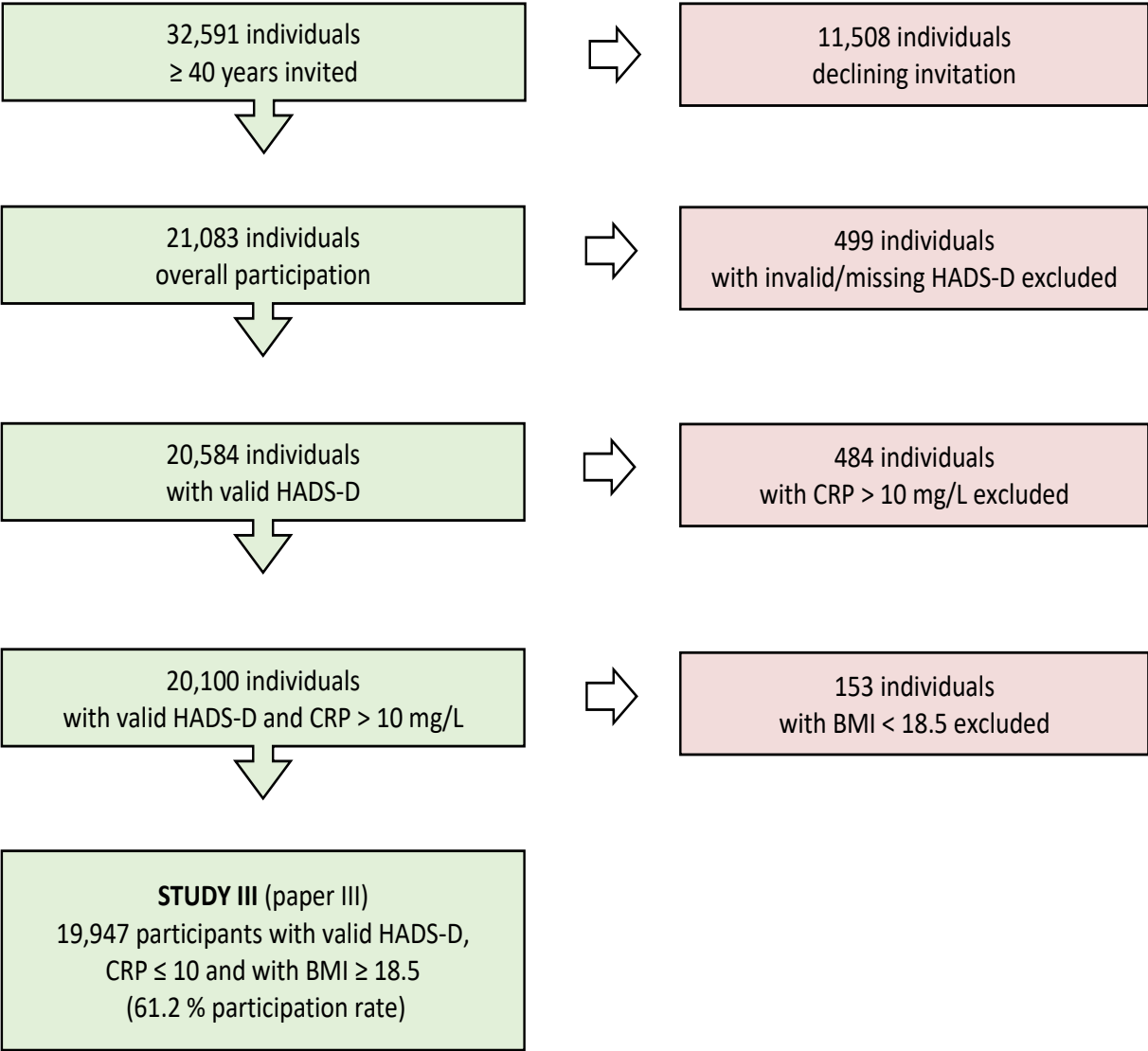Figure 2: Flow-chart inclusion/exclusion Study I & II

Figure 3: Flow-chart inclusion/exclusion Study III

7.5   Questionnaires

Study I & II: Within three days after admission, the patients were interviewed, using questionnaires, about their health status (physical and mental) and life style, incl. physical activity, education, smoking, alcohol, and diet/dietary supplements. Furthermore, by reviewing the medical records, data for the following variables were analysed: Age, sex, number of previous hospitalizations, reason for admission, length of stay, marital status, somatic disease, BMI, and medication.

Study III: Two questionnaires, Questionnaire Q1 (4 pages)[i] and Questionnaire Q2[j] (58 pages), were administered to all participants. Questionnaire Q1 covered health status (physical and mental), use of health services, medication, use of alcohol and tobacco, diet, physical activity, health anxiety, cancer, education & income, and social support. Questionnaire Q2 covered many of the same topics as Q1, but with a much broader and deeper scope, in addition to several special topics, such as headache, sleep, use of complimentary & alternative medicine, women's health, mental trauma etc. Questionnaire Q2 also included the Hospital Anxiety and Depression Scale (HADS). HADS is a self-rating scale, consisting of two, seven item subscales; one for anxiety, HADS-A, and one for depression, HADS-D. For the purpose of study III, the following variables were analysed: Age, sex, daily smoking, current somatic disease, having a partner or close friend, educational level, and HADS-D.

7.6   Clinical interview, examination and psychometrics

Study I & II: All patients underwent clinical interviews and examinations according to the standard procedure at the hospital. Interviews of next of kin were also undertaken when appropriate. Based on the interviews, examinations, reviews of medical records and use of psychometric tools (e.g. MINI and others), psychiatric diagnostics were performed by an experienced clinician.

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[i] Q1: https://uit.no/Content/686864/cache=20201407122756/Sporreskjema.Q1.engelskTromso7.pdf

[j] Q2: https://uit.no/Content/709325/cache=20202011171303/FINAL%20Q2%20translation20190307.pdf

Study III: All participants went through a basic examination, consisting of anthropometric measurements (height, weight, waist and hip circumference), blood pressure, heart rate and oxygen saturation, and biological sampling. Biological sampling included a broad spectrum of blood samples/analyses, for example CRP, and in sub-samples, nose-throat samples and saliva. In addition, a clinical examination of pain sensitivity was conducted on all participants, and a clinical dental examination was conducted on a sub-sample.

7.7   MINI (Study I and II)

The Mini International Neuropsychiatric Interview (MINI) was designed as a brief structured diagnostic interview for the major psychiatric disorders in the WHO diagnostic system ICD-10, and the American DSM-IV (120). MINI assesses the 17 most common psychiatric disorders, and is a shorter version of the somewhat more comprehensive MINI+, which includes 26 diagnostic items. In comparison to the *Structured Clinical Interview* for DSM-IV (SCID-IV) and the *Composite International Diagnostic Interview* for ICD-10 (CIDI), the MINI has reportedly good validity, with the exception of generalized anxiety, agoraphobia and bulimia (120).

7.8   Montgomery-Åsberg Depression Rating Scale (Study I and II)

The Montgomery–Åsberg Depression Rating Scale (MADRS) is a ten-item questionnaire used by clinicians to estimate the severity of depression. The questionnaire was originally developed for use in clinical trials, to monitor changes in depressive symptoms (121). The MADRS is relatively quick to administer, and focuses on core mood symptoms such as sadness, tension, lethargy, pessimistic thoughts, and suicidal thoughts. Each of the 10 items in MADRS can be graded from 0 (no symptoms) to 6 (most severe symptoms). MADRS has been validated as a screening instrument for depression in late life, with a suggested cut-off point of 16/17 (122).

7.9 Cornell Scale for Depression in Dementia (Study I and II)

The Cornell Scale for Depression in Dementia (CSDD) is a form used to screen for symptoms of depression in individuals with dementia (123). Unlike other scales and screens for depression, the CSDD takes into account signs of depression that might not be clearly verbalized by a person suffering from cognitive impairment. The rationale for the construction of the CSDD is that the assessment of depression in people with dementia is challenging due to deficits in concentration, memory and judgement, hence making it difficult to formulate a valid and precise response in a clinical interview. The CSDD is based on an interview and an observation of the patient, as well as information from the patient's caregiver. The scale consists of 19 items, and each item can be rated as *absent* = 0, *mild or intermittent* =1 and *severe* = 2. A total score of 8 and higher has been reported as a cut-off point for depression in older adults, both with and without dementia (124, 125).

7.10 Mini Mental State Examination (Study I & II)

The Mini Mental State Examination (MMSE) is a 30-point questionnaire to estimate cognitive impairment, commonly used to screen for dementia (126). The MMSE explores the following cognitive functions: orientation (time & place), registration (repeating named prompts), attention and calculation, recall (registration recall), language (naming an object), repetition (repeating a phrase), and ability to follow simple commands (drawing a figure shown). A score of 23 or lower has been reported to indicate the presence of cognitive impairment. The validity is considered satisfactory, with a high level of sensitivity for moderate-to-severe cognitive impairment and lower for mild degrees of impairment (127).

7.11 Clock-Drawing Test (Study I and II)

The Clock-Drawing Test (CDT) is a screening tool for cognitive impairment(128), where the patient is asked to draw a clock (or central parts of a clock). Several versions of the CDT have been developed. In our study, we used a Norwegian version of CDT, with a predrawn circle without numbers or hands; the patients were asked to set the time to ten past eleven. This

version has a maximum score of 5. The CDT has demonstrated a high correlation with MMSE and other cognitive tests (128), and has been validated in hospital settings and in older general population settings (129-131).

7.12 Hospital Anxiety and Depression Scale (Study III)

Originally developed for the assessment of psychological distress in non-psychiatric patients, the Hospital Anxiety and Depression Scale (HADS) is a self-rating scale, consisting of one 7-item subscale for anxiety, HADS-A, and one 7-itme subscale and one for depression, HADS-D (132). A Norwegian version of the HADS scale was used in the present study (133). HADS has been extensively validated in various populations, including the general population of older adults (134, 135).

In accordance with findings of previous studies, a score of HADS-D ≥ 8 and ≥ 11 was chosen as cut-offs for depression (i.e. all levels of depression) and moderate-severe depression, respectively (133, 136).

7.13 Laboratory analyses

7.13.1 Study I & II

Blood samples from the patients were analysed shortly after admittance for electrolytes, liver enzymes, blood cells, thyroid hormones etc. according to standard hospital procedures. In addition, plasma was analysed for 27 cytokines within the first three days after admittance, and at the day of discharge. Plasma for cytokine analysis were obtained from blood collected into EDTA-tubes, immediately placed on crushed ice, and then placed in a refrigerated centrifuge for 15 minutes at 3000 rpm. The samples were the rapidly frozen to -70 °C, until analysed using an immunoassay method, i.e. a Multiplex Analyser with a predefined kit (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories Inc., Hercules, CA).

The multiplex method, i.e. a fluorescent bead-based immunoassay, provides the opportunity to investigate multiple analytes at the same time, thus making it faster and cheaper than

ELISA (enzyme-linked immunosorbent assay), the gold standard of cytokine analyses. In addition, the multiplex method has demonstrated good correlation with ELISA (137, 138). Furthermore, it has acceptable variance coefficients (CV) for intra- and inter-assay analysis (139), as well as for intra-batch variance, but somewhat higher CV for inter-batch analyses (140). Therefore, all analyses were undertaken in one batch.

The multiplex technique requires experience and strict adherence to protocol, and multi-site comparisons have shown variability between different laboratories in their ability to quantify cytokines (141, 142). Still, there was good correlation between the different labs, in terms of the direction of change and the relative changes in cytokine levels. Consequently, the inter-batch and inter-lab variance makes comparisons between studies a precarious undertaking. Nevertheless, the method is well suited for estimation of relative, rather than absolute, changes in cytokine levels(143), and is as such, suitable for the purpose of study I and II.

The following cytokines were analysed: IL-1β, IL-1 receptor antagonist (IL1-ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ, interferon-inducible protein (IP-10), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1α, MIP-1β), platelet derived growth factor-BB (PDGF-BB), regulated upon activation T cell expressed and secreted (RANTES), tumour necrosis factor (TNF), and vascular endothelial growth factor (VEGF).

7.13.2 Study III

Blood samples from the participants were analysed for C-reactive protein (CRP), electrolytes, liver enzymes, kidney function tests, blood cells, thyroxine, etc. CRP in serum was analysed using the high-sensitivity technique, at the Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway. The analyses were executed by employing a particle enhanced immunoturbidimetric assay on a Modular P autoanalyzer (Roche Diagnostics, Mannheim, Germany), with a detection limit of 0.12 mg/L. CRP measured at this detection level is commonly referred to as *high-sensitivity C-reactive protein* (or just *hs-CRP*), as the standard detection limit in clinical settings is 5 mg/L. The method is the most widely

used analytic technology in clinical chemistry, with analytic performance well suited to determine levels of CRP with adequate precision (144, 145).

CRP value of 3 mg/L is broadly accepted as a cut-off point between normal and elevated serum levels, i.e. the majority of healthy adults have a serum concentration below 3 mg/L (146). Accordingly, CRP ≥ 3 mg/L was defined as elevated, signifying the presence of a low-grade, systemic inflammation (69). At the other end, participants with CRP > 10 mg/L (N = 484) were excluded due to the risk of an ongoing infection or a highly inflammatory condition, that eventually could skew the statistics (147, 148). In addition underweight individuals (N = 153), i.e. individuals with BMI < 18.5, were excluded, as underweight is known to compromise the immune system, thereby leading to aberrant inflammatory markers, such as CRP (149).

7.14   Statistical analyses

The IBM Statistical Package for the Social Sciences, Version 23 (SPSS Inc., Chicago, Illinois, USA) software was used in the statistical analysis.

7.14.1 Study I

Frequency tables were used to describe characteristics of the patients. The Kolmogorov-Smirnov test was used to evaluate distribution of the data; the data were not normally distributed and several groups had unequal variances. Accordingly, nonparametric tests were applied. The Spearman rank correlation coefficient was used to analyse differences between the rankings of two variables. The Mann-Whitney U or the Kruskal-Wallis tests were applied when comparing ranks of two or several subgroups. To examine possible relationships between depression/no depression, i.e. the dependent variable, and cytokines and other variables, i.e. the independent variables, we used a binary logistic model to calculate odds ratios. Due to multiple statistical analyses, 0.01 was selected as significance level. In addition, false detection rate adjusted p-value (FDR-p) was calculated and applied to all analyses related to the cytokines. A small group of patients had levels of cytokines below the measurement threshold of the instruments. Data for these so-called 'non-detects' were made by using the random number generator of SPSS, creating a random value between

zero and the lower detection limit, with a uniform distribution.

7.14.2 Study II

Frequency tables were used to describe characteristics of the patients. A chi-square test was applied to determine if there was a significant difference between two sets of data. The Kolmogorov-Smirnov test was used to evaluate the distribution of the data; the data were not normally distributed and several groups had unequal variances. Accordingly, nonparametric tests were applied. The Spearman rank correlation coefficient was used to analyse differences between the rankings of two variables. To test deviations from a theoretically expected distribution of a dichotomous variable, a binominal test was used. A p-value of 0.05 was defined as the threshold for statistical significance, but due to multiple statistical analyses, false detection rate adjusted p-values were calculated and applied to all analyses related to the cytokines. A small group of patients had levels of cytokines below the measurement threshold of the instruments. Data for these so-called ´non-detects´ were made by using the random number generator of SPSS, creating a random value between zero and the lower detection limit, with a uniform distribution.

7.14.3 Study III

Frequency tables were used to describe the characteristics of the patients. A chi-square test and a t-test were applied to determine if there was a significant difference between two sets of data. Test score reliability for the HADS-D scale was assessed by estimating Cronbach's alpha. Multicollinearity between the covariates was tested by creating a correlation matrix, as well as applying linear regression to calculate estimates of variance inflation (VIF). A binary logistic model, i.e. calculating odds ratios, was used to test associations between systemic inflammation/elevated CRP, i.e. the dependent variable, and depression/moderate-severe depression and age/age-groups, i.e. the two primary independent variables, and the various covariates.

8 Results

8.1 Study I

Paper 1: Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole Kristian Grønli: **Cytokine profiles and diagnoses in elderly, hospitalized psychiatric patients.**

BMC Psychiatry, Volume 18, September 2018.

Link: https://bmcpsychiatry.biomedcentral.com/articles/10.1186/s12888-018-1900-y

The aim of this study was to investigate the relationship between a panel of 27 cytokines and psychiatric diagnoses, as well as demographic, life-style, clinical, and biological data in diagnostically unselected psychiatric in-patients aged 60 years and older, admitted to a psychiatric hospital (N = 98).

The most common diagnosis was Recurrent depressive disorder (26.5%), the second most common was dementia in Alzheimer's disease (20.4%). The most frequent somatic disease was cardiovascular disease (28%).

We found no statistical association ($p < 0.01$) between cytokines and psychiatric diagnoses, gender, age, BMI, anti-inflammatory drugs, psychotropic drugs, reason for admittance, smoking, vitamin supplements, alcohol consumption, length of stay or somatic disease (present/not-present). However, when comparing depressed patients with non-depressed patients, we found higher levels of 10 cytokines in the non-depressed group (FDR-$p < 0.0044$[k]). Possibly, this could be explained by the higher prevalence of cardiovascular disease (CVD) and dementia in the non-depressed group, as these factors were significant predictors of patients being categorized as non-depressed in a logistic regression. There is also the possibility of a dilutional effect created by a general increase in the cytokines, triggered by the high frequency of psychiatric and somatic comorbidity in the subjects, consequently masking the effects of any one factor, depression included, on the cytokine levels.

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[k] In study I, we have called the adjusted p-value *False Detection Rate* p-value (FDR-P). *False Detection Rate* is synonymous with *False Discovery Rate*.

In conclusion, we found no significant difference in the cytokine levels between various psychiatric diagnoses in hospitalized elderly psychiatric patients. This indicates that previous findings of correlations between cytokines and homogenous psychiatric diagnoses - i.e. any somatic and psychiatric comorbidity excluded - might not be applicable to diagnostically unselected, elderly psychiatric inpatients, i.e. "real-life" patients.

8.2   Study II

Paper 2: Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole Kristian Grønli: **Changes in cytokines during treatment of elderly, hospitalized psychiatric patients – a naturalistic study**. Psychoneuroendocrinology, volume 108, October 2019.

Link: https://www.sciencedirect.com/science/article/pii/S0306453019304019

The aim of this study was to investigate changes in a panel of 27 cytokines during the treatment of diagnostically unselected psychiatric in-patients aged 60 years and older, admitted to a psychiatric hospital (N = 49). Next, to see if changes in cytokine levels during admission could be related to self-reported clinical outcome (improvement/no-improvement) and diagnoses, as well as demographic, life-style, clinical and biological data.

We found that the improvement-group were more likely to have a fall in cytokine levels than the no-improvement group (p < 0.032). There was also a positive correlation between clinical improvement and falling cytokine levels (p < 0.033), irrespective of psychiatric diagnoses, somatic diagnoses, and other variables.

I conclusion, we found that a broad range of cytokines fell during treatment, and the fall was associated with clinical improvement, but not with other variables. This indicates that exploitation of specific cytokines as biomarkers of clinical traits might be of limited use in a general population of elderly psychiatric in-patients as the field stands now.

8.3 Study III

paper 3: Erlend Bugge, Rolf Wynn, Tom Eirik Mollnes, Solveig Klæbo Reitan, Maria I. Lapid, Ole Kristian Grønli: **C-reactive protein levels and depression in younger and older adults - a study of 19,947** individuals. The Tromsø Study. Submitted.

The aim of this study was to investigate the association between systemic inflammation and two levels of depression, as well as selected covariates, in a community-based sample of 19,947 individuals, as we compared two age groups; ≥ 60 years (N = 8010) versus 40 - 59 years (N = 11 939). Systemic inflammation was defined as a CRP level in serum ≥ 3 mg/L. Depression (i.e. all levels of depression) and moderate-severe depression was defined as a score of HADS-D ≥ 8 and ≥ 11, respectively.

Participants with elevated CRP had a higher proportion of participants aged 60 and above, as well as a higher mean age, than those with normal CRP. Furthermore, participants with elevated CRP had a higher mean HADS-D, and a higher proportion of depression and moderate-severe depression, than participants with normal CRP.

In general, participants with depression, i.e. all levels of depression, and moderate-severe depression, were significantly more likely than non-depressed participants to have an elevated CRP, even when adjusting for several covariates (depression OR 1.62, CI 1.40 - 1.86, moderate-severe depression OR 2.00, CI 1.54 - 2.61).

Participants aged ≥ 60 years with depression, i.e. all levels of depression, and moderate-severe depression did not have higher odds of elevated CRP than participants without depression, when adjusted for covariates (depression OR 0.99, CI 0.75 - 1.30, moderate-severe depression OR 1.50, CI 0.87 - 2.58).

Participants aged 40 - 59 years with depression, i.e. all levels of depression, and moderate-severe depression, had higher odds of elevated CRP than participants without depression, when adjusted for covariates (depression OR 1.51, CI 1.24 - 1.84, moderate-severe depression OR 1.50, CI 1.03 - 2.17).

In conclusion, we found that depression in younger older adults is associated with an elevated CRP, while this is not the case for those participants aged 60 and older. The former is in accordance with previous studies, the latter indicates that inflammation is not a

prominent part of late-life depression in a general population of older adults.

9   Discussion – findings

9.1   Inflammation or no inflammation – that is the question

In study I, based on a real-life population of geriatric in-patients, we found no correlation between any of the cytokines and the clinical variables or diagnoses. There was, however, a correlation between *no depression* and 10 cytokines. It does not seem likely that *no depression*, i.e. the absence of depression, is related to inflammation. A more plausible explanation is that this correlation is an indirect effect of a higher frequency of cardiovascular disease and dementia in the *no depression* group, as both conditions are known to be associated with inflammation (114, 150), possibly with dysmetabolic processes as the common denominator (151, 152). In study II, based on the same population as study I, an intra-group comparison demonstrated a fall in a broad range of cytokines during treatment. At the same time, this decline in cytokine levels was associated with clinical improvement, irrespective of psychiatric and somatic diagnoses. In other words, study II, contrary to study I, indicates that there *is* a correlation between psychiatric disorders and inflammation, in elderly psychiatric patients. Specifically, the results of study II point to falling systemic cytokines as a possible indicator of clinical improvement. However, reviewing the results of study III, a population survey including almost 20,000 individuals aged 40 years and older, provides yet another result. In a first step, an overall logistic regression model demonstrated a correlation between depression and systemic inflammation, even when adjusting for several covariates. But, a separate analysis for participants 60 years and older, whom in general had higher levels of CRP than younger participants, provided *no* correlation between depression and systemic inflammation in a multi-adjusted model. Weighing the studies against one another, study I and II should be considered methodologically weaker than study III. Especially the fact that study II included only 60 % of the original group of patients, cast uncertainty over its results. Thus, the scale is tipping in favour of *no correlation* between psychiatric disorders - depression in particular - and inflammation in older adults.

Comparing the conflicting findings of this thesis with other studies does not provide any immediate clarity. As far as clinical studies on depression go, Thomas et al found a correlation between interleukin-1β (IL-1β) and depression in a group of 19 elderly patients,

but not in in the 41 controls. They also found that higher levels of IL-1β strongly correlated with depression severity (153). Similar findings were made by Diniz and colleagues in a group of 23 antidepressant-free elderly patients with late-life depression (154). In a study of 64 elderly inpatients with unipolar major depression, scheduled for electroconvulsive treatment, Gaarden and co-workers also demonstrated an association between several pro-inflammatory cytokines and depression (155). Nevertheless, all of the aforementioned clinical studies have, just like study I and II, some important shortcomings. Aside the small sample sizes, the risk of confounding is perhaps the most prominent limitation, as several important covariates are not included in their analyses; all of the aforementioned three studies - unlike study I and II - only partially adjust for covariates like somatic diseases, BMI, education, and marital status. Besides, there are other clinical studies that did not find any depression-inflammation correlation. Brambilla & Maggioni could not demonstrate any difference in plasma concentrations of pro-inflammatory cytokines between 10 elderly depressed women and 20 controls (156), neither could Saraykar & Diniz in 74 depressed older patients (157). However, a systematic review including depressed elderly patients from various settings (herein nursing homes, for instance) by Smith and co-workers, concluded there is a correlation between depression and markers of systemic inflammation, particularly cytokines like IL-8, IL-6, and TNF (158). There is, nonetheless, a problematic heterogeneity amongst the six studies included in this review, in terms of inflammatory parameters, mean age, covariates included, diagnostic method and follow-up period. For instance, two of the studies only include a one or two comorbid somatic disorders as covariates (159, 160), despite the fact that the majority of people over 65 years have more than two chronic somatic diseases (10). Another example from the review by Smith and colleagues, is that the mean age of the included studies ranges from 61 (159) to 85 years (161). This age range is likely to reflect very different levels of immunosenescence, with possibly very different effects on the level of systemic inflammation, perhaps even to the extent that the two populations should not be grouped together in a review focusing on inflammatory changes.

Auditing population-based studies produces somewhat similar results. Tiemeier and colleagues published a report from the Rotterdam Study, a population-based cohort study including 3884 adults aged 60 and over (106 participants with depressive disorder), stating

no association of CRP with depression after adjusting for covariates (162). This finding is in line with the results of study III, though the Rotterdam Study also reports of an adjusted relation between increased levels of IL-6 and depressive disorders. The authors themselves question this finding, though, as they recognize an important weakness of the study; only stroke is included as a somatic confounder. The conclude that "... *the associations of acute phase proteins with depression in this population-based study could be explained by confounding*". However, a later Dutch population study, the Longitudinal Aging Study Amsterdam, maintains the conclusion; an elevated plasma level of IL-6 *is* associated with an increased prevalence of major depression in older people, even when adjusted for covariates (163). In this study of 1285 participants aged 65 and over (38 participants with depressive disorder), variables like chronic somatic conditions and medications were included. Interestingly, the same study still aligns with study III on one point, in the sense that it did not find a connection between elevated plasma levels of CRP (above 3.2 mg/L) and depression. Neither did Gallagher and co-workers, reporting from ELSA, the English Longitudinal study of Ageing: CRP levels were not related to depression (164). The study included a sample of 807 participants aged 50 years and over (total number of participants = 7610), and the statistical analyses encompassed important covariates like BMI and chronic medical conditions. Then again, in another publication from ELSA, this time with 5909 participants ≥ 60 years, an adjusted analysis of depressive symptoms and their possible relation to inflammation reports *"… a significant dose-response association between C-reactive protein and the symptoms of fatigue (P < 0.001), restless sleep (P = 0.03), low energy (P = 0.02) and feeling depressed (P = 0.04), but not other symptoms*" (165). This study, focusing on selected depressive symptoms, echoed the result of at large Danish population study of 73,131 adults aged 20 – 100 years, concluding that "*Elevated levels of CRP are associated with increased risk for psychological distress and depression...*" (166). That being said, the Danish study did not investigate age-adjusted models, nor use validated diagnostic scoring scales for psychological distress and depression. Rather "… *symptoms of psychological distress possibly related to depression*" were targeted in the questionnaires, by using two questions: "*Do you have the feeling that you have not accomplished very much recently?*" (yes or no) and "*Do you feel like giving up?*" (yes or no). In addition, participants were asked if they used antidepressants or if they had been hospitalized for depression.

Though these questions are relevant to a possible depressive state, their sensitivity and specificity in terms of catching depression, are not known. At that, the questions are not tapping into the symptom spectre of geriatric depression (somatic symptoms, cognitive symptoms etc.), and are unlikely to outperform validated depression questionnaires. In total, this study shed little light on the inflammation-depression link in older adults. That being said, the dichotomous nature of most depression psychometrics (depression present/not present), might not be the only way to gauge depressive symptoms. Dimensional and combinational analyses of symptoms could catch important categories of depressed patients otherwise missed or misclassified by the use of psychometric tools alone. One study did both, i.e. apply the dichotomous categorization of depression (present/not present), as well as dimensional parameters of depression; the Brazilian Longitudinal Study of Adult Health (167). Though not specifically targeting late-life depression - the age span of the participants was 35 - 74 years, approx. 51 % were middle-aged between 45 - 58 years of age - nor performing subgroup analysis based on age, the study did analyse elevated levels of CRP in relation to various levels of depression, as well as depression with and without somatic symptoms. The latter is of particular interest, as somatic symptoms are more common in older depressed adults compared to younger adults (32, 168). 14,821 participants were assessed for depression by the use of the Clinical Interview Schedule-Revised (CIS-R), resulting in 626 participants with different levels of depression (mild, moderate, severe). Smoking, BMI, cardiovascular disease, kidney disease, cancer and diabetes were amongst the covariates included in the analyses. The result was negative: After adjustments for confounders, neither different levels of depression, nor depression with somatic symptoms, was statistically associated with elevated CRP levels. Then again, in regard to geriatric depression, the relevance of the study is uncertain, as it did not include age stratifications or subgrouping in the statistics.

The only review and meta-analysis focusing on inflammation in gero-depression apparently resolves the question, as the authors state "*We found evidence to indicate a weak but positive correlation between CRP and depression*…" in older adults (169). Yet, looking at the heterogenous cross-sectional studies included, their conclusion seems a bit overstretched, as 7 of the 17 studies that examined the least-adjusted relationship, and only 3 of the 17 studies that examined the most-adjusted relationship, found a positive association. In

addition, there are concerning methodological issues:

- Missing data on CRP, ranging from 17 to 42 %.

- Different prevalences of depression, even amongst studies using the same diagnostic tools, spanning from 2.7 to 27.3 %.

- Different CRP levels used as definition of an ongoing inflammation.

- Different ways of structuring the data (incl. various stratifications, groupings etc) in the statistical analyses, without any obvious clinical or scientific rationale.

- Different laboratory methods used to estimate CRP levels, e.g. some analyses were undertaken in plasma, others in serum, and some of the studies used immunoassay methodology better suited for intergroups comparisons rather than comparisons between studies (see section 7.13).

- Somatic disorders not included as covariates, alternatively including just one or two disorders, despite multimorbidity affecting the majority of people over 65 years (10).

In summary; both clinical and community-based studies on the association between inflammation and depression in older adults, are comparably few and far between, and their results conflicting. Like study I and II, the clinical studies tend to be small and prone to confounding, and do not inspire confidence in any one conclusion. In general, the community-based studies are methodologically sounder than the clinical ones, but are for the most part still characterized by significant methodological shortcomings (as outlined in the section above), leaving a question mark over their generalisability. Study III is, to our knowledge, the largest cross-sectional community-based study of inflammation in depression with comparative analyses of age-groups to date, with a total of 19,949 participants, of which 8010 were aged 60 and older. CRP analyses were undertaken for all participants, using well-established methodology and a broadly accepted cut-off value for inflammation (146). Depression were estimated by the use of HADS-D, which has a proven record in community studies (134), including studies on older persons (135). Furthermore, a spectre of relevant covariates was included in study III, herein a broad range of somatic disorders (alcohol and psychotropic drugs were excluded in the stepwise selection of covariates, as they did not turn out to be impacting factors). Not without its limitations, as I will discuss in chapter 10, study III still outperforms most other comparable studies, thus

substantiating that there is no association between depression and inflammation in the majority of depressed elderly.

9.2 There is nothing more deceptive than an obvious fact[I]

Despite the lack of evidence for inflammation as a vital part of most cases of depression in older adults, there are still findings to support that inflammation can be a factor in subgroups of depressed elderly, perhaps with differential roles and effects throughout the pathogenesis. The most compelling indication is perhaps that recent larger, longitudinal studies have found a correlation between inflammation and depression in older adults. For instance, the ARIC study (Atherosclerosis Risk in Communities Study Description), including 4,614 participants, found that chronic or repeated inflammation in the decades leading up to older adulthood is associated with late-life depression, even when adjusted for covariates like chronic somatic disorders (170). Comparable results were produced by Bondy and colleagues, reporting that baseline IL-6 and CRP were associated with elevated depressive symptoms in 1072 older adults at the follow-up sessions 4 and 6 years later (171). These findings point to inflammation as a risk factor, possibly even an aetiological factor, for late-life depression.

On a related note; inflammation lies at the heart of all neurodegenerative disorders, including the dementias (172) and there is a substantial neurobiological and immunological overlap between the neurodegenerative disorders and geriatric psychiatric disorders. For instance, neuronal dysregulation of the $Ca2^+$-signalling pathway has been implicated in the development of Alzheimer disease, late-onset bipolar disorder, and schizophrenia (173). Another example; depression is a common symptom in patients with Alzheimer's disease, Parkinson's disease and Huntington's disease, and there are several similarities between the neurobiological alterations in depression and these neurodegenerative disorders, including neuroinflammatory events contributing to neuronal atrophy in all these disorders (174). To provide a more specific example; patients with major depression and Alzheimer's disease both exhibits inflammatory changes mainly characterized by Th1 and Th17 inflammatory

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[I] Sir Arthur Conan Doyle: *The Adventures of Sherlock Holmes: The Boscombe Valley Mystery* (1891)

responses, with elevated levels of cytokines such as IL- 1β, TNF-α, IL-6, IL-18, IL-17A and IL-10 (175). Moreover, recurrent depression appears to be a neurodegenerative disorder in its own right, with decreased number of astrocytes, elevated inflammatory cytokines and activated microglias at the heart of the process (176-178). There are, of course, differences between most psychiatric disorders and the neurodegenerative disorders, but at some level there are common pathophysiological mechanisms, of which inflammation is one.

The numerous reports that have demonstrated a correlation between inflammation and various psychiatric disorders in younger adults also represents indirect evidence to support the inflammation hypothesis in at least subgroups of geriatric depression. Indeed, the research indicates that most, if not all, psychiatric disorders of a certain seriousness are accompanied by inflammatory changes (179). The most extensively researched condition is depression:

- Systemic inflammation, both humoral and cellular, is observed in depression (69, 180).
- Central inflammation, e.g. activation of microglias, is observed in depression (181).
- Patterns of inflammatory markers correlate with certain depressive phenotypes (182).
- Systemic inflammation in depression correlates with changes in specific brain regions (183).
- Systemic inflammation is a risk factor for depression (184).
- Level of systemic inflammation is correlated to the severity of the depression and reversely correlated to the treatment response (185).
- Remission of depression is accompanied by a normalization of inflammatory markers (186).

Adding to this chain of circumstantial evidence is the fact that inflammation is the primary defensive tool in all mammals; we share critical immunological mechanisms - the activation of white blood cells, the release of cytokines, the production of antibodies etc - to protect our biological machinery (187). Certainly, when inflammation occurs in younger adults with depression and other mental disorders, as well as in all humans irrespective of age in response to other threats (tissue damage, infection, etc), it seems unlikely that inflammation does not play any part, at any stage, in any form of depression in older adults.

9.3   Geriatric depression – same, but different?

One of the likely contributory factors to the heterogeneity of the results is - besides the sparsity of studies - differences in methodology, including study settings, subjects and procedures. For instance, the studies span from clinical research in hospitals and nursing homes, to community surveys, with an entry age of participants ranging from 50 to 85 years. As previously mentioned, a difference in participant age of 35 years, is likely to represent very different levels of age-related immune changes and inflammation. The use of different procedures and tools for diagnosis and assessment may also provide different results. To illustrate, here are the psychometric tools most commonly used to assess depression: HADS-D, MADRS (Montgomery and Åsberg Depression Rating Scale), HDRS (Hamilton Depression Rating Scale), GDS (Geriatric Depression Scale), CES-D (Centre for Epidemiological Studies-Depression scale), CIS-R (Clinical Interview Schedule-Revised), GHQ-30 (General Health Questionnaire), BDI (Becks Depression Inventory) etc. It is also worth mentioning that, with the exception of GDS, none of these psychometric methods are specifically designed to target late-life depression. Taking the HADS-D scale as an example. Used in study III to identify depression, the HADS-D scale is an effective screening tool for depression in general. However, HADS-D might not be ideal choice for identification of depression in elderly, as it does not include somatic symptoms (188), common in depressed older adults (32). Even so, the prevalence of depression in participants 60 years and older in study III (5,9 %) is comparable to prevalence estimates of geropsychiatric depression in general populations (189).

Pertaining to possible age-specific symptoms, there are reports of a differential association between inflammation and depressive symptoms, with somatic symptoms being the primary driver of the association (190), especially with increasing age (191). Aside the fact that the term *depression* covers a variety of depressive syndromes, this also raises the question whether depression in older adults is a different condition than that in younger adults. Phenomenologically related, but still different. For example, the differences in symptoms also includes higher rates of comorbid anxiety and cognitive impairment in elderly depressed individuals, as well as a more heterogenous aetiology with a lower genetic contribution (192). Another clue to geropsychiatric depression being a distinct subgroup of the depressive

disorders, perhaps primarily in aetiopathophysiological terms, comes from brain research; geriatric depression is associated with decreased brain volumes, particularly in frontal and temporal areas (193). Specifically, aging is accompanied by a disproportionate decline in volume and structural integrity of the striatum and frontostriatal circuits (194, 195), structures critical to the reward circuitry of the brain (196). The very same circuitry is affected both by depression and inflammation (197-199).

A short detour; some authors stress the distinction between depression debuting in old age, versus depression in older adults that originally debuted when they were younger. Accordingly, they call the first variant *late-life depression* (or *unipolar late-life depression*) or *geriatric depression*, and the second variant, simply, *depression*. The problem with insisting on *late-life depression* or *geriatric depression* as unique diagnostic entities, is that there is no consensus in terms of their threshold age, as the demarcating age in the literature spans from 50 to 65 years of age (158, 200, 201). Adding to this inconsistent brew, are other authors using the very same labels synonymous with any depressive state in older adults, regardless of the original debut age (202). Certainly, it might be very well justified to distinguish between depressive disorders based on the age of debut, but for now, there is no established nomenclature in the literature. Accordingly, I am using all the aforementioned labels interchangeably.

The reciprocity between depression and age-related processes also adds to the list of arguments for geriatric depression as a subcategory of depression. For one, it is well documented that a previous history of mental health problems is associated with subsequent medical disorders and accelerated aging (203, 204). Characteristically, depression lead to a functional decline typical of ageing, and a shortening of the telomere length – the very hallmark of aging (205, 206). Telomere shortening in aging is also closely linked to inflammation (207). Moreover, depression represents a risk factor for several age-related medical conditions, such as senile frailty, obesity, diabetes, cardiovascular and cerebrovascular disease (208-211). The inverse relation is also true; medical conditions represent risk factors for later depression, particularly medical conditions associated with activation of inflammatory pathways (184, 205, 212).

In summary, these findings support a hypothesis of geriatric depression being a subgroup of

depressive disorders, with age related degenerative processes and diseases, as well as immunosenescence and altered functioning of the psychoneuroimmunological system, as its unique constituents (some age-related changes in the immune system are briefly described in chapter 9.4).

9.4   Psychoneuroimmunology in older adults - a Pandoras box

If inflammation – of some kind, on some level, in some sub-groups – is connected with geropsychiatric disorders, what does that mean? For instance, how does the inflammation in geriatric depression relate to immunosenescence? To answer this question, one has to consider that immunosenescence might very well be an adaptive remodelling of the immune system in a trade-off for longevity. Data suggest that, without the existence of immunosenescence, the human life-span would be greatly shortened (213). Specifically, inflammaging triggers an anti-inflammatory response that can counteract the age-related pro-inflammatory environment, thereby prolonging life-span (214). To paraphrase; in an aging body, with a couple of chronic diseases, several degenerative processes, lots of senescent cells, a progressively leaky gut etc - a young, sprightly immune system would mobilize a massive inflammatory process to potentially lethal effect. The inhibitory effect of inflammaging could also affect a depression-related inflammation, i.e. dampening the inflammation, even to the extent that its systemic markers are not detectable. Subsequently, it is challenging for the researcher to identify the inflammatory contribution of a psychiatric disorder to a milieu already ripe with inflammation. In such a milieu, the modest inflammatory input of most depressions will be hard to measure, especially if the immunosenescence try to keep the overall inflammation at a minimum. The very same mechanism could be the reason why there is no correlation with depression (or other diagnoses) and inflammatory markers in neither of the three studies in this thesis. Taking study III as an example; a possible depression-contingent stimulus to increase the level of inflammation/CRP, is eventually nullified by the countering mechanisms of immunosenescence.

The use of indiscriminatory inflammation markers, such as CRP, IL-6, TNF, IL-1β and INF-γ, in ours and most other studies, does not make interpretations of results any easier. These molecules are the most commonly used inflammatory indicators in research, but they tend to be elevated in all stages of most inflammatory processes. Accordingly, the presence of one of these markers tells you that there is some sort of inflammation going on, but nothing about its origin or its stage. To remedy this, efforts have been made to connect specific patterns of inflammatory markers to specific mental disorders, or to establish state-related markers (e.g. acute versus chronic) or trait-related markers (e.g. suicidality)[72-74]. This approach may eventually pay dividends, but as of yet, we have not established any inflammatory fingerprints, e.g. specific patterns of markers, that is pathognomonic for depression or any other mental disorder, nor for any specific trait or state [179, 215]. If any such patterns are finally established, then there is the question if the immunological patterns of younger psychiatric patients are identical to those of older patients. The complexity does not end there. First, different inflammatory agents, including e.g. TNF and IL-6, have both pro-inflammatory and anti-inflammatory properties, depending on the stage of the inflammation and the tissue/cells they are acting upon. The effects of IL-6 also change as we age; parallel to the increasing levels of IL-6 as we grow older[m], IL-6 seem to take on a more anti-inflammatory role compared to that of younger individuals [216]. Second, there is the question of how the interaction between the immune system and the nervous system alters as we age, keeping in mind that neurons and microglias have receptors for cytokines, and immune cells have neuroreceptors [217-219]. For instance, numerous studies have demonstrated a transformation in the neuroimmune function accompanying aging, including a functional change in the blood-brain-barrier. Examples includes reduced microglial process speed [220], with weakened ability to clear misfolded proteins associated with neurodegeneration [221], easier passage through the aging blood-brain-barrier [222], and a cognitive decline promoted by circulating pro-inflammatory factors [223]. Further interactions and complications could have been added, as the psychoneuroimmunology of older people is a Pandoras box of connected and relevant aspects, angles and biases, several of which I have not yet addressed (immunomodulatory properties of drugs commonly used

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[m] IL- 6 has been called *"a cytokine for gerontologists"*.

by elderly, is one). This complexity makes research on depression in elderly particularly challenging, as illustrated by the partly conflicting results of this thesis.

9.5   Knowledge grows fast, understanding grows slow

Despite psychoneuroimmunology being a fast-growing field in psychiatric research, with ever finer details of its inner workings revealed, our overall understanding is growing disproportionally slow. We seem to know more and more about the minute particulars of the brain-immune system interaction, but our overall understanding of the interplay between the emotions, brain and the immune system is still at a rudimentary level (224). This discontinuity between how much we know, and how much we understand, is illustrated by the fact that no immunotherapy is yet established in psychiatry. Sure, several psychotropic drugs have immunomodulary effects, but if and how this adds to their effect, we do not know. Besides, for a large number of patients, the effect of psychotropic drugs is insufficient (225, 226). Certainly, there are some promising studies on the use of anti-inflammatory drugs against depression (227). Then again, a large randomised controlled trial of its kind, two drugs with anti-inflammatory properties failed to separate from placebo in reducing depressive symptom scores in a sample of patients with bipolar depression (228). Thirty years after the ground-breaking article "*The macrophage theory of depression*" by Robert S. Smith, we know more, a lot more, about the psychoneuroimmune system, but not nearly enough for it to have a clinical impact. To an even greater extent this is true for older adults; we know relatively less about the aged immune system, and even less about the immunology of geropsychiatric disorders. Indeed, we know less about any medical condition in older adults, than we do in younger adults, due to the fact that older individuals tend to be excluded from research.

At some level, it is puzzling that older adults are underrepresented in medical studies, given the fact that diseases in general, including e.g. cancer and heart disease, is closely related to age. Perhaps expectedly, this should lead to a prioritization of older adults in research. The explanation for why this in not the case, is most likely multifaceted. The possibility of discrimination based primarily on age, so-called ageism, is an explanation that cannot be

excluded. Possibly more importantly are the scientific standards of medical research itself. In order to limits the risk of biases, the ideal research group in a clinical trial, for instance, is drug-free, healthy and well-functioning. Naturally, recruiting sufficient number of candidates in their 70ties or 80ties that meet these requirements quickly becomes a challenge for even the most ardent gero-focused researcher. Besides, even if the older subjects seemingly are bursting with health, this does not exclude the inherent risk of aberrant physiological processes related to old age itself, eventually interfering with the results of a well-planned study. Consequently, medical studies tend to, by default, exclude older participants to maximise bias-control. This is particularly true for the very old, i.e. people 85 years and older, for which scientific reports are an exotic beast. The unintended consequence of this demand for scientific rigor is that the research findings may have uncertain relevance for those whom the findings are most likely to be applied on. Nevertheless, it would be a risky strategy to address this issue by soften the methodological requirements of medical research. Consequently, purposeful research adaptations are necessary in order to learn more about psychoneuroimmunology in elderly (see chapter 12 Future directions).

10 Discussion - methodology

10.1 Sampling and study design

As an introduction to the methodology of the studies, and particularly their validity and potential biases, a brief review of the sampling and study design is in order.

Study I & II: Methodologically, the sampling was of a non-probability nature, i.e. the sample was not randomly selected; the population was a purposive quota sample, in the sense that we selected a certain number of patients with certain characteristics, based on what we wanted to investigate. In terms of design, the studies had an observational, naturalistic design, which means that the study population represented a real-life population of geropsychiatric patients, receiving treatment as usual, without any further interventions, (notwithstanding that the study itself might be considered an intervention). No control groups were used in study I and II, mainly because the inter-batch variance of the cytokine analyses precludes reliable comparisons of different populations (se section 7.13.1). Furthermore, as data were collected at single point in time, the design was cross-sectional (thus precluding any causal analyses). Study II could also be characterized as a longitudinal study, as plasma cytokines were measured at admission and discharge, though the direction of the change in each plasma cytokine, i.e. *rise* or *fall*, were used as a dichotomous variable in the cross-sectional analyses.

Study III: Methodologically, the sampling was of a probability nature, i.e. that a large sample of a general population was randomly selected based on a single criterion (a cohort aged ≥ 40 years). The design of study III was observational, i.e. that no interventions were applied, and cross-sectional, as data were collected at single point in time (precludes any causal analyses).

NB! *Probability sampling* means that every member of a population has, in principle, an equal chance of being selected, i.e. participants of study III were selected *randomly* in methodological terms. That does not mean that it is absolutely random who, in the end, decides to participate. But, *ante factum* – imagining that we know nothing about the characteristics of a certain population we are planning to survey, except that all individuals

are 40 years and older, and we are inviting the entire population to participate – then all invited individuals would have, on a nominal level, an equal chance of participating. Consequently, the sample mode is labelled *random.* The fact some individuals are more likely to participate than others, is a problem that relates to results of the actual *selection process* (i.e. a selection bias), and not to the chosen *sampling method* per se. Another important point; the terms *random* and *randomly* used about the sampling method of a population study, must not be confused with the term *randomization* used about case-control studies. The former terms generally denote passive inclusion methods in an entire population (or large parts of an entire population), without the application of a specific design or technique for the selection/deselection or grouping of participants (NB! There are probability sampling methods that do use specific selection techniques, but that is outside the purview of this thesis). The latter denotes an active selection process in an already preselected group of participants, whereby a specific design, method or technique (e.g. toss of a coin) is applied to categorize or select/deselect participants, for instance to select participants for a treatment or a non-treatment group. In any case, the use of the terms *random*, *randomly* and *randomization* in medical research only reflect various degrees of arbitrariness, and not something that is truly random.

10.2   Validity and Bias

Some degree of error and bias is virtually unavoidable in science, particularly when studying humans, but the effects can be minimised by robust scientific methodology. Bias can be defined as "*systematic error introduced into sampling or testing by selecting or encouraging one outcome or answer over others*" (229). In other words, bias is the result of a systematic, not random, error in the design or conduct of a study. *Internal* and *external validity* are commonly used terms to describe the bias.

10.2.1 Internal validity

Definition: The internal validity of a study refers to how well it is conducted, that is, on its structure and procedures, and how rigorously it is performed. A high degree of internal validity means that the results are representative for the study population at hand. A

sufficient internal validity is a precondition for external validity. Three types of biases commonly affect the internal validity: **selection bias**, **information bias** and **confounding.**

SELECTION BIAS

Definition: Selection bias is present when "… *when individuals have different probabilities of being included in the study sample according to relevant study characteristics - namely, the exposure and outcome of interest*" (230). As a consequence, the relationship between exposure and disease differs between those included in the study and those potentially eligible for the study (including non-participants or non-responders).

Study I: The subjects of the study (N= 98) consisted of consecutively admitted patients to a gero-psychiatric ward during an 18-month period running from March 2010 until November 2011. However, patients admitted during the period of July were not included, due to the vacation of the researchers. Given the total duration of the inclusion period, and the fact that fewer patients are generally admitted in July, the inclusion pause of July is not likely to distort the main results of the study. Patients unable to cooperate and communicate, i.e. patients with severe dementia, were also excluded from the study, and a small number of patients (N = 5) did not want to participate. Given the relatively long period of inclusion (18 months), and the fact that only few patients declined to participate, the selection bias is presumably small.

Study II: The subjects of the study (N = 81) were drawn from the subjects in study I, but 17 patients did not have a cytokine analysis at discharge, as some declined, while others were lost due to insufficient procedures (particularly during the holidays). As far as the self-reported psychiatric status form is concerned, approximately 60 % (N = 49) of the patients completed the form at discharge. It is uncertain why so many patients (N = 32) did not fill out the form, but the assumption is that some of the patients declined to do so, while others just slipped through the cracks of an insufficiently established routine at the ward.

Compared to the total population (N=81), the population that completed the psychiatric status form at discharge (N=49) had a higher proportion of female patients, somewhat more patients with previous psychiatric hospitalization and a higher rate of patients with somatic

disease. This difference in group profile, combined with the 60 % participation rate, provides a selection bias risk, though we cannot estimate its potential direction and size.

Study III: The overall attendance rate in the Tromsø 7 survey was 65 % (21,083 participants of 32,591 invited - all inhabitants ≤ 40 years were invited to participate). The attendance rate in study III was 61.2 % (19,947 participants of 32,591), due to the exclusion of 1136 participants: 499 individuals with an invalid or missing score on the subscale for depression of the Hospital Anxiety and Depression Scale (HADS-D), 484 individuals with a serum C-reactive protein (CRP) higher than 10 mg/L and 153 individuals with BMI < 18.5.

In general, a large number of participants tends to decreases the likelihood of a skewed inclusion. Nevertheless, a large number of participants does not, irrespective of participation rate and participants profile, prevent selection bias. Pertaining to the profile, it is well known that women, married/cohabitants, people from higher socio-economic classes, and healthier people, are more likely to attend population surveys (231). As far as sex differences are concerned, the Tromsø 7 survey (N = 21 083) had an overall higher attendance of women (67.0 %) compared to men (62.4 %), resulting in a distribution of 52.5 % women and 47.5 % men. The sex distribution in our study (N = 19,947) was almost identical (52.3 % women, 47.7 % men) to the overall distribution. Clearly, this sex difference could represent a selection bias, and may have impacted the analyses, and in a logistic regression model, we did find that being male is associated with lower odds of systemic inflammation. This is, nevertheless, in concordance with most other studies, demonstrating that various populations of men tend to have lower CRP-levels than women (232-234).

The biggest difference in participation rate between men and women was found is in those aged 80 and older, as 2-3 times more men than women attended in this age group. Still, constructing a logistic regression model, excluding participants 80 years and older, did not affect the main findings of the study. However, the participation rate of those being 80 years and older was small, just 39.1 %, as was their proportion of totals participants; 3.6 % (proportion of people ≥ 80 years as a percentage of all invited inhabitants = 5.9 %).

In any case, the focal point of the study is to compare younger with older participants, and the difference in attendance rate between these age groups was less than 1 %; 61.7 % in

individuals ≤ 60 years and 60.8 % for individuals aged 40 – 59 years.

We do not know the profile of the non-attenders in the Tromsø 7 survey. From the Tromsø 2 survey (participation rate 77.5 %), we learned that the non-attenders had higher prevalence of psychiatric disorders, particularly substance abuse, than the attenders (235). A study of non-attenders in the Tromsø 6 survey (participation rate 66 %) demonstrated a that they were younger, with a lower proportion of married/cohabitants, compared to the attenders (236). It is reasonable to assume that these selection biases, to some extent, are present in the Tromsø 7 survey as well. But, for those below 60 years of age, it is not very likely that a somewhat higher proportion of lonely people with failing health (physical and mental) and lower education would have changed the overall outcome of our study, because these factors were indeed independently associated with elevated CRP in the study (see table 2 in paper 3). This finding is also in accordance with previous studies (237, 238). So, if more non-attenders had attended, this would probably just feed into the pre-existing findings - more of the same would produce the same.

For those 60 years and older, it is difficult to make predictions about the impact of more non-attenders being included in the study, one reason being that we know less about older non-attenders, than we do younger non-attenders. For example, the study of non-attenders in the Tromsø 2 study did not include people 55 years and older. Still, other studies inform us that the older non-attenders have one thing in common with younger non-attenders; they tend to have more health problems (239, 240). No surprise then, that hardly any institutionalized, very old people, i.e. people with ill-health in need of constant care, took part in the Tromsø 7 survey, as illustrated by the declining participation rate as people get older: 53 % in age group 80 – 84 years, 30 % in age group 85 – 89 years, and 16 % in age group 90 – 94 years. Any effects of adjusting for this selection bias is hard to predict, meaning, that is hard to estimate how a higher participation rate of the oldest old[n] - with multiple sources of inflammation, but counteracting immunosenescence - would affect a possible inflammation-depression link. On one hand, the immune system of the oldest old deals with multimorbidity and senescent cells, on the other hand, the very same system try

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[n] Older adults are sometimes classified in three groups, according to age: the *youngest-old*, aged 65 to 74 years, middle-*old*, 75 to 84 years; and *oldest-old*, ≥85 years.

to keep a balanced inflammatory response to avoid detrimental consequences (241). The net effect of this on the systemic CRP level is very difficult to estimate, thus leaving us without any inkling as to what a higher fraction of participating elderly, particularly the oldest-old, would mean to the overall result of the study.

The challenges facing the immune system of the oldest-old, also raises the question whether the oldest old should be considered a research population of its own in inflammation studies, rather than being incorporated in any population being defined as "old" or "older".

INFORMATION BIAS

Definition: Information bias can be defined as "… *a systematic tendency for individual selected for inclusion in the study to be erroneously placed in different exposure/outcome categories, thus leading to misclassification*" (230).

Study I & II: With the exception of the self-reported psychiatric status at discharge, the classification of the patients was undertaken by experienced investigators, well versed in the methodology of the study, including the diagnostic and psychometric procedures.

The Norwegian version of the diagnostic interview, MINI, has not been validated in the Norwegian population of elderly, but it has been used in several Norwegian studies (242, 243). Still, MINI was just one of several sources in the diagnostic process, as information from next of kin, staff, and medical records were all used to establish a psychiatric diagnosis. In addition, other psychometric tools such as MADRS, MMSE, Clock drawing test etc. were used in the clinical assessment. On top of this, the limited number of patients should prima facie also mean that it is easier to uphold a high and stable standard in the clinical assessments. Hence, the likelihood of diagnostic misclassification is relatively small.

Data for patient characteristics (somatic diseases, previous hospitalizations, drugs etc) were retrieved from medical records, in conjunction with interviews of the patients.

Information about daily smoking was attained during clinical interviews with the patients. Daily smoking was categorized as a dichotomous variable (present daily smoking yes/no), and is not considered to be prone to misclassification (very low recollection bias, for

instance).

The self-reported psychiatric status was reported using a self-composed questionnaire (as described in section 6.2 Subjects in study II). The questionnaire does not have any prior merit or testing, and consequently, we do not know its properties (validity and reliability), and in particular how it performs in comparison to similar, well-proven questionnaires. Nevertheless, the self-report form is extremely short and simple, with only five relatively unambiguous categories, and in the analyses, the five categories were collapsed into two mutually exclusive categories, thereby reducing the risk of misclassification.

Recall bias may represent a challenge in geropsychiatric research due to primary or secondary affection of cognitive capability. In study I and II, however, most of the information in these studies was collected from multiple sources and records, during a median length of stay of 34 days, and accordingly the risk of information bias is generally considered low.

Study III: Misclassification is an important issue in large population surveys, as most of the information tends to be based on questionnaires. Questions from a more distant past or concerning minor events are more vulnerable to recall/reporting bias, even when the subjects are conscious about the bias risk (244). The risk of recall/reporting bias also depends on the nature of the questions. Questions about the presence of a specific disease (e.g. *Do you have diabetes?*) is less susceptible to bias than questions about the amount of vegetables eaten per week.

The self-reported variables in study III are daily smoking, current somatic disease, partner or close friend and level of education. This type of information is cognitively readily accessible to most participants, and the questions are not of a particularly sensitive nature for someone who has made a conscious decision to partake in a health survey (245). In conclusion, the risk of information bias in study III must be considered low.

The HADS depression subscale (HADS-D) was used to determine presence and level of depression. HADS is one the most widely used questionnaires for the assessment of anxiety and depression in medical research. Based on previous studies, a score of HADS-D ≥ 8 and $\geq$ 11 was chosen as cut-offs for *depression,* i.e. all levels of depression, and *moderate-severe*

depression, respectively (133, 136). A cut-off score of ≥ 8 on the HADS-D has been observed to have a sensitivity of 74 - 80 % and a specificity of 84 - 88 % (136, 188), whilst a cut-off score of 11 provides a sensitivity of 38 - 44 % and a specificity of 95 - 97 % (188, 246)*. Ergo, there is a risk that a quarter of all depressed individuals (HADS-D ≥ 8) went undetected in the study. Then there is the question of how suitable HADS-D as a depression screening tool in older adults. Though HADS-D has been validated in populations of elderly (134, 135) , it does not include symptoms frequent in in older depressed adults, particularly somatic symptoms, such as fatigue, pain and sleep disturbance (188, 247). Combined with the sensitivity issues of HADS-D, this leaves a question mark over the suitability of HADS-D in geropsychiatric research. That being said, the overall prevalence of depression in participants 60 years and older in this study (5,9 %) does not deviate from prevalence estimates of geropsychiatric depression in general populations (189).

CONFOUNDING

Definition: Confounding can be defined as "… *distortion of an exposure-outcome association brought about by the association of another factor with both outcome and exposure*" (230) - "another factor" is normally referred to as a confounder. Confounding may take the effect of weakening, strengthening, inducing or eliminating an association between two variables. In experimental research, confounding is typically counteracted by randomization, whereas in observational studies, confounders can be adjusted for by stratification of data, and adjustments in multivariate analytic models.

Study I & II: The research populations were non-random samples. In the statistical analyses in study I, we made stratifications and various bivariate correlations. Based on the bivariate analyses, we constructed a logistic regression model. A specific analysis for confounding between the variables in the model demonstrated a low risk of confounding (less than 10 % change in odds ratio). In study II, we also made stratifications and various bivariate analyses, but as we found significant correlations only between two variables, no multivariate analytic model could be made.

For study I and II, the major risk of confounding is presumably related to unidentified

factors/confounders, as the studies are naturalistic with few exclusion criteria. The naturalistic design provides a research sample more likely to be representative of real-life populations, but the broad inclusion entails a risk of a highly heterogenous sample, herein the possibility of unidentified confounders being present. This is a particular risk in research on older adults, where comorbidity is common and there is a greater variability in health status (248-250) .

In conclusion, there is a risk of confounding in study I and II, as the naturalistic design involves lesser control of confounding variables.

Study III: In the statistical analyses, stratification, bivariate analysis and two multivariate models, i.e. logistic regression models, were applied. In the logistic regression model, a specific analysis for confounding between the variables in the models demonstrated a low risk of confounding (less than 10 % change in odds ratio). Accordingly, the risk of confounding is considered low.

10.2.2 External validity

Definition: The external validity of a study refers to how applicable the findings of the study are to the real world, or more specifically, how the findings are applicable to populations comparable to the study population. The term *generalisability* is a term commonly used as a synonym to external validity. External validity assumes sufficient internal validity.

Study I & II: Participants of study I and II were recruited from both rural and urban areas of the regions of Finnmark, Troms and Ofoten. These regions include approximately 53 % of all inhabitants of Northern Norway[o], and is demographically and culturally comparable to the rest of Northern Norway (251). The number of patients included in the studies (study I, N = 81, study II, N = 81/49) are relatively small, which is intrinsically a methodological weakness. Still, since the receiving ward is the only one of its kind in the area, it is likely that most regional gero-psychiatric patients in need of admittance during the 18-month inclusion period, were actually received at our ward.

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[o] Northern Norway is comprised of the counties of Nordland, Troms and Finnmark. The region of Ofoten - the northernmost part of Nordland - was included in study I and II.

In 2012, the Norwegian Board of Health published a mapping study of geropsychiatric wards and outpatients' services in Norway (31). The distribution of men/women, common diagnoses and age in this national study reflects the participant profile of study I. The exception, of course, are patients with severe forms of dementia, as these patients were excluded from study I.

The initial population of study II is identical to that of study I (N = 81), but the so-called *outcome population* (N = 49, i.e. those competing the self-reported psychiatric status form) differs in at least two aspects from the national statistics; there is a higher proportion of women and patients with affective disorders.

Table 5 presents patient profiles in the Norwegian mapping study, as well as in study I and II (*Table 5: Patients profiles - Norwegian mapping study, study I & II*).

Based on this information, it is a reasonable assumption that the results from study I are, at the very least, applicable to geropsychiatric in-patients of Norther Norway, and possibly to geropsychiatric in-patient of Norway in general. Extrapolation of the results to other countries is a precarious undertaking, as differences in health care services, life-style, culture, morbidity etc. may impact the characteristics of the patients.

As for study II, it is uncertain whether the results have generalisability beyond the actual group investigated. First, there are important differences between the participants of the study and the national population of geropsychiatric in-patients, and second, the attendance is rather low (60 %). It is worth noting, though, that the major finding of study II – falling levels of inflammatory markers correlating with clinical improvement – is in concordance with results from most other studies on various disorders and treatments in younger adults (252-257). This could be coincidental, but it its nevertheless interesting that the inflammatory changes during treatment of elderly reflects that of younger adults.

Study III: Study III is based on data from the Tromsø study. The population of Tromsø municipality is younger and somewhat better educated than the Norwegian average, but is roughly equivalent to the national statistics in terms of the unemployment rate, the proportion of disability pensioners, income per capita, and the proportion living in urban areas (258). The comparably younger population of Tromsø is not going to affect the

generalisability, in view of the fact that the study only includes those aged 40 and older, and that the statistical analyses were based on age stratified or grouped data. A slightly better educated population will arguably lower the morbidity rate, but is unlikely to nullify or reverse the direction of the results, as demonstrated in the multivariate analysis. Thus, it is a reasonable conjecture that the findings of study III have external validity on a national level, most likely/perhaps also on a Scandinavian level, assuming basic cultural and socio-economic similarities between the countries. Beyond that, caution should be exercised when extrapolating the results to other populations and regions.

Table 5: Patients profiles - Norwegian mapping study, study I & II (percent)

	Norwegian in-patients*	Study I participants	Study II participants**
Age ≥ 80 years	36	39.8	38
Women	65	61.2	71
Affective disorders	43	41.8	53
Dementia	53	20.4	21

* From a national mapping study of geropsychiatric wards and outpatients' services in Norway
* * Outcome population (N = 49)

11 Clinical implications

In terms of clinical implications, this thesis demonstrates that systemic inflammatory markers are not yet applicable as tools in the diagnosis and assessment of psychiatric disorders, especially depression, in older adults. Correspondingly, no specific part of the inflammatory process stands out as a target for therapeutic interventions. Certainly, a similar conclusion is also valid for younger adults and even many medical disorders, i.e. we have known for a long time that inflammation is a part of just about any disease, but this knowledge has to varying degree assisted us in clinical management. In psychiatry, we have certainly gained a lot of psychoneuroimmunological knowledge in the past decades, but there are still many missing pieces of the puzzle. For one, we do not know the extent of inflammation in various groups of psychiatric patients. Taking depression as an example, there is evidence that the inflammatory level varies between patients with depression, possibly reflecting differences in for instance phenotype, aetiology, severity and age (69). Consequently, the benefit of anti-inflammatory strategies could depend on the features of the patient, the disorder and the inflammatory process. In other words; inflammation could be a target for both preventive and therapeutic interventions in psychiatry, but it is not likely that anti-inflammatory strategies are going to be a clinical panacea.

Sticking to depression, there are some preventive, anti-inflammatory strategies that could be useful. An anti-inflammatory diet - i.e. vegetables (particularly leafy green), nuts, fatty fish, berries, low intake of red meat etc - may protect from depression, as there is evidence to support an association between a pro-inflammatory diet and risk of depression (259). Calorie restriction and fasting may also be beneficial (260), though their general applicability is perhaps questionable. The trio sleep, exercise & social activity tend to be regulars at the lists of good things to do for your health, including mental health. Their antidepressant effects are, at least partially, contingent on their immunomodulatory effects (237, 261, 262). The problem with several of the cross-sectional studies on positive health factors are the clustering effect of health promoting behaviours, socio-economic factors and epigenomics, making it difficult to single out the effect of a single factor. Hence, all the aforementioned anti-inflammatory strategies are likely to have some positive and additive effects on both

the mental and physical health, leading to the superfluous conclusion that living healthy is god for you.

Studies on anti-inflammatory drug treatment of depression (aside the anti-inflammatory properties of some psychotropics) have shown promising results. RCTs have shown that NSAIDs, particularly the COX-2 inhibitors, have effect on remission and response rates of depression, both as monotherapy and in combination with antidepressants (263). Cytokine-inhibitors, and drugs with anti-inflammatory properties such as statins (lipid-lowering drugs) and minocycline (a tetracycline antibiotic), have also shown antidepressant effect in RCTs (227, 264). It should be noted, though, that the studies are small and the antidepressant effect of these drugs is modest. In addition, the largest RCT to date, including 1542 patients with bipolar depression, found no evidence of minocycline or celecoxib being superior to placebo (228). The question is, of course, whether the inflammatory levels are different between bipolar depression and major depression, the latter being the focal point of most clinical trials.

In short, anti-inflammatory drugs show some promise, but there is still a way to go before targeted immunomodulatory drug regimens are available in psychiatry. To a greater extent, this is true for the older adults, as their representation in research is still lagging behind that of the younger adults.

12 Directions for further research

12.1 Research is formalized curiosity[p]

Undoubtedly, animal models have been, and will continue to be, very useful in research on ageing and immunology. In fact, most of our understanding of the immune system comes from animal models. However, results from studies on genetically manipulated, laboratory bred, ultra-hygienic mice - the most commonly used experimental animal - cannot be uncritically translated to humans. There are numerous lessons of the inadequacy of animal models in human medicine (265, 266), exemplified by the fact that corticosteroids are widely teratogenic in animals, but not in humans (267), and that thalidomide is teratogenic in humans, but not in rodents (268). In any case, results from animal studies has to be confirmed by human research. This is particularly necessary in geropsychiatric research, for at least a couple of reasons. First, there is the long-term exposure to a broad range of pathogens and environmental substances during the life span of a human, that is difficult to replicate in short-lived animals. Second, it is virtually impossible to create a reliable animal model of the human interaction between the social, psychological, neuronal and immunological factors. This does not mean that useful approximations cannot be made by using more "natural" animal models, like the use of *dirty mice* (269), but here, I will confine myself to human research. Evading the myriad of related technical, methodological and economic issues, including which variables to select, I will focus on depression, as I will try outline a few principal design features of future research. These suggested design features and methods are meant to remedy *some* of the major shortcomings of present observational studies, without pretending to be an exhausting account of design challenges, and unanswered research questions.

12.2 Longitudinal studies

To learn more about the psychoneuroimmunology of older adults, we have to start with younger adults, i.e. we have to do longitudinal studies to track changes in the

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[p] Hurston, Z. N. (1944). Dust tracks on a road, an autobiography. London, New York etc.: Hutchinson & co.

psychoneuroimmune system as a whole, as well changes in its constituent part, from adulthood into old age. This will enable us to discriminate between normal age-related changes, and changes originating from aberrant, pathological processes, as well as map out the sequence of events and interactions. A longitudinal study will also contribute to counteract reverse causality (Is depression causing inflammation, or inflammation causing depression?). The entry age of a longitudinal study should be no more than 40 years. Preferably entry age should be lower than 40 years of age - the younger, the better - but an entry age higher than 40 years, involves an increasing risk of senescent transformations, particularly in the brain (270). Ideally, the studies should be life-long, i.e. until participants decease, with a post-mortem investigation of relevant tissues to get a definitive end-point. At the very least the studies should run until the age of 70 years, because this will ensure that significant senescent changes are present in all participants. In addition, this will enable us to find out more about healthy aging – mentally, cerebrally, immunologically - as a proportion of the septuagenarians will still be in good health (271).

A single-time point measurement of a small number of immunological indicators could be misleading, due to temporary fluctuations in inflammation caused by environmental factors and circadian rhythm, regardless of somatic health status. Consequently, a longitudinal study must involve a spectre of indicators measured at multiple time points, in order to accurately assess inflammatory status.

12.3   Diagnosis, trait and state

Variability in the diagnosis of depression is a major contributor to the contrasting findings in the research literature. This variability is partly originating from the extensive use of diagnostic tools primarily developed for younger adults, that does not encompass symptoms more common in depressed elderly, like agitation and somatic symptoms (32). Therefore, assessment of depression and depression severity should include typical symptoms of geropsychiatric depression, as well as atypical symptoms of depression (hypersomnia, weight gain etc.). Another source of research heterogeneity is that depression is not *one* disorder, but a group of disorders. Hence, lumping all patients into one case group labelled

depression will deprive us of the opportunity to differentiate between state (degree of severity), trait (constellation of symptoms) and single symptoms, and their possible correlation to systemic/central inflammation, endocrine and neurobiological functioning. Thus, instead of just defining a dichotomous threshold for depression (present or not), an array of depressive symptoms should be monitored. Related to this, is should be pointed out that that some depressive symptoms seem to correlate stronger with inflammation than others (272).

12.4   Some aspects of selected variables

A longitudinal study should include a selection of static (structural) and dynamic (functional) variables that encompasses the brain (including the blood-brain-barrier), the immune system (molecules and cells), the endocrine system (HPA-axis), the somatic status (biometric data, diseases, medication, drugs etc) and the mental health of the participants (including stress and cognitive functioning). Ideally, life style factors, sociodemographic/-economic data, environmental factors, and genetic factors, should also be mapped and monitored. Monitoring these variables over time, could enable us to see the temporality of events, the precedents and the antecedents, the interactions, the mediators and the moderators.

A mediator-moderator model is presented in figure 4 (*Figure 4: Mediator-moderator model*).

The statistics of a mediator-moderator model are described in section 9.6.5.

For risk-factors of depression known to be associated with a specific genotype, a Mendelian randomization should be considered (273). Depression itself is a polygenic condition influenced by many genetic variants, each of small effect (274).

The challenge is, of course, what variables to choose, as every research project has its limitations, being scientific, practical or economic. In the end, a limited number of variables has to be picked, but as for inflammation, using only a small set of pan-inflammatory markers (e.g. CRP, IL-6, TNF) is unlikely to be sufficient, as this will make it difficult to entangle specific changes from the general ones. Currently, most studies only look at a small subset of cytokines and/or cytokine receptors. Hence, there is, a need for comprehensive analyses of cytokines and cytokine receptor, as we do not know of any specific marker(s)

sensitive primarily to age-related inflammation, or inflammation connected to psychiatric disorders.

Inflammatory markers in blood reflects the overall health of a person, but do not allow for a more precise estimate of the inflammatory status of the brain/CNS. Hence, inflammatory markers should be measured in both blood and cerebrospinal fluid to get a more complete picture of the immunological status of participants.

Multiplex technology is the most widely used method for the estimation of humoral inflammation markers, as it makes it possible to measure several analytes in a single sample (50 analytes is offered by some manufacturers). There is, however, a problematic inter-lab variability that makes direct comparison between research findings challenging. For one, there is a lack of standards in both material, methods and measurements in the development of the tests, resulting in tests from different manufacturers not correlating with one another. Further complications arise from the fact that strict adherence to prescribed protocols is necessary to get a correct result, as some cytokines are sensitive to sample handling and processing methods. New and revised ELISA-methods have been developed, now allowing for a handful of cytokines to be analysed at one time (Aldo et al., 2016; Liu et al., 2021), and it may catch up with multiplex, eventually. Until that happens there is a need for scientific and industrial standards to be established - possibly proficiency training for laboratories, as well – so that results from different studies can be confidently compared.

In regards to the brain, static investigative technology, such as cerebral CT, is of course useful, but it does not provide the particulars about the process behind observed structural changes. For that, functional imaging, such as fPET or fMRI, are necessary. Especially interesting is the recent development of technology capable of simultaneous dynamic PET/MRI acquisitions. Combination of multi-modal fMRI measures and neuroreceptor PET gives us an unprecedented opportunity to study neurotransmission through multiple lenses in the living brain (275, 276), and may represent the future for dynamic studies on neurotransmission and neurometabolism in depression (277).

12.5   Computer modelling

As previously mentioned, we have attained a large body of knowledge about the brain and the immune system, but we have not yet been able to assemble this knowledge into an (comprehensive) overall psychoneuroimmunological model. A longitudinal study has the potential of getting us a step further, as this design has the ability to distinguish between age and cohort effects, detect development and patternicity, provide insights into causal mechanisms and processes, reduce recall bias etc. Analyses of longitudinal data is, however, complicated, particular when handling data of a multifaceted mediator-moderator model over time. To achieve this, a statistical model has to allow time-varying variables (e.g. age, BMI) and time-invariant variables (e.g. gender, genotype), and handle irregularly timed and missing data, without the need for explicit imputation. Two candidates that fulfils these statistical criteria's are the *generalized estimating equations model* and the *mixed effects model* (278). But, despite the usefulness of statistical methods, they will only bring us so far, as to construct a holistic understanding of the psychoneuroimmune system. Provided the stupendous complexity of the mechanisms involved, statistics can deliver the building blocks of understanding, but cannot themselves create a comprehensive conceptualisation. To do this, we will need the help of computer technology, i.e. construct computer models based on generated data from studies, and then apply learning algorithms to elucidate how the systems interacts (279). This will enable us to make sense of large amounts of data in complex, integrated systems, for instance the identification of complex and high-dimensional patterns in immune responses. Over time, as we put in more data and tweak the models, the models will become more accurate and reliable. In time, hopefully, the computer models themselves can be applied in research, for instance to provide easy and fast predictions of interventions by the use of artificial intelligence.

Figure 4: Mediator-moderator model
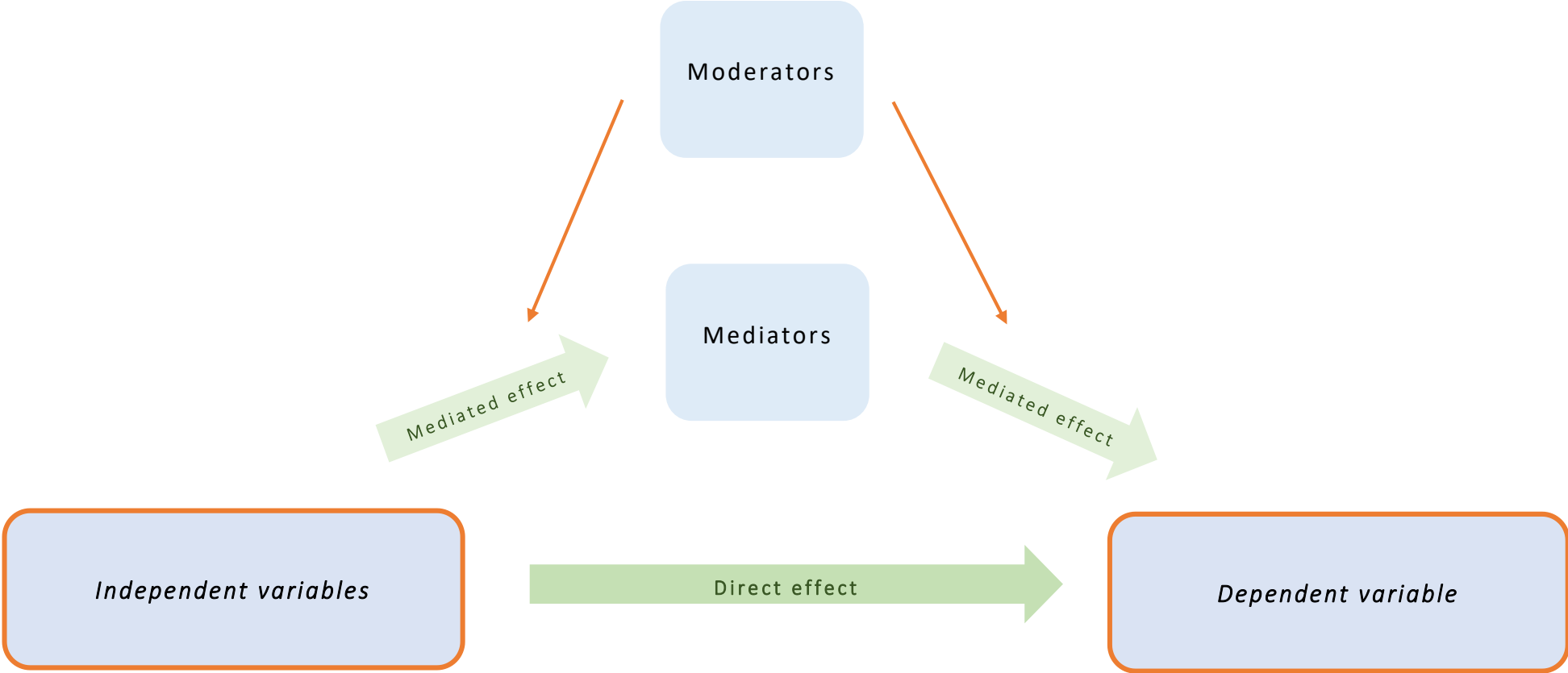
13 Conclusion

Collectively, the studies of this thesis do not support the notion that biomarkers of systemic inflammation are associated with diagnoses and other characteristics of older adults with psychiatric disorders. Notably, we did not find any association between systemic inflammation and depression in the elderly participants.

Further studies should have a longitudinal design, with purposely adapted approaches to target depressive symptomatology and inflammatory changes in the older population.

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15 Papers

Paper I

Cytokine profiles and diagnoses in elderly, hospitalized psychiatric patients.

Paper II

Changes in cytokines during treatment of elderly, hospitalized psychiatric patients

– a naturalistic study.

Paper III

C-reactive protein levels and depression in younger and older adults

- a study of 19,947 individuals.

Paper I

Cytokine profiles and diagnoses in elderly, hospitalized psychiatric patients

BMC Psychiatry

Cytokine profiles and diagnoses in elderly, hospitalized psychiatric patients

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Abstract

Background: There is a paucity of studies on inflammatory markers in elderly psychiatric patients. Hence, our study was undertaken to investigate cytokines as biomarkers in diagnostically unselected elderly patients admitted to a psychiatric hospital.

Methods: Demographic data, clinical data and blood samples, including 27 cytokines, were collected from 98 patients above 60 years, consecutively admitted to a psychiatric hospital in Tromsø, Norway (69°N).

Results: The most common diagnosis was Recurrent depressive disorder (26.5%), the second most common was dementia in Alzheimer's disease (20.4%). The most frequent somatic disease was cardiovascular disease (28%). No statistical association ($p < 0.01$) was found between cytokines and gender, age, BMI, anti-inflammatory drugs, psychotropic drugs, reason for admittance, smoking, vitamin supplements, alcohol consumption, length of stay, somatic disease (present/not-present) or psychiatric diagnoses. However, when allocating patients to two groups, *depression* and *no depression*, we found higher levels of 10 cytokines in the *no depression* group (FDR-*p* < 0.0044). Possibly, this could in part be explained by the higher prevalence of cardiovascular disease (CVD) and dementia in the no depression group, as these factors were significant predictors of patients being categorized as not depressed in a logistic regression. In addition, other unknown factors might have contributed to the association between no depression and elevated cytokines. On the other hand, the high level of psychiatric and somatic comorbidity in the study population may have led to increased levels of cytokines in general, possibly diluting the potential effect of other factors, depression included, on the cytokine levels.

The size of the study, and particularly the size of the subgroups, represents a limitation of the study, as do the general heterogeneity and the lack of a control group.

Conclusions: There was no significant difference in cytokine levels between various psychiatric diagnoses in hospitalized elderly psychiatric patients. This indicates that previous findings of correlations between cytokines and various psychiatric disorders in highly selected adult cases might not be applicable to elderly psychiatric inpatients. Further immunological studies are needed on gerontopsychiatric patients in general and gerontopsychiatric patients with specific disorders, preferably with patients that are physically healthy.

Trial registration: Retrospectively registered in the ISRCTN registry study, with study ID ISRCTN71047363.

Keywords: Psychogeriatric, Gerontopsychiatric, Cytokine, Depression, Neuroimmunology

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Background

Several studies have demonstrated an association between psychiatric disorders and biomarkers of inflammation, particularly cytokines. Primarily, these studies have focused on specific disorders and selected sets of cytokines. Depression and schizophrenia seem to dominate this research, both disorders repeatedly demonstrating elevated levels of pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, and TNF [1–3]. Though some of these studies include elderly patients [4–6], most studies have been conducted on younger adults. Thus, there is a paucity of studies on elderly psychiatric patients and particularly elderly psychiatric in-patients. Besides the fact that populations are ageing in most countries [7], the elderly are of particular interest because they are more likely to represent biological diversity due to age-related neuroimmunological changes [8] and higher frequencies of comorbid conditions. Consequently, findings of cytokine changes in younger adults do not readily translate to elderly psychiatric patients. Hence, our study was undertaken to investigate cytokines as biomarkers in diagnostically unselected elderly patients admitted to a psychiatric hospital.

Methods

Population

The population has been described in a previous publication [9]. Demographic data, clinical data and blood samples were collected from 98 patients, 60 years and older, consecutively admitted to a psychiatric hospital in Tromsø, Norway (69°N). The catchment area of the hospital was approximately 250,000 citizens. Exclusion criteria comprised inability to communicate and cooperate, e.g. due to a severe psychiatric condition like severe dementia or confusion/delirium, or a medical condition likely to significantly affect the blood/plasma analysis like severe dehydration or ongoing infection. The reasons for referral included a variety of psychiatric conditions, spanning from anxiety to psychosis, with depression (42%) and dementia (26%) being the most common. In terms of gender, age and diagnostic distribution, the study population was quite similar to the general population of patients admitted to gerontopsychiatric units in Norway [10], the possible exception being a lower proportion of dementia. However, the Norwegian national data included patients with severe dementia, whereas these patients were excluded from our study.

Clinical assessment

The following instruments were applied to assess the psychiatric and cognitive status of the participants (N = number of patients): the MINI International Neuropsychiatric Interview, $N = 43$ [11], the Montgomery and Aasberg Depression Rating Scale, $N = 76$ [12], the Cornell Scale for Depression in Dementia, $N = 22$ [13], the Mini-Mental State Examination, $N = 92$ [14] and the Clockdrawing Test, $N = 90$ [15]. In addition, clinical interviews and reviews of medical records were undertaken by experienced clinicians in assessment and diagnostics, according to ICD-10 research criteria. Interview of next of kin was also undertaken when appropriate.

Blood samples

During the first 3 days of admittance, morning blood samples (before 10 AM) were obtained for a range of analyses, e.g. electrolytes, liver enzymes, blood cells and thyroid hormones. In addition, plasma samples from EDTA-tubes were successively and rapidly frozen to − 70 °C, until analysed for cytokines in one batch. The analyses were performed by multiplex technology on a Multiplex Analyser with a predefined kit, according to the instructions of the manufacturer (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories Inc., Hercules, CA, USA). The assay was set up to detect the following interleukins, chemokines and growth factors: IL-1β, IL-1 receptor antagonist (IL1-ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ, interferon-inducible protein (IP-10), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1α, MIP-1β), platelet derived growth factor-BB (PDGF-BB), regulated upon activation T cell expressed and secreted (RANTES), tumor necrosis factor (TNF), and vascular endothelial growth factor (VEGF).

GM-CSF and IL-15 had a high frequency of non-detectable levels, i.e. below the lower detection limit, and were therefore excluded in the statistical analyses. Another eight cytokines had a small number of patients with cytokine levels below the lower detection limit (number of patients with non-detectable levels): IL-2 (4), IL-10 (11), IL-13 (1), IL-17 (4), bFGF (2), G-CSF (2), PDGF-BB (2), and VEGF (4). Data for these patients were imputed using SPSS, see Statistical analyses section.

Statistical analyses

Most of the data were not strictly normally distributed, as demonstrated by the Kolmogorov-Smirnov test, and several groups had unequal variances. Thus, nonparametric tests were applied. The Spearman rank correlation coefficient and Kendall Tau coefficient were used to analyse differences between the rankings of two variables. The Mann-Whitney U or the Kruskal-Wallis tests were applied when comparing ranks of two or several

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subgroups, subsequently. Goodness of fit was assessed by binary logistic regression. To examine whether the raised cytokine levels and the other variables could predict depression, we performed logistic regression analyses with depression/no depression as dependent variable. Patients were allocated to the depression group if they had been given depression as a primary or secondary diagnosis, or the no depression group if they had not been given a depression analysis.

Due to multiple statistical analyses, 0.01 was selected as significance level. In addition, false detection rate adjusted p-value (FDR-p) was calculated and applied to all analyses related to the cytokines. IBM Statistical Package for the Social Sciences, Version 23 (SPSS Inc., Chicago, Illinois, USA) software was used in the statistical analysis.

A small group of patients had no or very low levels of certain cytokines (which is a common finding for most cytokines in healthy adults), but the actual value could not be computed by the instrumentation; they are so-called non-detects (NDs). Accordingly, data from the NDs could hold valuable information, and in order to include them in the statistical analyses, we did single imputations, i.e. the NDs were substituted with a random value between zero and the lower detection limit, with a uniform distribution, using the random number generator of SPSS [16].

Results
Population characteristics
Population characteristics are presented in Table 1.

Diagnoses
The main diagnostic groups are presented in Table 2. The most common diagnosis was Recurrent depressive disorder (26.5%), the second most common was dementia in Alzheimer's disease (20.4%). Considering depression as a separate clinical entity, depending on whether the patients had been given depression as a primary or secondary diagnosis or not, the majority of patients could be allotted to the depression group, see Table 2. Selected features of the groups depression and no depression are presented in Table 3.

Distribution and correlation analysis
The cytokine values of the patients are presented in Table 4. In this group of diagnostically unselected elderly in-patients, no statistical correlation or unequal distribution was found between cytokines and gender, age, BMI, anti-inflammatory drugs, psychotropic drugs, reason for admittance, smoking, vitamin supplements, alcohol consumption, length of stay, somatic disease (present/not-present) or psychiatric diagnoses, dementia included. However, a correlation (FDR-p < 0.0044) was found

between *no depression* ($N = 39$) and raised levels of several cytokines, see Table 5.

While none of the raised cytokine levels predicted depression in a logistic regression model, cardiovascular disease (CVD) and dementia were predictors of patients being categorized as not depressed/no depression (Table 6). However, none of the cytokines came out as predictive in logistic regression models with CVD/no CVD, or dementia/no dementia, as dependent variables.

Looking at distributional data, CVD and dementia were more prevalent in the no depression group, compared to the depression group, 46.2% versus 15.3%, and 51.3% versus 22.0%, respectively.

Table 1 In-patients' characteristics

Characteristics	
Age, median/SD (years)	76/7.3
80 years and older (%)	39.8
Women (%)	61.2
Men (%)	38.8
Length of stay, median/SD (days)	34/25
Living alone (%)	53
Previous hospitalization (%) [a]	49
Two or more previous hospitalizations (%) [a]	38
No known somatic disease (%)	21
Cardiovascular disease (%)	28
Pulmonary disease (%)	10
Thyroid disease (%)	10
Previous stroke (%)	10
Rheumatic disease (%)	3.5
Other somatic diseases (%)	17.5
Potentially anti-inflammatory drug (%) [b]	55.1
Daily smokers (%)	29.6
BMI, median/SD (kilos)	24/5.3

[a] Psychiatric hospitalization
[b] The most common drug in this category is acetylsalicylic acid in low dose as prevention of cardiovascular events ($N = 30/68.2\%$)

Table 2 Distribution of diagnoses

Diagnoses	ICD-10	%
Organic, including symptomatic, mental disorders	F00–09	37.8
Mental and behavioural disorders due to psychoactive substance abuse	F10–19	1
Schizophrenia, schizotypal and delusional disorders	F20–29	12.2
Affective disorders	F30–39	41.8
Neurotic, stress-related and somatoform disorders	F40–48	7.2
Depression/No depression		60.2/39.8

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Table 3 Selected features of depressed and non-depressed patients

Comorbidities/features	Depression (%)	No depression (%)
Somatic disease (any)	76.3	82.1
Cardiovascular disease	15.3	46.2
Dementia	22.0	51.3
Antidepressants	74.6	25.6

Discussion

To our knowledge, this is the first study to explore cytokine levels in diagnostically unselected elderly psychiatric in-patients. Using an immunoassay method, we analysed 27 plasma cytokines in 98 patients, 60 years and older, admitted to a gerontopsychiatric unit.

The results demonstrated that cytokine levels did not correlate with variables such as age, gender, psychiatric diagnoses, somatic disease (present/not present), and the use of anti-inflammatory and psychotropic drugs. Considering each of these factors separately, this does not seem to match previous findings, as prior studies have shown positive associations between for instance Alzheimer's disease and increased levels of several cytokines [17], and between aging and increased levels of IL-6 and TNF-α [18]. Then again, our heterogeneous study population differs substantially from most of the diagnostically uniform populations previously studied.

The high frequency of somatic and psychiatric comorbidity in the study population may have contributed to the increased levels of cytokines in general, masking possible correlations between any single factor and changes in levels of cytokines. On the other hand, there is a possibility that altered immune activity in psychiatric patients is a general phenomenon, not restricted to specific diagnoses. Such a hypothesis can be bolstered by the fact that research has shown raised levels of inflammatory markers in several psychiatric disorders, ranging from schizophrenia to anxiety disorders [19, 20].

Contrary to some prior studies, we did not find any correlation between cytokines and depression. Given the fact the majority of our depressed patients were diagnosed with recurrent depressive disorder, it could be

Table 4 Serum levels of cytokines (pg/ml) in elderly psychiatric in-patients

Cytokine	Median	SD*	Minimum	Maximum	P 25**	P 75***
IL-1b	3.00	4.65	0.53	38.00	1.58	5.00
IL-1ra	158.00	769.64	31.00	7396.00	84.00	268.75
IL-2	9.00	17.97	0.01	147.00	3.00	16.00
IL-4	3.00	2.55	1.00	13.00	2.00	4.00
IL-5	5.00	5.65	0.73	28.00	3.00	9.00
IL-6	11.00	15.39	3.00	119.00	7.00	18.75
IL-7	21.00	22.83	0.25	104.00	11.00	36.00
IL-8	13.00	9.35	3.00	47.00	8.00	19.00
IL-9	18.00	47.03	3.00	441.00	11.00	28.00
IL-10	8.50	16.92	0.01	102.00	2.00	16.00
IL-12	27.00	51.60	0.05	381.00	11.00	46.75
IL-13	7.00	15.51	0.50	96.00	4.00	14.00
IL-17	42.50	66.57	0.35	371.00	15.25	88.25
Eotaxin	93.00	257.20	28.00	2286.00	65.50	157.75
bFGF	46.00	48.58	1.16	259.00	21.00	79.75
G-CSF	52.00	52.92	1.53	316.00	29.25	82.75
INF-g	184.00	217.26	20.00	1179.00	94.75	280.75
IP-10	1015.50	744.58	216.00	5075.00	762.25	1338.00
MCP-1	19.00	16.04	4.00	128.00	13.25	26.00
MIP-1a	10.00	9.82	2.00	58.00	6.00	15.00
MIP-1b	44.00	24.77	17.00	198.00	34.00	56.00
PDGF-BB	137.50	325.59	1.03	1651.00	34.25	308.00
RANTES	6642.00	12,393.18	532.00	60,319.70	2876.00	16,859.75
TNF-a	92.00	137.85	8.00	1173.00	43.50	127.25
VEGF	21.50	25.94	1.90	130.00	10.00	36.00

* Standard deviation. ** 25-percentile. *** 75-percentile

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Table 5 Correlation between cytokines and *No depression* (p-level > 0.01 excluded)

Cytokine	Correlation coefficient*	p	FDR-p**	Significant***	Eta[2]****
IL-2	0.268	0.008	0.0060	No	0.072
bFGF	0.271	0.008	0.0056	No	0.074
IL-1ra	0.271	0.008	0.0052	No	0.074
IL-1b	0.283	0.005	0.0048	No	0.080
IL-5	0.288	0.004	0.0044	Yes	0.083
IL-12	0.289	0.004	0.0040	Yes	0.083
IL-6	0.290	0.004	0.0036	Yes	0.084
TNF-a	0.295	0.004	0.0028	Yes	0.087
IL-7	0.306	0.002	0.0024	Yes	0.093
IL-10	0.321	0.001	0.0020	Yes	0.103
G-CSF	0.322	0.001	0.0016	Yes	0.104
INF-g	0.334	0.001	0.0012	Yes	0.111
IL-4	0.348	0.001	0.0008	Yes	0.121
IL-8	0.349	0.000	0.0004	Yes	0.122

*Spearman Rho; **False detection rate adjusted *p*-value, q-level 0.01, based on *p*-values of all 25 cytokines; ***FDR-adjusted p-criterion of 0.0044 ****Effect size. Based on Mann-Whitney Z-statistics of all 25 cytokines

hypothesized that relapsing depression in the elderly represents a somewhat different immunological process compared to depression in younger patients, on whom most studies have been conducted. On the other hand, our intra-group comparison could be the main explanation why the depressed patients did not have comparably higher levels of cytokines, considering that the overall level of cytokines was high regardless of diagnosis.

One relevant point to consider is that anti-depressants have exhibited anti-inflammatory properties in both clinical and experimental studies [21, 22]. Accordingly, the high percentage of antidepressant use in the depression group (74.6% versus 25.6% in the no depression group) might have been a factor in terms of lowering the cytokine levels amongst the depressed patients, cancelling out the association between depression and cytokine levels.

We did observe a correlation between *no depression*, a term denoting patients without significant depression irrespective of other clinical features, and several cytokines (FDR-*p* < 0.0044). Presumably, this correlation is not explained by the lack of depression per se. Rather, it is more likely that other factors contribute to increased

cytokines in the no depression group. CVD and dementia are two possible contributing factors, as they are more prevalent in the no depression group, hence becoming predictors in a binary logistic model. Both CVD and dementia have been linked to increased levels of cytokines [23–26]. Yet, the fact that none of the cytokines correlated with dementia or CVD, in addition to the absence of predictive power of any of the cytokines for both dementia and CVD in binary logistic regression, indicate that other, unrecognized factors also contribute to the association between no depression and elevated cytokines.

Finally, it should be taken into account that the total level of psychiatric morbidity in the no depression group (e.g. organic mental disorders) was probably just as high, perhaps higher, than in the depression group, hence diluting the potential effect of depression on the cytokine levels. There might also be a proportion of the no depression patients with dementia that actually was depressed, as it cannot be ruled out that patients with dementia express depressive symptoms in a way that is less likely to be recognized by the clinician. Then again, altered cytokine levels in psychiatric patients

Table 6 Logistic regression model assessing predictors of patients categorized as *no depression*[a]

Predictors of *no depression*	Log odds	SE[b]	Wald Chi[2]	P-value	Odds ratio	95% CI[c] for OR Lower - Upper
Dementia (N = 20)	1.422	0.484	8.651	0.003	4.147	1.607–10.700
Cardiovascular disease (N = 18)	1.664	0.515	10.441	0.001	5.279	1.924–14.479
Constant	−1.409	0.344	16.745	0.000	0.244	

[a]Nagelkerke R Square 0.253, Cox & Snell R Square 0.187
[b]SE: Standard error
[c]CI: Confidence interval

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may be a general phenomenon, possibly being primarily dependent on the severity of the disorder, and not the diagnosis.

When interpreting the result of our study, we need to be cautious, given the size of the study, and particularly the size of the subgroups. It should also be noted that though we chose 0.01 as significance levels due to multiple comparisons, and calculated FDR-p for the cytokine statistics, the risk of spurious correlations is still present. On this point, it worth mentioning that a Bonferroni correction would have required a significance threshold of 0.002. Moreover, applying few exclusion criteria may have provided a study population that resembled real-life gerontopsychiatric in-patients, but heightened the risk of confounders due to the general heterogeneity of the group, i.e. differences in age, socioeconomic background, lifestyle factors etc. Adding to this risk was the possibility of greater variability in health status, including immunological functioning, in the elderly compared to younger adults. Another possible source of cytokine variability could be that not all blood samples were fasting (35.7% non-fasting). Though the studies are somewhat conflicting, most seem to indicate that fasting has a certain anti-inflammatory effect [27–29]. A correlational analysis between fasting and cytokine levels in our population demonstrated nevertheless no correlation. Furthermore, a control group of healthy elderly would have made a statistical comparison possible, but at the time, we did not have such data available. Finally, it should be mentioned that single imputation of data to remedy NDs (see section Statistical analysis) may confer a risk of distorting the statistics, in particular when the number of NDs are high. In our study, the number of NDs are small (four at the most) and running the statistics without the NDs did not produce any significant change.

The plasma levels of cytokines observed in this group of elderly patients were the results of complex immunological processes, where different cytokines might have played different roles at different stages. Several interplaying factors, such as age, life style factors, somatic health, genetics, drugs and the psychiatric disorders per se, may have contributed in these processes. Besides, it is still unclear how an increased level of cytokines in systemic circulation relates to neuroimmunological processes in psychiatric disorders. Adding to the complexity is the application of various methods of cytokine analysis and methodological issues [30, 31]. Hence, caution should be exercised when interpreting data and making inferences about cytokine profiles and biomarkers in psychiatry, and perhaps particularly in the elderly population. This also begs the question as to what extent findings in younger adults, with uniform diagnostic profiles and no comorbidity, have bearings on real-life elderly patients.

Though the field of old age neuroimmunology has made great advances in the last decade, there is still a lot to be learned about the immunology of gerontopsychiatry. Further studies are needed on gerontopsychiatric patients in general and gerontopsychiatric patients with specific disorders, preferably with patients that are physically healthy. Clinical studies are necessary to gauge the immunological effects, both cellular and humoral, of various forms of treatment. As for our patients, the question is if psychiatric treatment can impact the high, probably multi-etiological, levels of cytokines. Finally, genomic and proteomic studies are required to uncover the immunological underpinnings of psychiatric disorders affecting the elderly, including longitudinal studies of healthy populations at risk.

Conclusions

There was no significant difference in cytokine levels between various psychiatric diagnoses in elderly psychiatric in-patients. However, when patients were allocated to two groups, *depression* and *no depression*, irrespective of diagnoses and other clinical features, we found higher levels of certain cytokines in the *no depression* group compared to the *depression* group. This might be due to a higher frequency of CVD and dementia in the *no depression* group, as well as other unknown factors.

Authors' contributions
EB designed the study, analyzed the data, drafted the manuscript, revised the manuscript, and approved the final version. OKG designed the study, analyzed the data, drafted the manuscript, revised the manuscript, and approved the final version. RW designed the study, analyzed the data, drafted the manuscript, revised the manuscript, and approved the final version. TEM analyzed the data, drafted the manuscript, revised the manuscript, and approved the final version. SKR analyzed the data, drafted the manuscript, revised the manuscript, and approved the final version. All authors have agreed to be accountable for all

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Paper II

Bugge, E., Wynn, R., Mollnes, T.E., Reitan, S.K., Lapid, M.I. & Grønli, O.K. (2019)

Changes in cytokines during treatment of elderly, hospitalized psychiatric patients - a naturalistic study

Changes in cytokines during treatment of elderly, hospitalized psychiatric patients – a naturalistic study

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ABSTRACT

Immunological abnormalities have been demonstrated in several psychiatric disorders. Predominantly, studies have focused on younger adults, and research on elderly psychiatric in-patients is scant. In this naturalistic study, we investigated changes in cytokine levels during the treatment of diagnostically unselected elderly psychiatric in-patients, and whether these changes could be related to clinical outcomes. Clinical variables, demographic data, lifestyle data, and blood samples, including 27 plasma cytokines representing a broad spectrum of inflammatory mediators, were collected from 81 patients, 60 years and older, at admission and discharge. A subgroup of 49 patients also completed a self-reported clinical, psychiatric status form, indicating their level of recovery during hospitalisation. Statistical analyses demonstrated that a broad range of cytokines fell during treatment, and the fall was associated with clinical improvement, irrespective of psychiatric and somatic diagnoses. Exploiting cytokines as biomarkers of clinical traits might to be of limited use in a general population of elderly psychiatric in-patients as the field stands now.

1. Introduction

Immunological dysfunction, including aberrant cytokine levels, has been demonstrated as an integral part of several psychiatric disorders, spanning from depression to autism (Bjorklund et al., 2016; Leighton et al., 2018; Trepanier et al., 2016). Specific cytokine patterns have also been suggested to provide clues to diagnoses, staging, treatment and prognosis (Black and Miller, 2015; Tatay-Manteiga et al., 2017). However, most of these studies do not include elderly psychiatric patients, and in particular elderly psychiatric in-patients, despite their growing importance due to the aging populations in most countries (Beard et al., 2016) and the high degree of health care utilization amongst elders (Ilinca and Calciolari, 2015). Furthermore, the studies have often been restricted to delineated psychiatric disorders, excluding patients with comorbid conditions. Though this might be considered beneficial from a methodological perspective, it leaves the question as to whether the research is relevant for the clinician working with elderly co-morbid psychiatric patients. A related issue is the naturally occurring age-related change in immunological competence, so-called immunosenescence or immune-aging (Weinberger, 2017). Accordingly, neuroinflammatory studies on younger adults are not necessarily applicable to elderly psychiatric patients (Bugge et al., 2018).

In this naturalistic study, we investigated changes in cytokine levels during the treatment of diagnostically unselected elderly psychiatric in-patients, and whether these changes could be related to clinical outcomes.

2. Material and methods

2.1. Ethics approval and consent to participate

All patients were presented oral and written information about the study. Consentual competency was assessed according to established guidelines (Pedersen et al., 2007). For patients who were considered

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unable to give individual consent due to their medical and/or psychiatric condition, information about the study was provided to their next of kin. All patients, and their next of kin when relevant, had to sign a written consent in order to participate in the study. Approval of the study was obtained from the Regional Committee for Medical and Health Research Ethics of Northern Norway (REC North, reg. nr. 2009/1388).

2.2. Declaration of interest

Declaration of interest: none.

2.3. Population

Eighty-one diagnostically unselected patients, 60 years and older, consecutively admitted to a psychiatric hospital in Tromsø, Norway (69 ˚N), during an 18-month period, were selected for inclusion. The patients were drawn from a catchment area of approximately 250 000 citizens. Patients that could not communicate or cooperate, for instance due to delirium or severe dementia, were excluded from the study, as were patients with medical conditions that could significantly distort inflammatory parameters, such as an ongoing infection or severe dehydration. Five eligible patients declined to participate, and two patients were excluded due to medical reasons. Demographic data, lifestyle data, clinical variables and routine blood samples including electrolytes, liver enzymes, blood cells and thyroid hormones were collected form all patients. In addition, 27 plasma cytokines representing a broad spectrum of inflammatory mediators were analysed at admission and discharge. At discharge, 49 patients (60% of all patients) completed a self-reported clinical, psychiatric status form, with five categories: Complete recovery, Almost complete recovery, Partial recovery, No recovery and Worsening.

2.4. Cytokines

Within the first three days after admittance, and at the day of discharge, morning blood samples were collected from all patients. Plasma for cytokine analysis were obtained from blood collected into EDTA-tubes, immediately placed on crushed ice, centrifuged, and rapidly frozen to -70 ˚C, until analysed in one batch, using an immunoassay method, i.e. a Multiplex Analyser with a predefined kit (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories Inc., Hercules, CA). The following cytokines were analysed: IL-1β, IL-1 receptor antagonist (IL1-ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ, interferon-inducible protein (IP-10), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1α, MIP-1β), platelet derived growth factor-BB (PDGF-BB), regulated upon activation T cell expressed and secreted (RANTES), tumour necrosis factor (TNF), and vascular endothelial growth factor (VEGF).

Two cytokines, GM-CSF and IL-15, had a high frequency of non-detectable levels. Consequently, these cytokines were excluded from further statistical analyses. An additional eight cytokines had a small number of patients with cytokine levels below the lower detection limit (number of patients with non-detectable levels): IL-2 (4), IL-10 (11), IL-13 (1), IL-17 (4), bFGF (2), G-CSF (2), PDGF-BB (2), and VEGF (4). In order to include these patients in the statistical analyses, data were imputed using SPSS, see the Statistical analyses section.

2.5. Clinical assessment

A battery of psychometric instrument were used in the clinical assessment of the patients (N = number of patients): the MINI International Neuropsychiatric Interview (Sheehan et al., 1998),

Table 1
Biological treatment during hospitalization.

Drugs	Number of patients	Percentage of patients
Antidepressants	54	67
Antipsychotics	40	49
Antidepressants + antipsychotics	26	32
Mood stabilizers	2	3
Paracetamol, Ibuprofen	13	16
Acetylsalicylic acid (low dose)	25	31
Steroids, cytostatic agent	8	10
Electroconvulsive therapy	12	15

N = 52, the Mini-Mental State Examination/MMSE (Folstein et al., 1975), N = 76, the Clockdrawing Test (Tuokko et al., 1992), N = 74, the Montgomery and Aasberg Depression Rating Scale/MADRS (Montgomery and Asberg, 1979), N = 60, or the Cornell Scale for Depression in Dementia (Alexopoulos et al., 1988), N = 20. The psychometric tests were applied at the discretion of the clinicians, based on the state and cooperability of the patient. Nevertheless, the majority of patients (N = 80) underwent at least one psychometric interview on depression and one test of cognitive impairment. In addition, experienced clinicians conducted standardized clinical interviews and reviews of medical records in assessment and diagnostics. When appropriate, interviews of next of kin were also undertaken. All patients were diagnosed according to the ICD-10 research criteria.

2.6. Treatment

Patients were treated with a combination of predominantly supportive psychotherapy, psychoeducation and biological treatment, including psychotropic medication. Table 1 provides an overview of the given biological treatment.

2.7. Statistical analyses

As demonstrated by a Kolmogorov-Smirnov test, the data were not strictly normally distributed. Accordingly, the Spearman rank correlation coefficient was used to analyse differences between the rankings of two variables. A chi-square test was applied to determine if there was a significant difference between two sets of data (Pearson Chi-square, asymptomatic 2-sided). To test deviations from a theoretically expected distribution of a dichotomous variable, a binominal test was used. Due to multiple statistical analyses, false detection rate adjusted p-values (FDR-p) were calculated and applied to all analyses related to the cytokines. IBM Statistical Package for the Social Sciences, Version 23 (SPSS Inc., Chicago, Illinois, USA) software was used in the statistical analysis.

As mentioned in the Cytokine section, a small group of patients had very low or undetectable levels of eight cytokines, i.e. the analyser could not determine the cytokines levels in these patients; they were so-called non-detects (NDs). This is a normal finding for several cytokines in healthy adults. Accordingly, data from these patients should not be reported as missing data, as they might provide valuable statistical information. For the purpose of including these patients in the statistical analyses, we did single imputations, i.e. using the random number generator of SPSS, we substituted the NDs with a random, uniformly distributed value between zero and the lower detection limit (Uh et al., 2008).

3. Results

3.1. Patient characteristics

Patients were referred by general practitioners for various psychiatric conditions, depression (54%), psychosis (17%) and dementia

Table 2
Distribution of major diagnostic categories.

Diagnoses	ICD-10	Main population (N = 81) %	Outcome population (N = 49) %
Organic, including symptomatic, mental disorders	F00-09	38	20
Mental and behavioural disorders due to psychoactive substance abuse	F10-19	1	2
Schizophrenia, schizotypal and delusional disorders	F20-29	12	16
Affective disorders	F30-39	41	53
Neurotic, stress-related and somatoform disorders	F40-48	7	8

(11%) being the most common. Women represented the majority of patients (61%). Median age was 76 year (SD 7.5). About half of the patients had undergone previous psychiatric hospitalization (47%), and a quarter of patients had two or more preceding hospitalizations. Most patients had a somatic disease (78%), cardiovascular disease being the most frequent (30%). The mean duration of admission was 39 days (median = 34 days).

Compared to the total population (N = 81), the population that completed the psychiatric status form at discharge (N = 49), henceforth the *outcome population*, had a higher proportion of female patients (71%), somewhat more patients with previous psychiatric hospitalization (57%) and patients with somatic disease (88%), but had otherwise comparable characteristics.

3.2. Diagnoses

The major diagnostic categories in the main population and the outcome population after assessment are shown in Table 2. The most common diagnosis in both populations was recurrent depressive disorder, though relatively more common in the outcome population (39%, versus 30% in the main population). The second most common in the main population was dementia in Alzheimer's disease (24%). In the outcome population, the second most common diagnosis was bipolar affective disorder (10%).

3.3. Self-reported clinical status

For analysing purposes, the five categories of the self-reported clinical, psychiatric status form, were combined into two main categories: Improvement (69% of patients, N = 49), containing patients who rated their status at discharge as Complete recovery (23%) and Almost complete recovery (46%), and Little/No improvement (31% of patients, N = 49), containing patients who rated their status at discharge as Partial recovery (21%), No recovery (8%) and Worsening (2%).

3.4. Changes in cytokine levels in the total population during treatment

In the total population (N = 81), there was a fall in cytokine levels, as median values fell for 19 cytokines and rose for three cytokines, whereas three cytokines were unchanged (Table 3). When categorizing each cytokine into two groups, Rise or Fall, depending on whether the majority of patients had a rise or fall in that cytokine, 20 cytokines were classified in the Fall-category, with a difference in median between rise and fall of 35% (difference calculated as a percentage of total number of patients, N = 81). The remaining five cytokines were classified in the Rise-category, with a difference in median between rise and fall of 9% (Fig. 1). Thus, most patients had a fall in cytokine levels during hospitalization, and the fall was more extensive than for the minority of patients that had a rise in cytokines.

Looking at the outcome population (N = 49), patients in the Improvement-group were more likely to have a fall in cytokine levels, than in the Little/no-improvement group (p < 0.032). Moreover, there was a positive correlation between clinical improvement and falling cytokine levels (p < 0.033) (Table 4).

Table 3
Change in serum levels of cytokines (pg/ml) during treatment (N = 81).

Cytokine	Median value at admission	Difference between median at admission and discharge	Percentage change in median (in-out)
IL-12	28.0	−9.6	−34
IP-10	1077	−368	−34
MCP-1	20	−6.7	−34
IL-17	43	−13	−32
RANTES	6767	−1 950	−29
IL-13	7.0	−2.0	−29
MIP-1a	11	−3.0	−27
Eotaxin	96	−23	−24
IL-10	8.0	−2.0	−25
PDGF-BB	138	31	−23
IL-5	5.0	−1.1	−22
IL-9	18	−3.0	−17
MIP-1b	45	−6.0	−13
INF-g	184	−23	−13
IL-7	21	−1.7	−8
bFGF	46	−3.1	−7
G-CSF	53	−3.0	−6
VEGF	22	−1.0	−5
TNF-a	92	−2.0	−2
IL-1ra	154	3.0	2
IL-8	13	1.0	8
IL-2	9.0	1.0	11
IL-4	3.0	0.0	0
IL-6	11	0.0	0
IL-1b	3.0	0.0	0

We did not find any significant difference in the distribution of the Little/No improvement patients among the rise and fall-category of individual cytokines. There was however, a significant difference in the distribution of Improvement patients, i.e. for 11 cytokines there was a majority of patients with clinical improvements in the fall-category (FDR-p): IL-17 (0.005), MIP-1α (0.007), INF-γ (0.009), MCP-1 (0.011), IL-6 (0.014), bFGF (0.016), IL-9 (0.018), PDGF-BB (0.020),G-CSF (0.023), IL-7 (0.025), IL-5 (0.027).

Adjusting for multiple analyses in the study population (N = 81), we did not find any statistical significant correlations (Spearman rho) between changes in plasma levels of any single cytokine during admission, and gender, age, marital status, psychiatric diagnosis, anti-inflammatory drugs, psychotropic drugs, electroconvulsive therapy, reason for admittance, smoking, vitamin supplements, alcohol consumption, length of stay, somatic disease, MADRS, MMSE and BMI. Likewise, we found no correlation between change in any single cytokine and self-reported clinical status at discharge.

4. Discussion

To the best of our knowledge, this the first study to explore changes in cytokine levels in a group of diagnostically unselected gerontopsychiatric patients in intramural treatment.

The main findings were, first, that for the majority of cytokines the plasma levels fell during hospitalization, indicating a reduction in inflammation, and second, in the outcome subpopulation, almost 70% of
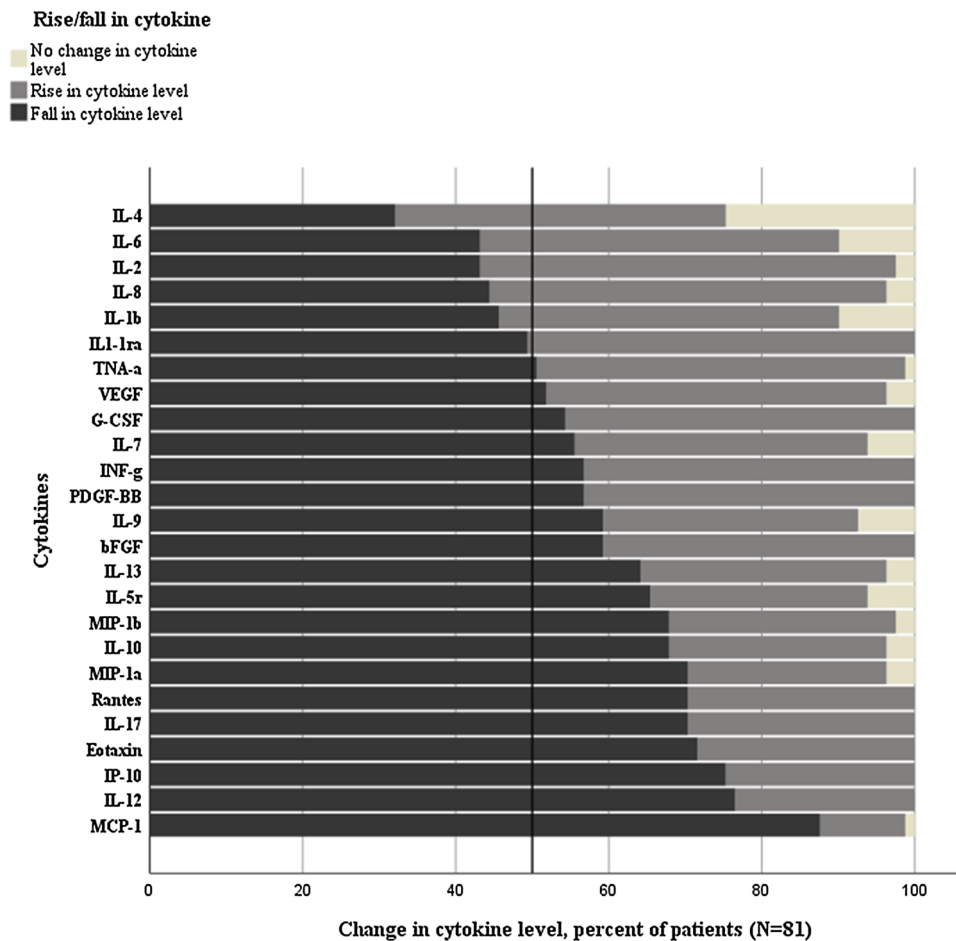
Rise/fall in cytokine
- No change in cytokine level
- Rise in cytokine level
- Fall in cytokine level

Fig. 1. Percentage change in cytokine level.

Table 4

Cytokine fall/rise in patients with Little/No improvement and Improvement (N = 49)[*].

		Rise or fall in cytokine (%)		
		Rise	Fall	Total
Clinical outcome	Little/no Improvement	12 (52 %)	11 (48 %)	23[**]
	Improvement	5 (22 %)	18 (78 %)	23[***]
	Total	17 (37%)	29 (63 %)	46
Pearson Chi-square 4.572		p < 0.032 (asymptomatic 2-sided, df = 1)		
Spearman's rho 0.315		p < 0.033 (2-tailed) p < 0.033 (2-tailed)		

* 25 cytokines analysed in each of the two clinical outcome groups.
** Two cytokines with equal number fall/rise excluded (VEGF, IL-8).
*** Two cytokines with equal number fall/rise excluded (IL-1ra, TNF-α).

the patients reported clinical improvement accompanied with a reduction in cytokines during the hospital stay. Thus, on a group level, there seems to be a correlation between clinical improvement and falling cytokines. On the other hand, we did not find significant correlations between change in any single cytokine and clinical improvement. Neither did we find significant correlations between cytokine changes and the various demographic and clinical variables, including diagnosis.

In principle, the treatment-related drop in cytokines seems to correspond to previous studies in both elderly and younger adults (Dahl et al., 2014; Hannestad et al., 2011; Hestad et al., 2003; Moreira et al., 2015; Tuglu et al., 2003). However, these studies have predominantly focused on selected cytokines in delineated psychiatric disorders, whereas the cytokine changes in our study appear to be broad-based and without any demonstrable patternicity. Pertaining to the latter, earlier research has hypothesised that specific cytokine patterns might provide markers of diagnosis, treatment options and prognosis (Goldsmith et al., 2016; Maes et al., 2012; Pedrini et al., 2012). Though this might be true at specific stages for some psychiatric disorders in younger adults, our study suggests that cytokine changes are widespread and non-specific in elderly psychiatric patients, perhaps due to the presence of several conditions. Multimorbidity, defined as the co-existence of more than two chronic diseases, is common in the elderly, with a prevalence of more than 60% for those aged 65–74 years and more than 80% for those aged ≥ 85 years (Salive, 2013). In our population, almost 78% of the patients had a comorbid somatic disorder, and more than 90% of these patients had more than one somatic disorder, i.e. several potential sources of inflammation. The picture is further complicated by the use of drugs with possible immunomodulatory effects (Baumeister et al., 2016), and by age-related immunological changes and variability (de Groot et al., 2004; Santoni et al., 2015). Consequently, exploiting cytokines as biomarkers of clinical traits might to be of limited use in a general population of elderly psychiatric in-patients as the field stands now. Likewise, the outcomes from our study indicate that extrapolating results from research on younger subjects to elderly patients, is a somewhat precarious undertaking.

The heterogeneity and the size of our population calls for caution when interpreting the results. The lack of a control group also represents a limitation of the study. Moreover, it should be noted that single imputation of data to substitute NDs (see the Statistical analysis section) could lead to skewed statistics, but analyses without the NDs

did not produce any significant change. We did not perform power analyses in advance, but in an exploratory study such as ours, power analyses are notoriously difficult. Furthermore, use of high-sensitivity C-reactive protein could have provide complementary information about the inflammatory processes. Finally, we did not have information about use of statins, a group of drugs known to exhibit anti-inflammatory and immunomodulary effects.

Clearly, the complexity of inflammatory processes in elderly psychiatric patients with comorbid disorders is difficult to untangle. This is not unique to the elderly, though, as we still have not mapped out the inflammatory mechanisms behind systemic cytokine changes in psychiatric disorders. Hence, more research is needed on both elderly and younger adults, including healthy individuals and individuals with psychiatric disorders. Longitudinal studies, including genomic and proteomic methodology, could also help to shed light upon the neuroimmunological processes that take place during the course of a psychiatric illness.

5. Conclusions

A broad range of cytokines fall during treatment of elderly psychiatric in-patients with multimorbidity, and the fall seem to correlate with clinical improvement, irrespective of psychiatric and somatic diagnoses.

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Paper III

Bugge, E., Wynn, R., Mollnes, T.E., Reitan, S.K., Lapid, M.I. & Grønli, O.K.

C-reactive protein levels and depression in younger and older adults - a study of 19,947 individuals. The Tromsø Study

Submitted manuscript

MAIN MANUSCRIPT

C-reactive protein levels and depression in older and younger adults

- a study of 19,947 individuals. The Tromsø Study

1.   INTRODUCTION

1.1 Depression and inflammation – research on older adults is scant

In recent years, a connection between depression and inflammation has been

established, with a range of immunological changes, both cellular and humoral,

presenting during depressive states (Beydoun et al., 2016; Haapakoski et al., 2015;

Wium-Andersen et al., 2013). Furthermore, there seems to be a dose-response

relationship between depression and inflammation, in the sense that the more severe

the depression, the higher the level of systemic inflammation markers, most notably

expressed as elevated levels of C-reactive protein (CRP) in peripheral blood (Kohler-

Forsberg et al., 2017). Accordingly, CRP has been suggested as a marker of

depression severity and depression subtypes, as well as an indicator of specific

symptom profiles (Jokela et al., 2016). Furthermore, inflammation has been

suggested as a target for treatment with immunomodulatory drugs (Alexopoulos &

Morimoto, 2011; Kohler et al., 2014).

However, the research populations are predominantly younger adults, mainly in

clinical settings, and there are few community-based studies providing comparative

analyses of age-groups, or focusing specifically on the older population. For those

that do, the results are inconsistent, as some demonstrate an association between

CRP and depression (White et al., 2017), while others do not (Bremmer et al., 2008). Thus, it is still unclear whether the inflammation in depression unfolds to the same extent in depressed older adults as in younger adults, and how the severity of the depression relates to inflammation in different age groups.

1.2 Confounding factors in older adults

One reason why results from studies on younger adults cannot be extrapolated to older adult, is the age-related changes in the immune system known as immunosenescence (Pawelec, 2018). Immunosenescence is characterized by two key features: a weakening of the inflammatory response and a low-grade inflammation (Fulop et al., 2017). The low-grade inflammation, called inflammaging, manifests for instance as an age-dependent increase in systemic CRP and certain cytokines, such as IL-6 and TNF (Franceschi et al., 2000). Inflammaging can start in early adulthood, and as such be a life style indicator, but the process seems to gain momentum and significance from the age of 60 and onwards (Malaguarnera et al., 2001).

Besides immunosenescence, other confounding factors to consider in older people are the high prevalence of somatic disorders (Salive, 2013), and the age-linked increase in BMI, both independent contributors to inflammation (Choi et al., 2013). Consequently, inflammatory findings in depressed younger adults, on whom most studies are undertaken, are not necessarily relevant to older individuals (Bugge et al., 2018).

1.3 Aim of the study

The aim of this large community-based study is to investigate the cross-sectional association between systemic inflammation and different levels of depression, as we compare individuals of two age groups; those 60 years and older versus those between 40 and 60 years. Systemic inflammation is estimated by measuring serum CRP, and depression by using the subscale for depression of the Hospital Anxiety and Depression Scale (HADS-D). Two levels of depression were analysed: depression, i.e. all levels of depression, and moderate-severe depression. The statistical analyses also included covariates previously found to be associated with elevated levels of CRP.

2. MATERIAL AND METHODS

2.1 Study population

The Tromsø Study is a community-based, prospective cohort study of inhabitants in the municipality of Tromsø, Norway. The study design includes repeated population surveys to which total birth cohorts and random samples are invited. The study was initiated in 1974 (Tromsø 1), with repeated health surveys in 1979 - 80 (Tromsø 2), 1986 - 87 (Tromsø 3), 1994 - 95 (Tromsø 4), 2001 (Tromsø 5), 2007 - 08 (Tromsø 6) and 2015 - 16 (Tromsø 7).

Our study consists of participants from the latest survey, Tromsø 7. 21,083 individuals $\geq$ 40 years participated, i.e. 64.7 % of eligible inhabitants at the time (all inhabitants $\geq$ 40 years were invited to participate).

The survey encompassed clinical interviewing and examination, biometric data, biological sampling, including CRP, and self-administered questionnaires, including HADS (The-Tromsø-Study, 2022) .

Participants in the Tromsø Study were informed that data would be treated in strict confidence. Written informed consent was obtained from all participants.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/the participants were approved by the Norwegian Data Protection Authority and the Regional Committee for Medical and Health Research Ethics (REC North, reference no. 2014/940 and 2020/88232).

2.2 The Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a self-rating scale, originally developed for the assessment of psychological distress in non-psychiatric patients (Zigmond & Snaith, 1983). It consists of two, seven item subscales; one for anxiety, HADS-A, and one for depression, HADS-D. HADS has been extensively validated in various populations, including the general population above 60 years of age (Djukanovic et al., 2017; Grønli et al., 2022; Mykletun et al., 2001). A Norwegian version of the HADS scale was used in the present study (Leiknes KA, 2016).

A score of HADS-D $\geq$ 8 and $\geq$ 11 was chosen as cut-offs, as previous studies have shown that these levels represent an optimal balance of sensitivity and specificity

when screening for depression (i.e. all levels of depression) and moderate-severe depression, respectively (Leiknes KA, 2016; Olsson et al., 2005).

All HADS-D questionnaires with three missing items or more were excluded from the study. Questionnaires with one or two missing items were included; for these questionnaires the scores were based on the sum of completed items multiplied by 7/6 or 7/5, correspondingly.

2.3 C-reactive protein (CRP) measured with the high-sensitivity technique

CRP is an acute phase protein playing a key role in infection, immunity and inflammation (Sproston & Ashworth, 2018). In peripheral blood it is a reliable indicator of inflammation, and as such, one of the most widely used biomarkers in medicine. CRP, in particular when detected with the high-sensitive technique, has repeatedly shown to correlate with inflammation in depression in younger adults, even when adjusting for various risk factors (Valkanova et al., 2013; Wium-Andersen et al., 2013), as well as being an indicator of depression severity and treatment response (Chamberlain et al., 2019; Yang et al., 2019). Additionally, CRP is associated with increased all-cause mortality (Proctor et al., 2015).

It should be noted that though the immune response in older people is somewhat slower and weaker compared to younger people, the elevation of CRP is a reliable indicator of trauma, infections, mortality etc. even in older adults (Alfaddagh et al., 2020; Ticinesi et al., 2017).

Participants with CRP > 10 mg/L (N = 484) were excluded due to the risk of an active infection or highly inflammatory conditions eventually distorting the results (Lelubre et al., 2013; Pepys & Hirschfield, 2003).

A CRP value of 3 mg/L is broadly accepted as a cut-off point between normal and elevated serum levels, i.e. the majority of healthy adults, irrespective of age, have a serum concentration below 3 mg/L (Kalogeropoulos et al., 2010; Kushner et al., 2006; Tang et al., 2018). Accordingly, CRP ≥ 3 mg/L was defined as elevated, signifying the presence of a low-grade, systemic inflammation (Osimo et al., 2019).

CRP in serum was analysed using the high-sensitivity technique, at the Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway. The analyses were executed by employing a particle enhanced immunoturbidimetric assay on a Modular P autoanalyzer (Roche Diagnostics, Mannheim, Germany), with a detection limit of 0.12 mg/L (Hafner et al., 1997; Roche-Diagnostics, 2019).

2.4 Covariates

Participants completed questionnaires about their health, diseases and medication, as well as various life style factors and socio-economic status. Based on previous studies, and using a stepwise selection process, the following variables were examined as covariates: Age, sex, obesity (defined as BMI ≥ 30), daily smoking, current somatic disease (including hypertension), having a partner or close friend, and higher education (i.e. a college or university degree).

Underweight individuals (N = 153), i.e. individuals with BMI < 18.5, were excluded

from the study, as underweight is known to compromise the immune system, thereby

leading to aberrant inflammatory markers (Brown et al., 2008).

The present analyses are based on data for participants with valid HADS-D,

CRP < 10 mg/L, and BMI ≥ 18.5. 19,947 participants were included in the final

analyses, i.e. 61.2 % participation rate (Figure 1).

2.5 Statistical analyses

IBM Statistical Package for the Social Sciences, Version 26 (SPSS Inc., Chicago,

Illinois, USA) software was applied in the statistical analysis.

Frequencies were used to describe the population characteristics. Test score

reliability for the HADS-D scale was assessed by estimating Cronbach's alpha.

Possible multicollinearity between the covariates was tested by creating a correlation

matrix, as well as applying linear regression to calculate estimates of variance

inflation (VIF).

To test possible associations between systemic inflammation/elevated CRP, i.e. the

dependent variable, and depression/moderate-severe depression and age/age-

groups, i.e. the two primary independent variables, and the various covariates, we

used binary logistic regression to calculate odds ratios. First, two logistic regression

models were analysed: an *adjusted model*, including depression (HADS-D ≥ 8) and

moderate-severe depression (HADS ≥ 11), as well as 10 years strata and sex as

covariates, and a *multi-adjusted model*, including depression and moderate-severe

depression, as well as 10 years strata, sex, obesity, daily smoking, current somatic disease, higher education and having a partner/close friend as covariates. Second, the aforementioned adjusted and multi-adjusted models were utilized to compare two age groups; those 60 years and older versus those between 40 and 60 years. In these analyses, the covariate *10 years age strata* was removed from both models.

3.   RESULTS

The characteristics of the participants with normal and elevated CRP are presented in Table 1. Participants aged 40 – 59 years had a higher proportion of depression and moderate-severe depression, than participants aged 60 and above. Mean HADS-D, however, was not significantly different between the two age groups (Table 1).

Participants with elevated CRP had a higher proportion of participants aged 60 and above, as well as a higher mean age, than those with normal CRP. Furthermore, participants with elevated CRP had a higher mean HADS-D, and a higher proportion of depression and moderate-severe depression, than participants with normal CRP.

Cronbach's alpha for HADS-D was 0.73, indicating satisfactory internal consistency. For the regression models, low correlation coefficients and VIFs (range 1.02 - 1.16) signified that multicollinearity between the covariates was not a concern.

In general, participants with depression, i.e. all levels of depression, and moderate-severe depression, were significantly more likely than non-depressed participants to

have an elevated CRP, even when adjusting for several covariates (Table 2). Moreover, a 10-year increase in age produced a 16 % increase in the odds for an elevated CRP in the adjusted model, and an 11 % increase in the odds for an elevated CRP in multi-adjusted model, regardless of depression level (Table 2).

Participants 60 years and older with depression and moderate-severe depression had higher odds of elevated CRP than participants without depression when adjusting for sex, but not when adjusting for several covariates (Table 3). Participants between 40 and 60 years with depression and moderate-severe depression had higher odds of elevated CRP, even when adjusting for several covariates, than participants without depression (Table 3).

4. DISCUSSION

To our knowledge, this study is the largest cross-sectional community-based study of inflammation in depression with comparative analyses of age-groups, with a total of 19,949 participants, of which 8010 were aged 60 and older. The main finding is that, when adjusting for the covariates (as defined in 2.4), depression and moderate-severe depression was significantly associated with an elevated level of CRP in participants younger than 60 years, but not in those people aged 60 or above. The former is in accordance with most previous clinical studies and community-based studies. As for the latter, comparable studies are few and the findings conflicting. For instance, two Dutch population studies reported no correlation between CRP and depression (Bremmer et al., 2008; Tiemeier et al., 2003), whilst two studies from the English Longitudinal Study of Aging (ELSA) reported the opposite (Gallagher et al.,

2017; White et al., 2017). However, all of these studies have some significant limitations, of which missing data on CRP is perhaps one of the most prominent, ranging from 17 to 42 %. There is also a considerable variation in the prevalence of depression. For instance, Bremmer and colleagues reports a prevalence of 14.8 %, whereas Tiemeier and co-workers reports 2.7 %, despite the latter population being younger (mean age 61 versus 76) with a higher proportion of women, thus expected to have a higher rate of depression. Both research groups used the 20-item Center for Epidemiological Studies Depression scale (CED-S), with 16 points as cut-off for depression. Such methodological issues are common in this field of research, making comparisons and interpretations of the studies a precarious undertaking. Besides missing data and a substantial variety in the rates of depression and depressive symptoms, the issues include e.g. a large span in mean age, different cut-off scores for CRP, various methods of statistical structuring and analysis, and insufficient covariates analyses. For example, a mean age of 61 years (Stewart et al., 2009) versus 85 years (van den Biggelaar et al., 2007) are likely to reflect very different levels of immunosenescence in the two populations, with potentially dissimilar effects on the levels of CRP. Another example; despite multimorbidity affecting the majority of people over 65 years (Salive, 2013), some studies includes no chronic diseases as covariates, others only include one or two (Smith et al., 2018).

The absence of an adjusted correlation between depression and elevated levels of CRP in individuals aged 60 and older, signify that inflammation is not an intrinsic part of the pathophysiology of depression in a general population of older adults. Theoretically, there might be smaller subgroups of geriatric depression for whom inflammation are a key factor, but in terms of severity, we did not find any association

between neither depression in general (HADS-D ≥ 8), nor moderate-severe depression (HADS-D ≥ 11), with an elevated CRP.

An alternative or complementary explanation is that the inflammatory processes triggered by depression in older persons, are eventually blunted by immunosenescent mechanisms, even to the extent that no significant changes in systemic inflammatory markers (e.g. CRP) are detected (Shaw et al., 2013). A similar problem may arise because of the non-specific nature of CRP. CRP is elevated in inflammation of virtually all causes (with the exception of a few autoimmune diseases). In older people, there are generally several potent contributors to an elevated CRP, due to the high prevalence of somatic diseases, obesity, degenerative processes, senescent cells etc. Consequently, a possible contribution to a systemic elevation of CRP stemming from depression - expectedly modest in size - might be obscured by other inflammation sources.

Furthermore, depression is a heterogenous disorder, with important clinical differences between depression in the geropsychiatric population and depression of younger people (Hegeman et al., 2012). For instance, later life depression presents with higher rates of somatic symptoms, anxiety and cognitive impartment, compared to depression in younger individuals (Gottfries, 1998; Hegeman et al., 2012; Wu et al., 2021). Given that the HADS-D used in our study does not include somatic symptoms, it is conceivable that some of the depressed older adults are not identified in our study, or that their depression level is inaccurately estimated. That being said, the prevalence of depression in participants 60 years and older in this study (5,9 %)

does not deviate from prevalence estimates of geropsychiatric depression in general populations (CDC, 2021).

The main strengths of our study are the number of participants and the inclusion of most relevant covariates, including a broad range of somatic disorders. It is worth mentioning that alcohol consumption and psychotropic drugs were amongst the covariates excluded from the final analyses, as they did not turn out to be impacting factors in the stepwise selection process of covariates. Pertaining to alcohol, this could be explained by the J-shaped relation between alcohol and inflammation, i.e. moderate drinkers have been shown to have a lower level of inflammation compared to occasional drinkers, abstainers and heavy drinkers (Bektas et al., 2016). An analogous case could be made for psychotropic drugs, their effects on inflammation varying both between and within groups of drugs (Baumeister et al., 2016).

The participation rate of this study (61.2 %), as well as the recruitment from a single community, calls for caution in generalization of the results. The inclusion of important confounding variables is another strong point of the study, but the presence of unrecognized covariates contributing to residual confounding, cannot be ruled out. Besides, several covariates are based on questionnaires, which by itself involves an increased risk of misclassification.

Just like all community-based studies, our study runs the risk of a selection bias, as it is well documented that women, healthier people, people living with a partner, and people from higher socio-economic classes are more likely to attend population surveys (Galea & Tracy, 2007). In our study, the sex distribution was 52 % women,

and 48 % men. The statistical analyses showed that being male is associated with lower odds of systemic inflammation (Table 2). This is, however, in concordance with most other studies, demonstrating that various populations of men tend to have lower CRP-levels than women (Khera et al., 2009, Valentine et al., 2009, Choi et al., 2013).

We do not know who the non-attenders of our study are, but from the Tromsø 6 survey, we learned that attenders were older, with a higher proportion of married/cohabitants, compared to the non-attenders (Eggen et al., 2013). A study of non-attenders aged 55 years and younger in The Tromsø 2 survey, revealed that the non-attenders had a higher prevalence of psychiatric disorders, particularly substance abuse, compared with attenders (Hansen et al., 2001). It is reasonable to assume that these characteristics are, at least to some extent, also present in our study, though it is unclear what the effects, if any, might be on the overall outcome of the study. In any case, it is to be noted that the difference in attendance rate between the age groups was less than 1 %; 61.7 % in individuals ≤ 60 years and 60.8 % for individuals aged 40 – 59 years.

Finally, the cross-sectional design of the study does not allow for any hypothesis about the direction of the associations between CRP and depression, and thus, precludes information about causality.

In conclusion, we did not find an adjusted correlation between depression, and moderate-severe depression, with systemic inflammation measured by CRP, in individuals 60 years and older. This finding demonstrates that inflammation is not a prominent part of the pathophysiology of depression in most older adults, and thus, it

does not support previous theories about inflammation as an assessment tool or treatment target in geriatric depression.

Further studies should include purposely adapted approaches to target depressive symptomatology and inflammatory changes in the older population.

5.   DECLARATION OF INTEREST

None.

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FIGURES & TABLES

Figure 1: Flow-chart inclusion/exclusion

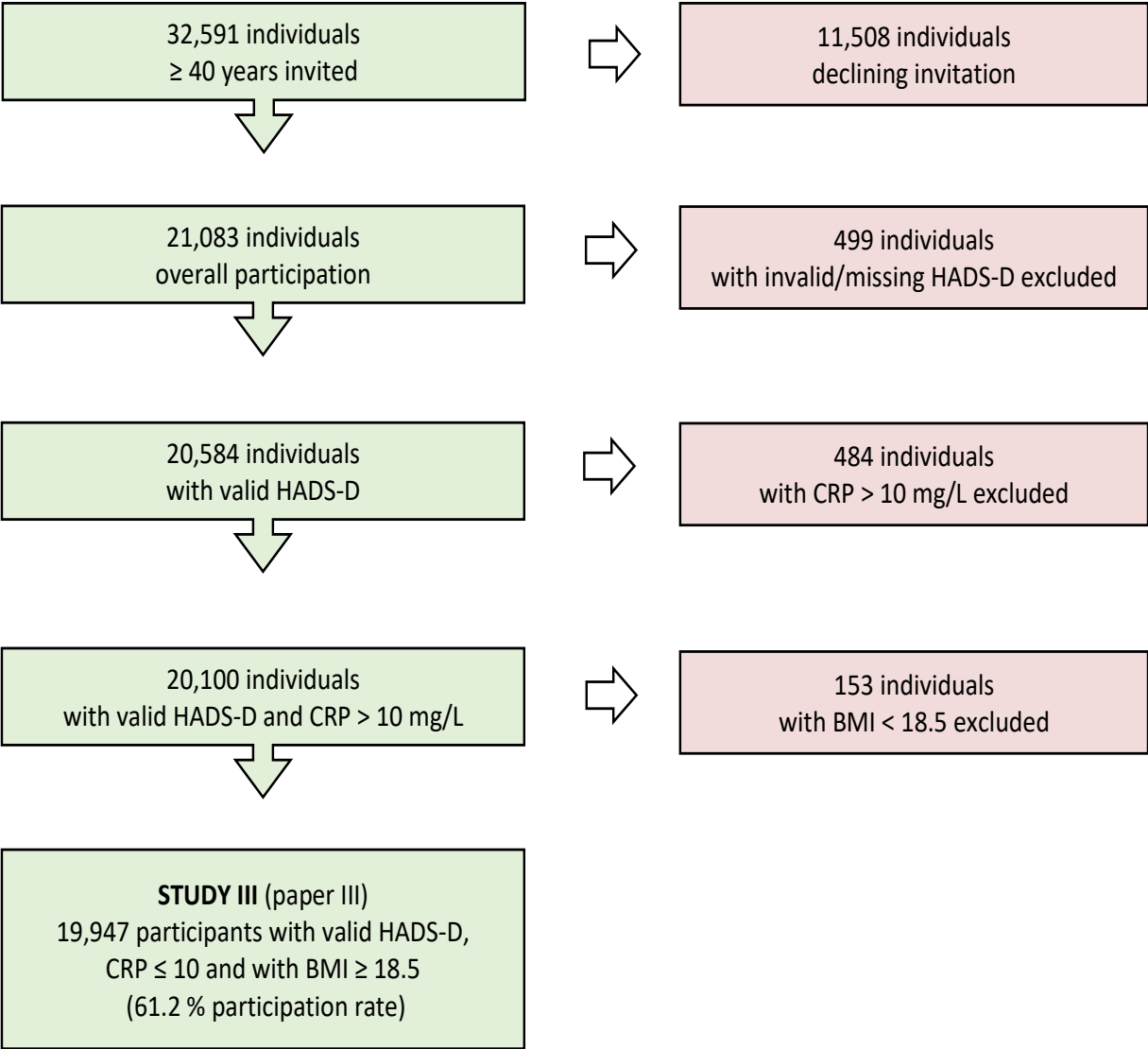|	
32,591 individuals ≥ 40 years invited	11,508 individuals declining invitation
21,083 individuals overall participation	499 individuals with invalid/missing HADS-D excluded
20,584 individuals with valid HADS-D	484 individuals with CRP > 10 mg/L excluded
20,100 individuals with valid HADS-D and CRP > 10 mg/L	153 individuals with BMI < 18.5 excluded
STUDY III (paper III) 19,947 participants with valid HADS-D, CRP ≤ 10 and with BMI ≥ 18.5 (61.2 % participation rate)	

Table 1: Population characteristics of participants aged ≥ 60 years versus 40 – 59 years, and with normal versus elevated CRP

N = 19,947	Age ≥ 60 years	Age 40–59 years	P-value X^2/t-test	Normal CRP 0 – 2.99 mg/L	Elevated CRP 3 – 10 mg/L	P-value X^2/t-test
N	8010	11,937	0.00	17,216	2731	0.00
HADS-D, mean (SD)	2.84 (2.6)	2.81 (2.8)	0.40	2.8 (2.7)	3.1 (3.0)	0.00
Depression*, N (%)	470 (5.9)	880 (7.4)	0.00	1088 (6.3)	262 (9.6)	0.00
Moderate-severe depression**, N (%)	96 (1.2)	225 (1.9)	0.00	247 (1.4)	74 (2.7)	0.00
Age, mean (SD)	68.7 (6.8)	49.3 (5.7)	0.00	56.8 (11.2)	58.8 (11.6)	0.00
Age ≥ 60 years, N (%)	-	-	-	6767 (39.3)	1243 (45.5)	0.00
Elevated CRP 3 – 10 mg/L, N (%)	1243 (15.5)	1488 (12.5)	0.00	-	-	-
Women, N (%)	4100 (51.2)	6333 (53.1)	0.01	8888 (51.6)	1545 (56.6)	0.00
BMI, mean (SD)	27.4 (4.3)	27.3 (4.5)	0.53	26.9 (4.1)	30.3 (5.3)	0.00
Obesity, N (%)	1864 (23.3)	2823 (23.6)	0.54	3388 (19.7)	1299 (47.6)	0.00
Daily smoking, N (%) Missing = 173	968 (12.1)	1743 (14.6)	0.00	2171 (12.7)	540 (20.0)	0.00
Current somatic disease, N (%) Missing = 780	4883 (61.0)	4224 (35.4)	0.00	7506 (45.4)	1601 (61.1)	0.00
Higher education, N (%) *** Missing = 319	2917 (36.4)	6840 (57.3)	0.00	8673 (51.2)	1084 (40.5)	0.00
Partner or close friend, N (%) Missing = 230	7718 (96.4)	11,597 (97.2)	0.81	16,718 (98.1)	2597 (96.9)	0.00

* HADS-D ≥ 8, i.e. all levels of depression. ** HADS-D ≥ 11 ***University or college degree

Table 2: Relationship between inflammation (CRP ≥ 3 mg/L) and two levels of depression – depression (i.e. all levels of depression) and moderate-severe depression

	Adjusted model			Multi-adjusted model		
	OR*	(95 % CI)	P-value	OR	(95 % CI)	P-value
*Depression***	1.62	(1.40 - 1.86)	0.00	1.31	(1.11 - 1.53)	0.00
10 years age strata	1.16	(1.12 - 1.20)	0.00	1.11	(1.07 - 1.16)	0.00
Sex (male = 1)	0.81	(0.75 - 0.88)	0.00	0.80	(0.74 - 0.88)	0.00
Obesity				3.60	(3.29 - 3.94)	0.00
Daily smoking				1.80	(1.60 - 2.02)	0.00
Current somatic disease				1.47	(1.34 - 1.62)	0.00
Higher education***				0.85	(0.77 - 0.93)	0.00
Having a partner/close friend				0.74	(0.56 - 0.97)	0.03
Constant	0.12		0.00	0.11		0.00
Hosmer-Lemeshow test	X^2 = 2.70 Sig. = 0.85			X^2 = 6.46 Sig. = 0.60		
*Moderate-severe depression*****	2.00	(1.54 - 2.61)	0.00	1.50	(1.11 - 2.04)	0.01
10 years age strata	1.16	(1.12 - 1.20)	0.00	1.11	(1.07 - 1.16)	0.00
Sex (male = 1)	0.81	(0.75 - 0.88)	0.00	0.81	(0.74 - 0.88)	0.00
Obesity				3.61	(3.30 - 3.95)	0.00
Daily smoking				1.80	(1.61 - 2.02)	0.00
Current somatic disease				1.48	(1.35 - 1.62)	0.00
Higher education**				0.84	(0.77 - 0.93)	0.00
Having a partner/close friend				0.73	(0.56 - 0.96)	0.03
Constant	0.12		0.00	0.11		0.00
Hosmer-Lemeshow test	X^2 = 1.71 Sig. = 0.94			X^2 = 12.87 Sig. = 0.12		

* Odds ratio. ** HADS-D ≥ 8. *** University or College Degree **** HADS-D ≥ 8.

Table 3: Relationship between inflammation (CRP ≥ 3 mg/L), depression (HADS-D ≥ 8, i.e. all levels of depression) and moderate-severe depression (HADS-D ≥ 11)

	Age ≥ 60 years			Age 40-59 years		
	OR[*]	(95 % CI)	P-value	OR	(95 % CI)	P-value
Depression, adjusted model[**]	1.29	(1.01 - 1.64)	0.04	1.85	(1.55 - 2.20)	0.00
Depression, multi-adjusted model[***]	0.99	(0.75 - 1.30)	0.91	1.51	(1.24 - 1.84)	0.00
Moderate-severe depression, adjusted model	2.06	(1.31 - 3.25)	0.00	1.95	(1.41 - 2.70)	0.00
Moderate-severe depression, multi-adjusted model	1.50	(0.87 - 2.58)	0.14	1.50	(1.03 - 2.17)	0.03

[*] Odds ratio [**] Adjusted model: Adjusted for sex. [***] Multi-adjusted model: Adjusted for sex, obesity, daily smoking, ongoing somatic disease, higher education, partner or close friend