From NEUROBIOLOGY, CARE SCIENCES AND SOCIETY
Karolinska Institutet, Stockholm, Sweden

INFLAMMATION AND SUBJECTIVE HEALTH: THE ROLE OF SICKNESS BEHAVIOUR

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Stockholm 2018

Published by Karolinska Institutet.
Printed by Eprint AB 2018

ISBN 978-91-7676-935-5

Inflammation and subjective health: the role of sickness behaviour
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To the memory of Per-Olof Hulth

ABSTRACT

Sickness behaviour refers to a set of coordinated behavioural and psychological changes in response to inflammation aimed to redirect available energy to the immune system to promote recovery. Sickness behaviour symptoms closely resemble important determinants of poor self-rated health, which in turn has been coupled to increased levels of inflammatory cytokines. However, it is not known if this association between inflammatory cytokines and self-rated health is mediated by sickness behaviour. Furthermore, it has not yet been established if other systemic and local inflammatory markers affect self-rated health, sickness behaviour and other patient reported outcome measures (PROMs).
This thesis main aim was to investigate sickness behaviour as a determinant of self-rated health and as a possible mediator in an association between inflammatory markers and subjective health perception in primary care patients and in patients with asthma.

In **paper I**, a questionnaire to measure sickness behaviour, SicknessQ, was developed and validated in two steps. First, sickness behaviour was experimentally induced by injecting endotoxin in healthy volunteers. The participants completed 37 items describing a broad range of sickness symptoms, items that responded to acute inflammatory provocation were selected and psychometric properties were tested in 172 primary care patients. The results demonstrated adequate psychometric properties for the resulting 10-item SicknessQ-scale and gave support for using SicknessQ as a brief instrument to assess human sickness behaviour.

In **paper II**, the relationship between inflammatory markers, health anxiety, sickness behaviour and self-rated health was investigated in 311 primary care patients, 172 of which were also part of the study population in paper I. Furthermore, mediation analysis was conducted to exploratory investigate if putative relationships between inflammatory markers and self-rated health were statistically mediated by sickness behaviour. The results showed that poor self-rated health was associated with increased sickness behaviour and higher health anxiety. In addition, elevated levels of IL-6 were associated with poor self-rated health in men, although this association was not mediated by sickness behaviour.

In **paper III and IV**, the longitudinal associations between self-rated health, sickness behaviour, asthma-related quality of life, inflammatory markers (paper III: F_ENO, ECP, EDN, IgE, paper IV: IL-5, IL-6) and lung function were investigated with repeated measurements in 181 patients with allergic asthma during 12-months. Poor self-rated health was associated with increased sickness behaviour, poorer asthma-related quality of life and high levels of seasonal IgE and food IgE but not total IgE or F_ENO, ECP or EDN. In men, a u-shaped relationship was found where both low and high levels of IL-6 were associated with increased sickness behaviour. Analysed over time, a worsening in sickness behaviour was associated with a worsening of self-rated health. Also, an improvement of asthma-related quality of life was associated with an improvement in self-rated health. In men, but not

women, increased lung function measured as FEV1 was associated with an increase in IL-6, better self-rated health and increased asthma-related quality of life over the year.

In this thesis, new knowledge is gathered to understand the underpinnings and interrelations between self-rated health, sickness behaviour and inflammation. More sickness behaviour emerged as a more consistent determinant of poor self-rated health compared to inflammatory markers, for which mixed results were found. The results of the thesis highlight the role of sickness behaviour in subjective health perception.

LIST OF SCIENTIFIC PAPERS

I. A global measure of sickness behaviour: Development of the Sickness Questionnaire

   Andreasson A, Wicksell R.K, **Lodin K**, Karshikoff B, Axelsson J, Lekander M. (2016). *J Health Psychol. 2016. Epub 2016/07/28.*

II. Associations between inflammation, self-rated health and sickness behaviour in primary care

   Lodin K, Lekander M, Petrovic P, Hedman E, Andreasson A. *Manuscript*

III. Associations between self-rated health, sickness behaviour and inflammatory markers in primary care patients with allergic asthma: a longitudinal study

   Lodin K, Lekander M, Syk J, Alving K, Andreasson A. *NPJ Primary Care Respiratory Medicine. 2017;27(1):67.*

IV. Longitudinal co-variations between inflammatory cytokines, lung function and patient reported outcomes in patients with asthma

   Lodin K, Lekander M, Syk J, Alving K, Predrag P, Andreasson A. *PLoS One, 2017. 12(9): p. e0185019*

CONTENTS

LIST OF ABBREVIATIONS

EDN	Eosinophil-derived neurotoxin
ECP	Eosinophil-cationic protein
F_ENO	Fraction of exhaled nitric oxide
FEV1	Forced expiratory volume during one second
ICS	Inhalation corticosteroids
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LPS	Lipopolysaccharide
LTRA	Leukotriene-receptor antagonist
mAQLQ	Mini-asthma quality of life questionnaire
NOAK	Optimization of the anti-Inflammatory treatment of asthma through exhaled nitric oxide for increased asthma-related quality of life
NOS	Nitric oxide synthase
PROM	Patient reported outcome measure
SIA	Self-rated health and inflammation at Älvsjö Vårdcentral
SicknessQ	Sickness Questionnaire
SRH	Self-rated health
T_H	T helper
TNF	Tumor necrosis factor

1 INTRODUCTION

Diffuse sickness symptoms such as fatigue, malaise, depressed mood, fever and increased pain sensitivity are common and routinely treated in primary health care [1]. Due to their non-specific nature, these symptoms are sometimes found difficult to interpret and treat satisfactorily by the clinician. They represent a large cost for the society and most importantly, they impair the subjective health status for the individual. In fact, many of these diffuse symptoms are associated with poor self-rated health [2]. Poor self-rated health has been shown to be a predictor of morbidity and mortality in a number of studies [3-5] though relatively little is known about its biological and psychological determinants [6]. Several investigations show that measures obtained directly from the individual subject are strong predictors of mortality [3, 5]. The importance of self-ratings of health have increased lately with the inclusion of patient-reported outcomes in health care [7], but neither the biological underpinnings of perceived health nor why it is associated with objective health outcomes is well known.

Inflammatory processes mediated by inflammatory cytokines are central to the pathogenesis of several maladies, for instance allergic asthma. Recently, the impact of inflammation on brain function has received much attention. Specifically, the brain has been shown to be partly regulated by peripheral signals such as inflammatory cytokines, which are central in orchestrating behavioural changes during acute inflammation by initiating a coordinated set of symptoms collectively referred to as sickness behaviour [8], including fatigue, pain and negative affect. Those symptoms closely resemble the main determinants of self-rated health which, in turn, are associated with poorer health [9], even when adjusted for diagnosis.

Biological as well as behavioural aspects of sickness may be key to the understanding of diffuse and unspecific symptoms underlying many states of ill health that are often the reasons why a patient seeks primary health care. However, it is not known whether the association between self-rated health and inflammation is mediated by sickness behaviour or whether there are other independent contributors. Furthermore, it has not yet been established whether the inflammatory markers elevated in chronic inflammatory disease like allergic asthma may have an effect on subjective health appraisal. In addition, disease-specific markers such as those increased in allergic asthma in comparison to pro-inflammatory markers are less well characterised in terms of their influence on brain and behaviour.

This thesis aims to investigate the importance of sickness behaviour for subjective health perception and if sickness behaviour mediates an association between inflammatory markers and subjective health perception in primary care patients and in patients with asthma. It was written in an effort to try deepen the knowledge of the underlying mechanisms behind diffuse symptoms of ill health with an ambition to ultimately be able to provide better care in the future for individuals who experience such symptoms.

2 BACKGROUND

2.1 PSYCHONEUROIMMUNOLOGY

It is now well established that communication between the brain and the immune system is reciprocal [10]. Neuroendocrine and autonomic pathways are activated by behavioural responses to infectious stimuli resulting in modulation of the immune system [11]. The immune system is highly integrated with several other physiological systems. Its lymphoid organs are innervated by sympathetic, parasympathetic and sensory nerves and the immune system is able to interact with almost every hormone in the human body. Thus the immune, endocrine and nervous systems communicate bi-directionally and are not isolated closed circuits working independently of each other [12]. The field of psychoneuroimmunology therefore has emerged as an interdisciplinary effort to delineate the links between brain, behaviour and the immune system and provides one framework that can be applied for increased understanding of subjective health perception.

As a short introduction to this interplay, the background section will be presented to explain a few basic principles of the immune system, the inflammatory process and how the immune system can communicate with the brain. This will be followed by a short background about self-rated health and sickness behaviour and finally, the chronic allergic inflammatory disorder asthma will serve as a model for investigating the influence of both local and systemic inflammatory factors on subjective health perception and sickness behaviour.

2.2 THE IMMUNE SYSTEM

The immune system consists of an innate (non-specific) and an adaptive (specific) defence arm. Every day we encounter a number of threatening pathogens such as viruses and bacteria which are repelled by the innate immune system, acting as first line of defence. Usually this combat takes place without our knowledge and the threat is warded off before we even can detect any signs of infection. The innate immune system protects our bodies through a number of defences including anatomic, physiologic and inflammatory barriers and through cellular defence mechanisms. Furthermore, the innate and the adaptive immune systems closely interact with each other. The adaptive immune response can be further divided into cellular and humoral responses where the cell-mediated immune response is best suited for eliminating endogenous antigens (residing within the host) and the humoral response is more effective for eliminating exogenous antigens (produced outside of the host cell) [13]. In contrast to the fast acting innate response reacting within minutes to a few hours, the adaptive immunity reacts slower and develops within 1-2 weeks [14].

All white blood cells involved in the immune system are developed from a pluripotent stem cell into either lymphoid stem cells or myeloid stem cells [13]. The lymphoid stem cells can be further divided into B-lymphocytes, T-lymphocytes and NK-cells. The lymphocytes are the only cells in the immune system that possesses the immunological attributes of diversity,

self/non-self recognition, specificity and memory [15]. The myeloid stem cell is the progenitor of several cells in the innate immune system such as granulocytes (neutrophils, eosinophils and basophils), macrophages (such as microglia), dendritic cells and mast cells [14].

2.2.1 The inflammatory response

If the anatomic and physiologic barriers are not enough to protect the body from infection, the innate immune system will begin its action by recognition of pathogen associated molecular patterns, for example the endotoxin lipopolysaccharide (LPS) which is a component of gram negative bacterial walls. When the pathogen associated molecular patterns binds to pattern recognition receptors, a cascade of reactions will be induced including vascular and cellular changes in response to infection [15]. However, the inflammatory response can also occur in the absence of a pathogen in response to tissue injury for instance, and the inflammation is then classified as sterile inflammation [16].

Commonly when there is an infection or tissue damage, the immune system reacts by initiating a local inflammatory response. Local inflammation is characterised by five cardinal signs of inflammation: dolor (pain), calor (heat), rubor (redness), tumor (swelling) and functio laesa (loss of function). These changes are due to a complex series of events orchestrated by the immune system including increased production of cytokines by activated immune cells and cellular defence mechanisms [13]. If this reaction is insufficient in preventing further infection or tissue damage, the inflammatory process spreads further into the blood and creates a systemic inflammation which can eventually lead to sepsis and death if not cleared by the immune response [14, 17].

The granulocytic cells of the innate immune system react fast and neutrophils and eosinophils are generally the first arriving cells at a site for inflammation. They act through phagocytosis and by release of cytotoxic proteins. One of the cytotoxic proteins secreted by neutrophils is nitric oxide [18]. The eosinophils on the other hand secrete other cytotoxic proteins like eosinophilic cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) [19-21]. The neutrophils are attracted by chemotactic factors such as IL (interleukin)-1 and IL-8, secreted from activated macrophages and a subset of T-cells called T-helper cells (T_H) whereas the eosinophils are attracted by different chemokines and eosinophil chemotactic factor released by mast cells [14]. Macrophages arrive to the site of inflammation a few hours later after having circulated the bloodstream as monocytes. Before arriving, the monocytes enlarge and migrate from the blood vessels to the tissue where they differentiate into tissue specific macrophages [13]. At the site of inflammation, the macrophages act by phagocytosis, and similar to the neutrophils they secrete nitric oxide and other cytotoxic proteins [14]. In addition, they function as antigen-presenting cells to the adaptive immune system, and secret IL-6 which further promote B-cell differentiation and activate the humoral defence [22]. Macrophages also secrete tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)

which have cytotoxic effects and further activate macrophages and neutrophils, thus enhancing phagocytic activity even more. The system is thus able to facilitate and direct the response by suppressing or enhancing the production of a variety of interferons and cytokines [14]. After the initial combat, T helper cells, T cytotoxic cells and B cells from the adaptive immune system arrive to provide help for driving and regulating the immune response [13]. The T_H-cells can further be divided into T_H1 and T_H2 depending on their actions. T_H1 cells secret cytokines such as IL-2 and IFN-γ, which activates cytotoxic T-cells and macrophages which act through the cell-mediated response suited for eliminating endogenous antigens. The T_H2 cells secrete IL-6 and IL-10 which promote B-cells to differentiate into antibody producing plasma cells thus directing the humoral defence system towards fighting extracellular pathogens and mediating allergic reactions [14]. Some of the T_H2 cytokines such as IL-4 and IL-5 induce production of immunoglobulin (Ig) E which is important in, for instance, allergic asthma [23].

Inflammation can be divided into acute and chronic inflammation based on time course and immune response patterns. The acute inflammation transforms into a chronic state as a result of failure to eradicate or shut off the acute inflammatory trigger, autoimmune responses or chronic irritants [17]. In many chronic disorders the initiating trigger is not well defined and multiple triggers may be present. Resolution of inflammatory responses is terminated when adaptive inflammatory responses are terminated [14, 17].

2.2.2 Immune system to brain communication

Cytokines can be produced both peripherally and in the nervous system [14]. They are generally considered to be either pro-inflammatory or anti-inflammatory. Pro-inflammatory cytokines can induce one or several of the five cardinal signs of inflammation: fever (calor), pain (dolor), vasodilation (rubor), swelling (tumor) and loss of function (functio laesa) whereas the anti-inflammatory cytokines are involved in downregulation of the inflammatory cascade [13]. To be able to maintain a proper immune response, a well-regulated balance between pro-inflammatory and anti-inflammatory cytokines is crucial [24]. However, cytokines do not act one by one on their own but rather in cascades. Furthermore, the cytokines have redundant functions. During normal conditions cells encounter a milieu of various cytokines and cytokine actions are thus context-dependent [24-26].

Cytokines mediate specific physiologic responses in a similar manner to hormones and neurotransmitters by receptor-ligand interactions with self (autocrine), local (paracrine) and distal (endocrine) effects [27]. Cytokines can be produced by a large variety of cells. In addition, several unrelated cell types can produce the same cytokine. The primary source of cytokines is immune cells but some non-immune cells such as neurons and muscle cells can also produce cytokines [14]. Cytokines can be produced peripherally or in the brain [24].

Because the brain is involved in immune regulation, and behavioural changes are necessary to promote recuperation, the brain and the immune system need to communicate [24, 28]. Cytokines are too large to freely penetrate the blood-brain-barrier. Cytokines access the brain through a number of pathways. So far, five separate pathways have been described, see Haroon for a review [12]. Cytokines can enter the brain (1) through leaky regions in the blood-brain-barrier such as circumventricular organs, (2) through binding to cytokine specific transport molecules, (3) activation of cells lining the cerebral vasculature, or (4) by recruitment of activated monocytes and macrophages from the periphery to brain tissue which then initiates release of inflammatory mediators and cytokines directly into the brain. Peripheral cytokines can also (5) bind to cytokine receptors at afferent nerve fibres which innervate bodily sites. At sites of inflammation in the periphery, afferent nerve fibres become activated and transmit cytokine signals to specific brain nuclei such as the nucleus of the solitary tract. In the nucleus of the solitary tract the signals are then transmitted to other brain nuclei including the paraventricular nucleus in the hypothalamus [8, 12]. Thus, in response to peripheral cytokines activating afferent nerves, induction of expression of brain inflammatory cytokines takes place [27].

In the brain, neurons and cells (such as microglia and astrocytes) express cytokine receptors, produce cytokines and amplify cytokine signals [13]. Microglia are the most active cytokine producing cells [29] and are the equivalent of macrophages in the brain. In response to peripheral inflammatory signalling, multiple cytokines such as IL-1β, TNFα, IL-6 and IFN-γ are expressed in the brain. Each of these cytokines also has the power to initiate or modulate neurochemical cascades that directly affect behaviour through the release of neurotransmitters including serotonin, dopamine, norepinephrine, monoamines and glutamate [12, 24]. In fact, this has been demonstrated by experimental acute or chronic administration of cytokines in numerous laboratory animal studies [30-32] and human studies [12, 26, 33]. Some of the resulting behavioural changes constitute a highly coordinated set of behavioural patterns referred to as sickness behaviour.

The cerebrospinal fluid communicates with all cell types in the brain [12]. Cytokines produced in the brain can re-enter the blood circulation through resorption from the subarachnoidal space. They leave the brain through neuronal extensions of the subarachnoidal space that surround nerves going in and out of the brain and thus enter the peripheral blood [28]. Through the acute release of inflammatory cytokines influencing the brain the inflammatory response will have widespread effects on both the nervous system and the immune system. The immune system thus regulates behaviour and brain activity including emotional and cognitive domains [34, 35].

2.3 SICKNESS BEHAVIOUR

Sickness behaviour refers to a set of coordinated physiological and behavioural changes in response to systemic inflammation. These changes constitute an active process and are thus

not only passive consequences in response to inflammation. The behavioural pattern in the sickness response is referred to as sickness behaviour and typically manifests as fatigue, lack of energy, increased pain sensitivity, depressed mood, sleep changes, malaise, chills, anxiety, anorexia, psychomotor retardation, fever and anhedonia (i.e. inability to experience pleasure), figure 1 [28, 36, 37]. Several of these symptoms including fatigue, lack of energy, pain and depressed mood are the main determinants for self-rated health [38, 39] and the sickness symptoms thus closely resemble the determinants for self-rated health [9].

The evolutionary biologist Paul Ewald theorised that host signs of infection could mainly function in three ways [40]. Firstly, host signs of infection could function to benefit the host. For instance, fever would be beneficial for survival of the host but not for the pathogen. Secondly, it could be of benefit for both the host and the pathogen. As an example, sneezing will help the host to get rid of the pathogen by exhaling both pathogen and mucus from the airways and at the same time it will help the pathogen to spread by aerosol to other hosts. Thirdly, it could benefit neither the host nor the pathogen. Ewald thus gave support to the idea that some behavioural patterns could be an adaptive response to fight infections. A couple of years after Ewald's 1980 paper Hart published his study " Biological basis of behaviour of sick animals" where he studied fever, a very energy intense state, in rats [41]. Hart argued that sickness behaviour could be understood as a coordinated response aiming at reducing costly energetic behaviour so that available energy could be redirected to raise the rats' core temperature instead. He concluded that this behaviour was not a maladaptive effect of illness. Instead, this effort could be viewed as highly organised and evolved strategy to help in producing fever to combat infection. These studies have been followed by a long series of studies on sickness behaviour supporting the idea that these behaviours represent genetic adaptations to increase fitness and introducing the idea that dysregulation is a risk for illness in itself. Sickness response thus benefits the host, but can when unabated contribute to development of disorders such as depression [42].

Immune responses are highly energy dependent and increase resting expenditure [43]. Many of the sickness symptoms serve to limit motor and brain activity as well to limit appetite and thereby redirect metabolic energy to combat the primary infection or inflammation. Sickness behaviour such as motor inhibition and increased sleep helps conserve energy needed for recovery to increase the survival of the organism [41, 43, 44].

Several studies have been conducted that support the notion that sickness behaviour is a motivational state. For instance, Aubert showed that female mice expressing sickness behaviour due to treatment with LPS (lipopolysaccharide, an endotoxin) diminished their nest building and pup retrieval compared to saline-treated controls [44]. The surrounding temperature was then decreased from normal 22° C to 6° C, threatening pup survival. Confronted with this threat, the LPS-treated mice ignored their sickness behaviour and increased their nest building and pup retrieval equalling the control animals [45]. In a study by Lasselin et al., healthy human subjects were injected with low-dose LPS to experimentally

induce sickness behaviour and then presented with a monetary reward task [46]. Despite the energy-saving behaviours expressed by the subjects, the incentive to receive a monetary reward altered the motivational state so that the task was carried out as long as the reward was considered to be worthwhile. The fact that sickness behaviour was suppressed when the evolutionary fitness was challenged contributed to the view of sickness behaviour as a motivational state, aimed at promoting recovery. Thus, sickness behaviour cannot simply be viewed as a sign of bodily weakness [42]. Besides experimental induction of sickness behaviour through administration of a pathogen it can be activated without involvement of a pathogen. For instance, sickness behaviour can be induced by conditioning [47] or by direct administration of LPS [48].

On the one hand, behavioural changes following sickness behaviour facilitate recovery from an acute illness. On the other hand, these behavioural changes are believed to contribute to ill health when they persist long after recovery or where recovery has not been completed [49]. Over-exaggerated sickness behaviour can thus be damaging. This is particularly true in the case of chronic inflammatory diseases where persistent sickness behaviour leads to ill health. For example, a study showed that individuals with medical conditions such as rheumatic disease or certain forms of cancer experienced fatigue, depression or pain as a result of the inflammatory response triggered by the disease [50]. Other areas where longer-term effects of sickness behaviour have received growing attention are psychiatric illnesses such as depression [36, 51] and psychosis [52], other chronic inflammatory disorders [53] and cytokine cancer treatment [54].

2.4 SELF-RATED HEALTH

Subjective measures, such as self-rated health, obtained directly from the individual patient constitute one of the strongest predictors of mortality [3, 5], even when controlled for diseases and objective medical health issues [2, 5, 57]. In fact, self-rated health is often a better predictor of mortality and morbidity than objective health estimates and risk factors [5] and it is a stable and fairly reliable factor over time [58, 59]. The importance of self-ratings of health has increased recently with the inclusion of patient-reported outcomes in health care [7], but neither the biological underpinnings of perceived health nor why it is associated with objective health outcomes is well known [39, 60]. Self-rated health refers to an individual's perception of his/her general health status assessed by using a single-item question, most often formulated as "How would you rate your general health status on a range from excellent to very poor?" It is, thus, the subjective view of one's own health status rated on a specific scale and conducted by the individual instead of the clinician. In spite of being a one item question only, it captures a surprising amount of information [61]. A main reason for the relevance of self-rated health lies in its ability to predict future ill health and death [4, 5, 62] and the predictive value in relation to morbidity and mortality as well as other clinical outcomes brings up the need to clarify its biological basis. Lekander et al. have argued, and -

Figure 1. *Sickness behaviour typically manifests as fatigue, lack of energy, increased pain sensitivity, depressed mood, sleep changes, malaise, chills, anxiety, anorexia, psychomotor retardation, fever and anhedonia [28, 55, 56]. The term behaviour is here used in its wider sense, including emotion and cognition, in addition to overt behaviours/motor responses. The phenomenon was first observed in mice and other animals, then referring to observable responses.*

presented evidence to support the idea, that the brain uses inflammation with its ensuing behavioural changes as a marker when health status is appraised [9, 63].

An association between inflammatory cytokines and poor self-rated health has for instance been found in the elderly [64, 65], care-givers with age-matched controls [66] and women with cardiovascular disease [67]. High levels of IL-1β, IL-1ra and TNF-α have been associated with poor self-rated health in women but not in men (for which the number of participants was low) in a primary health care population [6, 9]. In fact, in the study by Lekander et al. self-rated health was found to be an independent and more robust predictor of those cytokine levels than physician-rated health [9]. Higher levels of IL-6 were associated with poorer self-rated health in older adults in ten consecutive measurements [68]. Other studies have shown different results. For instance, in one sample of 116 relatively younger healthy individuals aged 23-62, no association was found between the inflammatory cytokines TNF-α or IL-6 and self-rated health [69]. Self-rated health has also been associated with other markers of systemic inflammations, such as C-reactive protein [66, 70-72], increased fibrinogen [73] and higher erythrocyte sedimentation rate [74]. Finally, it should also be noted that the inflammatory marker that has shown a significant association with self-rated health has varied somewhat across studies and results are thus not fully consistent in this respect.

2.5 PROMS - SUBJECTIVE MEASURES OF HEALTH AND DISEASE

When a clinician measures self-rated health or asthma-related quality of life no technical or expensive equipment is needed. Instead, these measures are reported directly by the patient without interpretation of the response by a clinician or anyone else. They are subjective measures of the patient's perceived health which cannot be objectively verified. Collectively, such measures are called patient-reported outcomes (PROMs). PROMs can be divided into generic PROMS considering general aspects of health (such as self-rated health and sickness behaviour), or disease specific PROMs tailored to capture disease-specific symptoms and impact on function (such as asthma-related quality of life).

PROMs have been indicated to increase cost-effectiveness and improve survival and are designed to capture the patients' views of their symptoms, their functional status and their health-related quality of life [75-77]. They were initially developed for use in research, but have recently gained interest also in clinical practice since most clinicians have now started to recognise the benefits of incorporating the views of patients in their recorded assessment of the patient's health status. The reliability of PROMs is similar to that of clinical measures such as blood glucose or blood pressure [78].

2.6 ASTHMA

Most studies on sickness behaviour and self-rated health have so far been conducted on acute inflammatory conditions. Less is known about the relationship between sickness behaviour,

self-rated health and inflammatory markers during states of chronic inflammation. In this thesis, asthma was chosen as a representative model of chronic inflammatory disease with both local and systemic inflammation.

Asthma is a chronic inflammatory respiratory disorder characterised by episodic and reversible airway obstruction and bronchial hyperresponsiveness. In the GINA guidelines (Global Initiative for Asthma), asthma is defined by "the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation"[79]. The disease usually starts in childhood although it can present at any age. Up to 300 million people worldwide are affected by asthma [80]. Asthma is the most common chronic condition to affect children [74]. The prevalence is approximately 5-10% in western countries [81]. In the West Sweden asthma study, consisting of a random sample of thirty thousand individuals aged 16-75 years living in West Sweden, Lötvall et al. reported an 8.3% prevalence of physician-diagnosed asthma in Sweden [82]. In another study, Wändell et al. reported that asthma was ranked number 17 of the most common groups of diagnoses in primary care during 2011 in Stockholm County [83].

Asthma is considered to be a heterogeneous disease and the emerging view is that asthma should be considered as a syndrome rather than a single disease [80]. Asthma has traditionally been divided into two clinical forms: allergic and non-allergic asthma. In recent years, clinicians have started to realise that dividing asthma into only two clinical forms is oversimplification and several phenotypes based on demographic, clinical and/or pathophysiological characteristics have now been recognised [79, 80]. However, the old division into two phenotypes is still of clinical value in a primary care setting and will therefore be used in this context.

The most common asthma phenotype is allergic asthma [79, 84]. In allergic asthma, the patients are IgE sensitised to an allergen and there is usually a clinical correlation between allergen exposure and symptoms [84]. These features are in contrast to non-allergic asthma phenotype which per definition has no IgE reactivity. Furthermore, patients with allergic asthma usually have higher levels of IgE compared to patients with non-allergic asthma and the onset of the disease is at a younger age [84]. The risk of developing asthma increases with multiple sensitisation to different allergen groups [85]. In addition, patients with allergic asthma have an increased prevalence of atopic dermatitis and up to 80% of the patients have concurrent allergic rhinoconjunctivitis [80, 86].

2.6.1 Pathophysiology in allergic asthma

Allergic asthma is characterised by T_H2-driven inflammation with recruitment of both eosinophilic granulocytes and mast cells [87]. It is a chronic inflammatory disorder of the airways where T_H2-cells produce IL-4, IL-5 and IL-13 [88]. This T_H2-dominated cytokine

balance drives the allergic inflammation, including a switch in antibody production from IgM to IgE, and is related to the clinical manifestation of asthma [89]. The hallmark of an allergic response is immediate hypersensitivity reactions mediated by IgE-antibodies attached to specific parts of the membrane of mast cells and basophils [90]. When a mast cell or basophil encounters an allergen, the allergen gets trapped by the IgE-molecule which is bound to the surface of the cell. The cross-linking of IgE-antibodies and the allergen triggers the cell to release its granular content such as histamine, ECP, EDN and other cytotoxic protein, resulting in an "early phase" allergic reaction [14]. The signs and symptoms of this reaction are dependent on where in the body the granular content is released. When it is released in the lungs it can cause asthma. Other locations can be in the skin causing atopic dermatitis or eczema, the nose (rhinitis) or the gut (food-allergic reactions) [14, 90]. The degranulation and liberation of cytotoxic proteins and cytokines initiate the "late phase" allergic reaction. In the "late phase" eosinophils and neutrophils are recruited and activated on inflammatory sites sensitive to allergens where they will contribute to disrupting the epithelial cells causing erythema and continued swelling [14]. Chronic inflammatory asthma results in airway hyperresponsiveness, increased mucus secretion and airway remodelling. These changes, in turn, lead to airway obstruction and reduced lung function [91].

In addition to the systemic inflammation with production of IgE and cytokines, there is also a prominent local eosinophilic inflammation [91]. When local eosinophils are activated they release granular proteins such as eosinophil-derived neurotoxin (EDN) and eosinophil-cationic protein (ECP) which act locally in the lungs but also contribute to the systemic inflammation [92]. Several studies have shown a correlation between eosinophilic activity in the lungs, plasma levels of EDN (p-EDN) and serum levels of ECP (s-ECP) [21, 93]. High s-ECP and p-EDN have been suggested to reflect asthma activity and severity [92, 94, 95]. The local eosinophilic inflammation in the airways is also reflected in the fraction of exhaled nitric oxide (F_ENO) [96-98], which is increased in patients with allergic asthma [99, 100]. Nitric oxide is produced by a reaction involving nitric oxide synthase (NOS) enzymes. NOS exists in three different isoforms: inducible (iNOS), endothelial (eNOS) and neural nitric oxide synthase (nNOS) [101]. Pro-inflammatory T_H-2 cytokines such as IL-4 and IL-13 upregulate iNOS in the respiratory epithelium which results in increased F_ENO [102, 103]. For a long time F_ENO has been considered to be a reliable marker of eosinophilic airway-inflammation. However, recent studies indicate that F_ENO actually is more representative of a T_H2-driven inflammation in the local bronchial mucosa rather than a general eosinophilic inflammation [102]. Furthermore, both IgE-antibody titres and positive skin-prick tests indicate that the degree of IgE sensitisation correlates to levels of F_ENO [104-106].

2.6.2 General PROMs and inflammation in asthma

Traditionally when a clinician has evaluated the health and quality of life of a patient with asthma the consultation has relied on clinical examination and objectively verified clinical asthma measures. Recently, many studies have shown a weak correlation between clinical

asthma measures and asthma-related quality of life, suggesting that quality of life cannot be inferred by clinical data only [107-109]. In light of this new knowledge, PROMs are gaining more clinical interest but the usage of general PROMs in asthma so far has still been limited to a few clinical studies.

The role of self-rated health and sickness behaviour in asthma, and its relation to chronic inflammation, are largely unknown. Syk and co-workers showed in a population-based study including 5355 persons from Stockholm County that persons with a diagnosis of asthma reported poorer self-rated health than persons without asthma[110]. Furthermore, self-rated health was associated with asthma at least as strongly as quality of life.

To the best of my knowledge there are no published studies where sickness behaviour has been investigated specifically in asthma. However, in a study by Sundbom et al. patients with asthma who reported presence of anxiety, depression and sleep disturbances had significantly lower asthma-related quality of life compared to a group of patients with asthma without those reported sickness symptoms [111]. Unfortunately neither self-rated health nor inflammatory markers were included in that study and only some aspects of sickness behaviour were among the investigated variables. The knowledge about the associations between sickness behaviour, self-rated health and the inflammatory markers IgE, ECP and EDN in patients with asthma is also limited and sparse and no studies have been published investigating the associations between either self-rated health or sickness behaviour in relationship to the inflammatory markers, IgE, ECP and EDN. However, ECP has been reported to correlate positively to subjective symptoms and objective measures of asthma activity in a young Swedish population, which in turn are believed to influence subjective health [112].

The association between inflammatory cytokines and allergic asthma has been more thoroughly studied. Allergic asthma is, as noted, characterised by a T_H2-dominated cytokine balance including the production of the inflammatory cytokines IL-4, IL-5 and IL-13 [88]. In addition, the pro-inflammatory cytokine IL-6 has been shown to be elevated in both serum and in bronchoalveolar lavage in asthmatic patients [113]. Serum levels of IL-6 have also been suggested to be an adequate measure of disease activity [114] and are also of importance in subjective health appraisal [64, 68]. The cytokines IL-1β and TNF-α have both been shown to be increased in asthma [115] and have both been associated with poor self-rated health [6, 9] and shown to be able to induce sickness behaviour [116, 117]

The relationship between F_ENO on the one hand and factors of relevance for subjective health on the other is another area where the knowledge is limited and conflicting results exist. For instance, one study reported no effect on F_ENO -levels during examination stress in asthmatic students but a small reduction in F_ENO -levels was observed in non-atopic students [118]. Other studies have reported associations between higher F_ENO -levels and negative affect, anxiety and acute stress [119-121].

2.6.3 Asthma-related quality of life - a disease-specific PROM

Asthma-related quality of life is a disease-specific PROM aimed to capture the perceived impact of asthma on a patient's quality of life. The concept of quality of life has become an increasingly used measure in clinical research. There is no exact definition of which parameters should be included in this term but the constructs that have historically been covered in quality of life measures are: "health status (most often symptom levels), functional status (activity capabilities or impairments) and the patient's perception of the impact of these impairments on the individual's quality of life" [108]. Focusing on the importance of the individual's perspective could attain a more complete description of the patient's perceived situation. The individual's assessment of quality of life could complement classical clinical outcome variables, which may only partly describe the burden of illness. In asthma, PROMs could thus potentially improve the clinician's assessment and treatment by including the patient's perspective of the disease [7]. There are several instruments measuring asthma-related quality of life [108]. All of the instruments are constructed to try to identify the problems that are most frequent and troublesome for the patient and to evaluate to what extent those problems affect daily life. This thesis will focus on one of these instrument called Mini-Asthma Quality of Life Questionnaire (mAQLQ) which is frequently used in clinical settings [122].

In contrast to the gap in knowledge regarding the associations between general PROMs and asthma, the association between the disease-specific PROM asthma-related quality of life and measures of subjective health is well established. For instance, asthma-related quality of life is closely associated with all items in the subjective Asthma-Control Test consisting of five items including subjective limitation of function, subjective dyspnea, subjective nocturnal symptoms, self-reported medication and subjective perception of asthma control [123-125]. Syk and co-workers conducted a large randomised controlled trial investigating whether a FENO-guided anti-inflammatory treatment algorithm could improve asthma-related quality of life [126]. The study was called the NOAK study and in this case NOAK was an abbreviation for "Optimization of the anti-inflammatory treatment of asthma through exhaled nitric oxide for increased asthma-related quality of life", not to be confused with the more commonly used NOAK-abbreviation referring to "new oral anticoagulants". The NOAK study included 187 primary care patients with asthma diagnosis (material from this study is also used in paper III and IV in my thesis). The results showed that although FENO-guided anti-inflammatory treatment improved asthma symptom control and reduced the exacerbation rate, asthma-related quality of life as measured by mAQLQ was not significantly affected. Data from this study were further analysed post hoc at baseline and after one year to investigate if optimisation with inhaled corticosteroids (ICS) and leukotriene-receptor antagonist (LTRA) according to either symptoms or F_ENO -levels would vary over one year when parameters such as asthma control questionnaire and levels of IgE were taken into account. In total, 158 patients with well-controlled asthma were included in the post hoc analysis. The results showed that a decrease in perennial, total and all specific IgE correlated significantly with a reduction in F_ENO -levels, improvements in asthma quality of life and

improved asthma control. Importantly, this study suggested that IgE could be reduced by LTRA and ICS and result in a clinically important effect on asthma control and asthma-related quality of life [127].

There are only a few studies where the relationship between asthma-related quality of life and inflammatory markers has been investigated. Sundbom et al. recently investigated variables with potential impact on asthma-related quality of life in patients in the MIDAS cohort consisting of 369 children and young adults (aged 12-35 years) with physician-diagnosed asthma [111]. The results showed that uncontrolled asthma as measured by low Asthma - Control Test score was the main predictor for impaired asthma-related quality of life. However, no association was found between mAQLQ and blood eosinophil count, levels of F_ENO, total IgE, lung function as measured by forced expiratory volume during one second (FEV1) or bronchial hyperresponsiveness which all are indicators of asthma control.

2.6.4 Coherency between subjective PROMs and objective measures of disease

More traditionally, objective measures such as clinical variables have been the first choice when trying to assess the patient's quality of life. However, objective measures do not include the patient's view of his or her own health status. Among the common objective asthma measures is lung function which can be measured as FEV1 by spirometry. Other relevant objective measures can be obtained through reversibility testing after bronchodilation or by measuring fraction of exhaled nitric oxide. Even though the use of either subjective PROMs or objective measures aims to capture information of the patient's health, the two types of information obtained often mismatch, suggesting that quality of life cannot be measured using clinical data only.

In a study by Ehrs et al. of 77 primary health care patients with mild asthma, no correlations were found between asthma-related quality of life (measured by AQLQ) and clinical measures of asthma, such as lung function, reversibility to bronchodilation or levels of F_ENO [128]. Sundell et al. investigated the health-related quality of life in 159 adolescents with asthma and found no correlation between quality of life and FEV1 [129]. In another study conducted by Wechsler et al. 46 patients with asthma where randomly assigned double-blindly to treatment with an albuterol inhaler, a placebo inhaler, sham acupuncture or no intervention [130]. The subjective patient-reported improvement was significant in all three of the intervention-groups when compared to the control group even though no objective improvement was found. Shingo et al. conducted a study where the correlation between airway obstruction and patient-reported outcomes in 1576 patients with asthma was investigated [131]. The results showed that FEV1 was only weakly correlated to physical dimensions of asthma-related quality of life such as daytime symptoms and "as needed" beta-agonist use. Taken together, these studies by Ehrs, Sundell, Wechsler and Shingo suggest that

there is a discrepancy between objective clinical asthma measure and subjective health appraisal in asthma.

2.7 SUMMARY OF THE BACKGROUND

With this background, a theoretical framework has been introduced highlighting an existing link between behavioural factors such as subjective health, different sickness symptoms and inflammatory markers, underlining the critical role of bidirectional communication between the immune system and the nervous system. Even though some parts of this intricate system have started to be explored, more knowledge is especially needed in patient populations where symptoms of sickness and subjective health factors are common complaints, such as in primary health care.

3 AIMS

The overall aim of this thesis was to investigate sickness behaviour as a determinant of self-rated health and as a possible mediator in an association between inflammatory markers and subjective health perception in primary care patients and in patients with asthma.

The specific aims of each paper were as follows:

I To validate the Sickness questionnaire (SicknessQ).

II To investigate the relationship between inflammatory markers, health anxiety, sickness behaviour and self-rated health in primary care patients. Furthermore, to investigate through exploratory analysis if putative relationships between inflammatory markers and self-rated health were statistically mediated by sickness behaviour.

III To investigate sickness behaviour, F_ENO, s-ECP, p-EDN and IgE as determinants for self-rated health in patients with chronic allergic asthma followed over 12-months.

IV To investigate IL-5, IL-6, FEV1, sickness behaviour and asthma-related quality of life as determinants of self-rated health in patients with asthma. In addition, to investigate the co-variation over time between inflammatory cytokines and subjective patient reported outcomes in relation to objective clinical measures.

4 METHODS

4.1 PARTICIPANTS AND STUDY DESIGN

Data from three separate studies are included in this thesis:
1) "Endotoxin study"
2) "SIA" (Self-rated health and Inflammation at Älvsjö primary health care centre)
3) "NOAK" (Optimization of the anti-inflammatory treatment of asthma through exhaled nitric oxide for increased asthma-related quality of life).

Paper I is based on the endotoxin and SIA studies, **paper II** is based on the SIA study and **paper III and IV** are based on the NOAK study. Descriptive information about participants and study design are given in table 1.

Table 1. Descriptive overview of paper I-IV

	Paper I	Paper I, II	Paper III	Paper IV
Study	Endotoxin	SIA	NOAK	
participants	healthy volunteers	consecutive patients from a primary care centre	Randomised primary care population with asthma-diagnosis	
n	52	311	181	
age	28.6 (20-47, SD 7.1)	51.0 (18-86, SD 16.6)	41.0 (18-64, SD 12.4)	
women	55.8%	65.3%	48.1%	
study type	experimental	cross-sectional	one-year longitudinal	
PROMS	SRH SicknessQ	SRH SicknessQ SicknessQ4 HAI	SRH Sickness-composite	SRH Sickness-composite mAQLQ
Systemic inflammatory markers	IL-6 IL-8 TNF-α	IL-6 IL-8 TNF-α	IgE ECP EDN	IL-5 IL-6
Local inflammatory marker		F_ENO	F_ENO	
Objective measure of disease				FEV1(% predicted)

4.1.1 Endotoxin study (paper I)

In this experimental study, healthy participants were recruited by advertising at university areas in Stockholm. In total, 23 men and 29 women without physical or mental health problems were recruited. For inclusion, participants had to be non-smokers and without previous history of inflammatory or psychiatric disorders. C-reactive protein was assessed to exclude an ongoing infection on the experimental day. Apart from the presently included paper I, other results from the same data collection have been reported in three other publications [132-134].

4.1.2 SIA study (paper I and II)

The SIA study was a cross-sectional study of primary health care seeking men and women attending Älvsjö primary health care centre located in an urban area in Stockholm County. A total of 311 patients were consecutively recruited from a drop-in clinic, serving patients with a wide range of acute medical problems. The study was conducted in a two-wave data collection during six consecutive weeks 2012 and six consecutive weeks 2013. During 2012, 179 patients completed the study (response rate of 83%) and during the second data collection, 132 patients completed the study (response rate 68%). Medical consultations regarding annual health examinations, prescriptions for addictive drugs, chronic pain conditions and extensions of sick leave or other certificates were referred to booked appointments instead of the drop-in clinic and were automatically ineligible. Pregnant patients, patients under 18 years of age and patients not able to speak and read Swedish were excluded.

4.1.3 NOAK study (paper III, IV)

The NOAK study was a 12-month longitudinal study. In total 181 patients (87 women) aged 18 to 64 years with physician-diagnosed allergic asthma and a confirmed IgE sensitisation to at least one airborne perennial allergen were recruited from 17 primary health care centres from November 2006 to March 2010. The health care centres were located in seven different county councils in central and southern Sweden. All participants were non-smokers since at least one year before inclusion and had a previous smoking history of maximum 10 pack-years. In addition, all patients had been on a medication with inhalation corticosteroids (ICS) since at least 6 months before the inclusion. The participants were randomised into two groups. The groups differed in how the anti-inflammatory treatment, dose of ICS and LTRA (montelukast 10 mg daily), was adjusted. The anti-inflammatory treatment was adjusted on the basis of F_ENO in the F_ENO -guided treatment group (n=93). In the control group (n=88), treatment was adjusted based on symptoms according to routine clinical practice [121]. In the papers included in this thesis, however, both treatment groups were collapsed into one group and followed over the one-year period. In total, the participants visited the health care centre six times during the study period.

4.2 ASSESSMENTS

The assessments investigated in this thesis can be divided into PROMs, inflammatory markers and an objective measure of disease.

4.2.1 PROMs

4.2.1.1 SRH

Self-rated health was assessed in all four studies using the SRH-5 question ”How do you rate your general health status”[38]. The response alternatives were rated on a five point Likert scale with the response alternatives: Very good (1), Rather good (2), Neither good nor poor (3), Quite poor (4) and Poor (5).

4.2.1.2 Sickness behaviour

Three scales were used to assess sickness behaviour (1) the Sickness Questionnaire (SicknessQ), (2) SicknessQ4 and (3) a composite measure of sickness behaviour called Sickness-composite. The Sickness Questionnaire (SicknessQ), used in paper I and II, consisted of ten questions rated on a four point Likert scale (with a maximum score of 30 points) [48]. In addition, a short form of SicknessQ called SicknessQ4 (with a maximum score of 12 points), consisting of four items from the original SicknessQ, was included in paper II. The data collection which resulted in paper III and IV was conducted before the development and validation of SicknessQ and at that time no validated measure of sickness behaviour existed. Therefore, in paper III and IV, a composite scale called Sickness-composite attempting to measure sickness behaviour was used instead. The Sickness-composite consisted of weighted means of the answers to the following questions “How satisfied are you with your situation regarding the following aspects: energy/sleep/fitness/appetite and memory” ranging from “very poor” (1) to “excellent, could not be better” (7). However, the scale was inverted during analyses to facilitate interpretation so that higher score corresponded to a higher degree of sickness behaviour. The items in Sickness-composite were originally derived from the ”Gothenburg Quality of life Instrument”[135]. The Sickness-composite measure is a slightly modified version of a composite sickness scale used in an earlier study by our research group [6]. The separate items of each sickness scale and the internal consistency between the different scales are presented in table 2.

4.2.1.3 Asthma-related quality of life

Asthma-related quality of life was assessed using the Mini-Asthma Quality of Life Questionnaire (mAQLQ) [122], developed by Juniper. The mAQLQ is a short 15-item version of the original AQLQ [136] (consisting of 32 items), aimed to measure functional problems (physical, emotional, social and occupational) that are most troublesome to adults with asthma. The responses on each item were rated on a Likert scale ranging from “all of the time/ totally limited” (1) to “none of the time/ not at all limited” (7).

Table 2. The included measures of sickness behaviour

	SicknessQ	SicknessQ4	Sickness-composite
	I want to keep still My body feels sore I wish to be alone I don't wish to do anything at all I feel depressed I feel drained I feel nauseous I feel shaky I feel tired I have a headache	I want to keep still My body feels sore I feel depressed I feel nauseous	How satisfied are you with your situation regarding the following aspects: -energy -sleep -memory -appetite -fitness
response alternatives	disagree(0) - agree(3)	disagree(0)-agree(3)	very poor(1)-excellent, could not be better(7)
Internal consistency (Cronbach's alpha)[1]	.86	0.60	0.72
Correlation with SicknessQ (Spearman's rho) [1]	Reference	0.91, p<.001	-0.06, p=0.441
Paper	I, II	II	III, IV

[1] SIA data used for analyses

4.2.1.4 Health anxiety

Health anxiety was measured using seven items from the 18-items "Short health anxiety inventory" [137]. The seven items included worries about their own health, awareness of bodily sensations or changes, relief when the doctors tells them there is nothing wrong, thoughts about having an illness they have heard about, concerns about what bodily sensations mean, feelings of being at risk for developing an illness and thoughts about whether to be able to enjoy life if having a serious illness. The items chosen included six items from the main section and one item from the negative consequences section. Items were rated from lowest rating of health anxiety (1) to highest rating of health anxiety (4). The internal consistency of the 7-item scale (Cronbach's alpha) was 0.82 and the correlation (Spearman's rho) between the original 18 items and the 7-item scale was 0.87 (p<.001).

4.2.2 Inflammatory markers

The systemic inflammatory markers included in this thesis were inflammatory cytokines, eosinophilic proteins and immunoglobulins. The local inflammatory marker was F_ENO.

4.2.2.1 Cytokines

In paper I and II, circulating levels of the cytokines IL-6, IL-8 and TNF-α were analysed from serum using Millipore's MILLIPLEX multi-analyte profiling (MAP) high-sensitivity human

cytokine kit (Millipore Corporation, Billerica, MA, USA) according to the manufacturer's instructions (paper I) or from plasma using high-sensitive enzyme-linked immunosorbent assay (Quantikine hs ELISA, R&D systems, Minneapolis, USA) (paper II). In paper IV, plasma levels of the circulating cytokines IL-5 and IL-6 were analysed using OLINK multiplex immunoassay (Olink Proteomics, Uppsala, Sweden).

4.2.2.2 IgE, ECP, EDN

In paper II and III, S-IgE and S-ECP were analysed in a Phadia 100 system with ImmunoCAP Phadiatop® (dog, cat, horse, birch, timothy, Dermatophagoides pteronyssinus, Dermatophagoides farinae, Cladosporium herbarum, mugwort) and ImmunoCAP fx5® (cow's milk protein, egg white, peanut, soy, wheat, fish) reagents (Immunodiagnostics, ThermoFischer Scientific, Uppsala, Sweden). Specific IgE antibodies were grouped into three categories: perennial (cat, dog, horse, mite x 2, cladosporium), seasonal (birch, timothy, mugwort) and food (cow's milk protein, egg white, peanut, soy, wheat, fish). Plasma samples for EDN were analysed in a sandwich-ELISA (Diagnostics Development, Uppsala, Sweden).

4.2.2.3 F_ENO

In paper III, F_ENO was measured according to standardised recommendation using NIOX MINO (Aerocrine AB, Solna, Sweden). The participant was asked to inhale to total lung capacity through the NIOX MINO followed by exhalation for 10 seconds at 50ml/sec (assisted by visual and auditory cues). Mean values from two successive measurements were used. F_ENO can be influenced by individual factors such as age, height and sex. Olin et al. reported that individuals aged above 64 years had 40 percent higher levels of F_ENO than those aged 35-44 years [138]. In the general population, women have been shown to have significantly lower F_ENO -levels compared to men [139-141]. Several reasons for sex differences in F_ENO -levels exist. According to Jilma et al. women have lower levels of plasma nitrate and therefore lower production of endogenous nitric oxide [142]. In addition, women have a lower airway diffusing capacity for nitric oxide due to smaller surface area of the conductive airways in relation to body size compared to men [143]. Thus, all mean F_ENO -values (parts per billion) were therefore divided by predicted normal F_ENO -values in non-atopic adult subjects adjusted for height and age.

4.2.3 Objective measure of disease

4.2.3.1 FEV1

In paper IV, forced expiratory volume in one second (FEV1) was assessed through spirometry (Vitalograph, Spirotrac IV, Buckingham, England). Hedenström's reference values were used to calculate percent of predicted FEV1 [144, 145].

5 ETHICAL APPROVALS

All studies were approved by the regional ethics committee in Stockholm, Sweden. The dnr-numbers for the approvals are listed below:

Paper I: 2008/955-31, 2009/1273-32, 2010/1362-32, 2010/1829-32 and 2011/1851-31/1
Paper II: 2011/1851-31/1, 2012/27-32, 2012/952-32
Paper III and IV: 2006/185-31/1

6 STATISTICAL METHODS

For all included papers in this thesis, STATA® 11.0 or 14.0 (StataCorp LP, Texas, USA) was used for all statistical analyses and an α-level of 0.05 was used to test for significance.

Below is a summary of the main statistical analyses used in the included papers.

6.1 DESCRIPTIVE STATISTICS AND BACKGROUND VARIABLES

Differences between genders were tested for all variables using Student's t-test (normally distributed interval or ratio scale data) or Mann-Whitney U-test (non-normally distributed ordinal scale data) when applicable in all studies.

The PROMs included in this thesis were assessed on ordinal scales. In most cases they were approximately normally distributed and thus, mean and standard deviation were used to describe the study group characteristics.

Two separate approaches were used to handle gender. In paper III, an interaction term between gender and the independent variable was included in analyses. By using this approach, gender was treated as an effect modifier rather than as a confounder. In paper II and IV, the analyses were stratified for gender. All models were adjusted for age and BMI as being possible confounders. In paper I, gender was used as a criteria validity variable.

6.2 MIXED EFFECT REGRESSION MODELS (PAPER I, II AND IV)

Mixed effect regression models with participant identity as random effect were used for repeated measurements. These models were used for three types of questions. Firstly, to test for correlations when data consisted of repeated longitudinal measurements for the same individual by including identity as a random effect (paper III, IV). Secondly, to test if

variables would change over time by adding time as a dummy variable in the model (paper III, IV). Thirdly, to test the effect of treatment for an outcome by including treatment and time as one interaction term (paper I).

6.3 PSYCHOMETRIC PROPERTIES (PAPER I)

In the process of developing SicknessQ, potential items to be included in the final scale which were significantly affected by endotoxin with a α-level below 0.05 were further analysed regarding psychometric properties in the first wave of the SIA-material of primary care patients. Specifically, response rate, response distribution, variability and inter-correlations were tested. Factor structure was then tested by principal component analysis and internal consistencies were measured by Cronbach's alpha.

Concurrent criteria validity refers to how well a comparison between the measure of interest and an outcome measured at the same time fits. As an example, negative affect is a symptom which is included in both sickness behaviour and in depression. If a patient reports a similar rating for an almost identical item regarding negative affect on both a depression scale and on the SicknessQ scale, the concurrent criteria validity is considered to be high. For the concurrent criteria validity analyses, bivariate relationships between the final set of items in SicknessQ, anxiety, depression and self-rated health as well as demographic variables were calculated using Pearson or linear regression.

6.4 SPEARMAN'S CORRELATIONS (PAPER II, III, IV)

Spearman's rank correlation is a non-parametric test for correlation. The test was chosen since the variables were ordinal and data non-normally distributed. Spearman's correlations were used to calculate cross-sectional crude and partial correlations between different parameters.

In paper III and IV, Spearman correlations were also used to correlate delta values between visit 1 and visit 5 to investigate if changes in inflammatory factors were associated with changes in PROMs and lung function. Spearman rank correlations were used because of the non-normal properties of the delta values.

6.5 MEDIATION ANALYSIS (PAPER I, II)

A Sobel-Goodman test was used to test indirect statistical effects of mediation [146]. This mediation analysis was based on a longitudinal regression framework to test sickness behaviour as a putative mediator of the effect of inflammatory cytokines on self-rated health. In the mediation models in paper II, elevated levels of inflammatory cytokines (x, causable

variable) are assumed to cause poor self-rated health (y, outcome variable). The effect of inflammatory cytokines (x) may be mediated by sickness behaviour (M, mediator, intervening variable). If the mediation is complete, x will no longer affect y after M has been controlled for. In more detail, the a-path (X to M), was the interaction effect of inflammatory cytokines on sickness behaviour. The b-path (M to Y) was the association between the putative mediator sickness behaviour and self-rated health controlled for inflammatory cytokines. The indirect mediated effect was calculated as the ab cross. The model is graphically illustrated in figure 2.

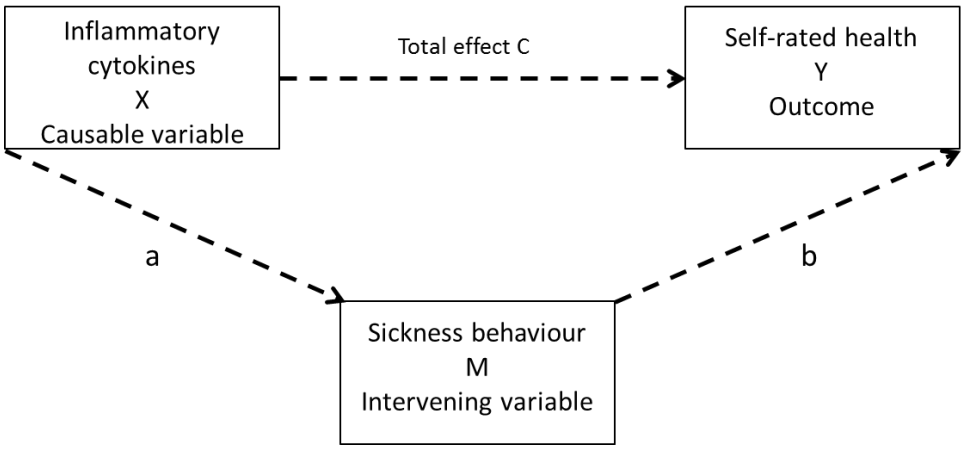

Figure 2. *Schematic model of the mediation analysis in paper II*

Mediation is a causal model where the mediator is assumed to cause the outcome, not vice versa. On a similar note, statistics can be used to evaluate a presumed mediation model but mediation is not defined statistically. A model such as the model used in paper II is further limited to provide evidence for causality since it was a cross-sectional study and the time criterion, was therefore not fulfilled [147]. To fulfil the time criterion, the exposure needs to be assessed before the mediator and the mediator needs to be assessed before the outcome.

6.6 CUBIC SPLINE TEST (PAPER IV)

In paper IV, a univariate cubic spline regression model with three degrees of freedom [148] was used to conduct an exploratory test for non-linearity in the associations between inflammatory cytokines and PROMs. This was done due to the fact that non-linear associations between inflammatory markers and self-rated health previously have been demonstrated [149]. As the associations between cytokines, self-rated health and sickness behaviour were found to be non-linear in the cubic spline regression analyses, IL-5 and IL-6 were divided into quartiles and the lowest category was used as reference in the analyses. To

further explore if the found relationships in fact were u-shaped (i.e. if quartile 2 and 3 differed significantly from quartile 4), quartile 4 replaced quartile 1 as reference.

6.7 Z-TRANSFORMATION (PAPER III, IV)

Crude values of IgE, ECP, EDN and cytokines were z-transformed to facilitate interpretation of regression coefficients (b) (paper III, IV) in the mixed effect regression models, where b represents the change in the outcome variable per one standard deviation increment in the exposure variable. In paper I and II, Pearson's and Spearman's rank correlations, respectively were used and z-transformation were unnecessary.

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6.8 BOOTSTRAPPING (PAPER I-IV)

Due to the non-normal properties of the parameters included in the mediation analysis (paper I, II) and in the mixed effect regression models (paper I, III, IV) all p-values were estimated by bootstrap with 1000 repetitions [150]. The resampling procedure produces a distribution, in this case of the standard error and p-value, that is non-reliant on the assumption of normal distribution.

7 SUMMARY OF STUDIES I-IV

7.1 PAPER I

Background and objectives
Sickness symptoms are common reasons for seeking in primary care. They impair the patient's subjective health and can be problematic to diagnose. Yet, comprehensive methods to measure sickness behaviour in humans are lacking. The aim of paper I was to develop and validate the questionnaire SicknessQ.

Method
The SicknessQ was developed and validated in two steps: Firstly, the participants in the endotoxin study completed the initial form of the SicknessQ with 37 items covering a broad range of sickness behaviours. The participants had blood samples drawn at baseline, at peak inflammatory response 90 minutes after injection, and 270 min after injection and analysed for circulating levels of IL-6, IL-8 and TNF-α. Secondly, 13 items that responded to an acute inflammatory provocation compared to placebo were selected and the psychometric properties were tested on 172 patients from the first data collection in the SIA study.

Results and conclusion
A one-factor solution (Cronbach's alpha=0.86) was indicated in the principal component analysis and a final 10-item questionnaire, SicknessQ, measuring sickness behaviour was presented. In the validation process, the scale correlated with self-rated health ($\beta=0.28$, $p<0.001$), a single item of feeling sick ($\beta=0.55$, $p<0.001$), depression ($\beta=0.41$, $p<0.001$) and anxiety ($\beta=0.36$, $p<0.01$).

The results from paper I demonstrate adequate psychometric properties for the 10-item SicknessQ scale and lend support for using SicknessQ as a brief instrument of human sickness behaviour.

7.2 PAPER II

Background and objectives
It is unclear if the previously described association between inflammatory cytokines and poor self-rated health is mediated by sickness behaviour. Furthermore, disease conviction or fear of disease is a core feature of severe health anxiety [151], and thus the reason why health anxiety was hypothesised to be associated with poor self-rated health in this population of primary health care patients. Although this objective was originally intended to be presented in a separate paper, the relevance of health anxiety became apparent also for the relation between inflammation, sickness and self-rated health. In paper II, the aim was to investigate the relationship between inflammatory markers, health anxiety, sickness behaviour and self-

rated health in primary care patients. We also aimed to investigate through exploratory analysis if putative relationships between inflammatory markers and self-rated health were statistically mediated by sickness behaviour.

Method

In total, 311 patients seeking primary health care were consecutively recruited from the drop-in clinic aimed for short consultations for patients with a wide range of medical problems. The participants had their F_ENO measured, blood drawn (analysed for IL-6, IL-8 and TNF-α) and completed questionnaires regarding self-rated health, sickness behaviour, health anxiety and background factors. Due to the development process of the SicknessQ, the 10-item version was available for the 179 patients from the first data collection only while SicknessQ4 was available for all participants. The reason was that the first preliminary 12-item solution was later revised, overlapping with the final 10-item solution by four items. In the statistical analysis, health anxiety was added as a covariate in an exploratory analysis as confounding was suspected.

Results and conclusion

Poor self-rated health was associated with both increased sickness behaviour as well as higher health anxiety. In men, elevated levels of IL-6 were associated with poor self-rated health as expected (rho: 0.26, p=0.009). The mediation analysis showed that sickness behaviour did not mediate this relationship (20.8% mediation, p=0.79). In women, the opposite relation was observed between IL-6 and self-rated health (rho:-0.15; p=0.04). However, the association was weak and rendered non-significant when adjusted for health anxiety (rho:-0.08, p=0.31). In conclusion, the study presents mixed results regarding the relation between inflammatory markers and self-rated health and does not support sickness behaviour as a mediator of the association between IL-6 and self-rated health. However, both sickness behaviour and health anxiety are suggested as behavioural determinants of self-rated health.

7.3 PAPER III

Background

Asthma is a chronic inflammatory respiratory disorder with eosinophilic inflammation in the airways, increased F_ENO and elevated levels of IgE. Little is known about co-variation over time between subjective health outcomes and inflammation in patients with asthma as well as in the general population. The aim of this study was to investigate sickness behaviour, F_ENO, s-ECP, p-EDN and IgE as determinants for self-rated health in patients with chronic allergic asthma.

Method

The relationship between local and systemic inflammatory parameters, self-rated health and sickness behaviour was investigated in 181 men and women with asthma over a 12-month

period during repeated measurements. The participants were randomised into two groups where the anti-inflammatory treatment was adjusted based on either the basis of F_ENO or adjusted based on symptoms. As there were no significant effect of treatment (inhaled corticosteroids and leukotriene-receptor antagonist on self-rated health, sickness behaviour nor any of the IgE-variables, s-ECP, p-EDN, groups were combined in all analyses in this present report and treatment was included in the analyses as a possible confounder.

Results and conclusion
Poor self-rated health was associated with high levels of food IgE and seasonal IgE, but not total IgE, ECP, EDN or F_ENO. Furthermore, an increase over one-year in perennial IgE was associated with a worsening of self-rated health. Poor self-rated health was associated with more pronounced sickness behaviour. A worsening over the year in sickness behaviour was associated with a worsening of self-rated health. Sickness behaviour and self-rated health co-varied over a one-year period, showing that sickness behaviour is a determinant of self-rated health in a group of patients with asthma. Future studies are needed to investigate the importance of specific IgE for perceived health.

7.4 PAPER IV

Background
In addition to the eosinophilic inflammation, increased levels of F_ENO and IgE, asthma is also associated with increased plasma cytokines, reduced lung function and poor quality of life. The aim of paper IV was to investigate IL-5, IL-6, FEV1, sickness behaviour and asthma-related quality of life as determinants of self-rated health. In addition, to investigate the longitudinal co-variation over time between inflammatory cytokines and subjective patient reported outcomes in relation to objective clinical measures.

Methods
Self-rated health, sickness behaviour, IL-5, IL-6, quality of life and FEV1 were assessed in 181 patients with allergic asthma with repeated measurements in a one-year longitudinal study. Similar to paper III, treatment groups were combined in all analyses in this present report and treatment was included in the analyses as a possible confounder.

Results and conclusion
More sickness behaviour and poorer asthma-related quality of life were associated with poorer self-rated health as hypothesised. In men, a u-shaped relationship was found where both low and high levels of IL-6 were associated with increased sickness behaviour. In addition, poor lung function was also associated with poor self-rated health. Over the year, improved asthma-related quality of life was associated with better self-rated health. Also, if sickness behaviour decreased, self-rated health improved, but only in women. In men,

increased FEV1 over the year was associated with an increase in IL-6, better self-rated health and improved asthma-related quality of life.

8 DISCUSSION

In this thesis, the associations between self-rated health, sickness behaviour, inflammatory markers and other PROMs as well as objective measures of disease have been investigated in both cross-sectional and longitudinal studies in healthy participants with experimentally induced sickness behaviour, primary care patients and patients with allergic asthma. In particular, an effort has been made to elucidate the role of sickness behaviour in the associations between inflammation and self-rated health. A global measure of sickness behaviour, SicknessQ, was developed and validated and was indicated to be an adequate instrument to measure subjective human sickness behaviour.

8.1 MAIN FINDINGS

8.1.1 Overall associations

The main finding among the overall associations was that poor self-rated health was associated with increased sickness behaviour (Paper II, III and IV). This association was valid regardless of whether SicknessQ, the short form SicknessQ4 or the Sickness-composite variable was used as a measure of sickness behaviour. Furthermore, poor self-rated health was associated with other PROMs such as poor asthma-related quality of life (paper IV) and increased health anxiety (paper II). Thus, in accordance with our hypothesis, sickness behaviour and asthma-related quality of life were determinants of self-rated health in both primary care populations (paper I, II) and in patients with allergic asthma (III, IV).

In addition to investigating the associations between subjective ratings of relevance for health and sickness, one of the main aims with this thesis was to delineate how these measures were related to inflammatory markers, such as cytokines (IL-5, IL-6, IL-8 and TNF-α) and other systemic or local measures of inflammation (IgE, ECP, EDN and F_ENO). The results showed that poor self-rated health was associated with increased levels of IL-8 (paper I), TNF-α (paper I), IL-6 (paper I, II), but not with IL-5 (paper (IV), in both women and men. Surprisingly, among the male population in paper IV, we also found that both low and high levels of IL-6 were associated with increased sickness behaviour in a u-formed fashion, a similar pattern was seen regarding self-rated health in men where the third quartile reported significantly better self-rated health than quartile one although no significant difference from quartile 4 was found. A non-linear relationship between IL-1β and long-term potentiation of the memory in rats has previously been indicated [34], perhaps suggesting that the involvement of e.g. IL-1β in basic physiological processes makes either too low or too high

levels coupled to sub-optimal functioning and thereby subjective health appraisals. More studies are needed in order to delineate if there is a similar non-linear relationship between IL-6 and sickness behaviour. There were no u-shaped association between levels of IL-6 and self-rated health. Poor self-rated health was further associated with an increase in the inflammatory markers seasonal IgE and food IgE in both men and women (paper III). However, the inflammatory markers ECP, EDN or FENO were neither related to poor self-rated health nor sickness behaviour (paper III).

One possibility why increased seasonal IgE and food IgE was associated with poor self-rated health is that patients who have to occasionally worry acutely about having an allergic reaction to allergens they encounter when they eat something unfamiliar or when they are exposed for a seasonal allergen would experience poorer health compared to patients having allergic reactions to perennial allergens that are present at all times during the year. It is possible that the latter group of patients have adapted to the constant danger of having an allergic reaction in a similar fashion as described during a "response shift". A "response shift" refers to when patients have adapted to their disease to such extent where they rate their health as good as before the onset of disease despite the presence of their disease [152-154]. Thus, patients with perennial allergy exposure might rate their health higher than those who occasionally experience fear of an acute allergic reaction and the relationship between levels of IgE and subjective health could potentially have a clinical importance. However, more studies are needed to investigate and further delineate the relationship between IgE, other inflammatory markers and subjective health perception. More specific, it would be interesting to conduct a study to investigate if there are any differences in self-rated health between patients with seasonal or perennial allergy.

In study IV, FEV1 was used as an objective measure of lung function in patients with asthma. In men, but not in women, improved FEV1 over one year was associated with better self-rated health, decreased sickness behaviour and increased asthma-related quality of life. No correlation was found between FEV1 and any of the PROMs in women. The phenomenon of discrepancy between PROMs and objective clinical asthma measures are well known and described in a number of studies [128, 130] Even so, FEV1 was one of the strongest physical predictors, and self-rated health the very strongest predictor (at least in men), of all-cause mortality in a large study of almost 500 000 UK Biobank participants [3]. Apparently, how subjective ratings relate to objective measures is an area still in its infancy, and I hope that small steps such as those taken in the present thesis can contribute to its gradual development. The obvious importance of outcomes such as morbidity and mortality and the way they are related both to self-ratings and more biomedical measures warrants further research. For example, how much can biomedical measures complement self-ratings of health to inform about the patient's health status, and are the relations between self-rated health and mortality due to a "true" evaluation of bodily state or rather reflecting the fact that self-rated health influence behaviours that in turn influences mortality rates? There is in fact support for both these and related explanations [4, 57], and future research needs to combine observational

and experimental approaches to understand the underpinnings of self-rated health and why it is connected to future objective health.

8.1.2 Longitudinal covariations

Both paper III and IV were bases on a longitudinal data collection conducted to investigate if and how the parameters co-varied over one year. In these longitudinal studies, increased sickness behaviour was associated with deterioration in self-rated health in both men and women (paper III) while this association was significant in women only in gender stratified analyses (paper IV). These results are new and give important information and may have reflected a causal relationship between increased sickness behaviour and poor self-rated health.

Increased levels of perennial IgE were associated with poorer self-rated health over the year. There were no longitudinal covariations between any of the PROMs and the other inflammatory markers (cytokines, ECP, EDN or F_ENO) in paper III and IV. This lack of covariation between PROMs and the remaining inflammatory markers could be due to several reasons, apart from being a true negative finding. First, the participants rated their health as "quite good" already at the start of the study which decreases the variation in the invested variables since the room for improvement is limited. Secondly, it is possible that inflammatory markers fluctuate more rapidly than is relevant for the subjective ratings. This would be congruent with some previous data where a link between self-rated health was concluded to have a trait- like component in addition to a relationship over very short time scales (hours to days) that has been demonstrated in experimental studies [68].

8.1.3 Mediation analyses

Analyses of statistical indirect effects of mediation were conducted in paper I and paper II. In paper I, all three cytokines (IL-6, IL-8 and TNF-α) were significant mediators of the experimentally induced effect of endotoxin on SicknessQ. In paper II, sickness behaviour was not indicated to mediate the association between increased levels of IL-6 and poor self-rated health in men in primary care. The direction of causation – if present at all – should be interpreted with caution, especially as the data in paper II were cross-sectional. For this and other reasons, more longitudinal studies are needed in order to try to better elucidate both the relation over time between inflammatory mediators and self-rated health and the putative role of sickness behaviour in this relation.

8.2 METHODOLOGICAL CONSIDERATIONS

8.2.1 Three ways of measuring sickness behaviour

One limitation in this thesis is that three separate measures have been used in an effort to measure human sickness behaviour in an adequate fashion. In paper III and IV, a Sickness-Composite scale was used since no validated measure of sickness behaviour existed when the study was planned. In paper I, a global measure of sickness behaviour was constructed and validated, suggesting a one-factor solution as indicated by a principal component analysis. This global measure of sickness behaviour, called SicknessQ, and a short form of SicknessQ (SicknessQ4) were used in paper II. The separate items of each sickness scale and the internal consistency between the different scales are presented in table 2. The association between SicknessQ and SicknessQ4 was satisfactory (Spearman's rho=0.91. Thus, the results suggest that the short form SicknessQ4 can be used as an alternative to SicknessQ. However, the association with the Sickness-Composite scale was low (Spearman's rho=.14) and is not interchangeable for SicknessQ or SicknessQ4.

The major difference between the SicknessQ, SicknessQ4 and Sickness-composite scale is that items associated with pain, depression, positive or negative affect are lacking in the Sickness-composite scale. Contentwise, this is a clear and obvious limitation since pain, depression, positive or negative affect have been shown to be of importance when measuring sickness behaviour [48, 149, 155, 156]. In paper I and II including a wide range of patients from primary care, muscle and joint pain was the reason for seeking medical care in 17% of the patients and 8.7% were seeking care for depression. Thus, a measure of sickness behaviour such as SicknessQ or SicknessQ4 which includes pain, depressive mood and affect would probably be more accurate to use not only in the primary care population but also in patients with asthma.

8.2.2 Measuring inflammation off-site from the region of interest

In this thesis, inflammatory markers were measured either in peripheral blood or in exhaled air. Measuring nitric oxide in exhaled air can be considered to be a reasonable approach to measure local airway inflammation since eosinophilic airway inflammation has proven to be reflected in F_ENO. Considering IgE, ECP and EDN, the highest concentration of those proteins would be located at the site of inflammation. In the case of allergic asthma, the highest concentration of inflammatory proteins supposedly would be located in the airways even though asthma has also proven to be a systemic inflammatory disorder [157]. Still, IgE, ECP and EDN were measured in peripheral blood. The same scenario was true for inflammatory cytokines. Circulating cytokines were measured in peripheral blood instead of cerebrospinal fluid. It should make sense to measure cytokines in cerebrospinal fluid by lumbar puncture since it is located on the inside of the blood-brain barrier and therefore in close proximity to the brain which has the capability to induce behavioural changes. The approach of measuring levels of circulating cytokines in peripheral blood was chosen

although knowledge about the exact relationships between differences in local and systemic concentrations of cytokines, interactions and impact on behaviour are still are partly unknown. However, even though the cytokines are measured from a distance from the brain which would appear to be the logical place to measure cytokines if trying to study the relationship between cytokines and behaviour, it is now well known that cytokines can enter the brain through several pathways [12-14]. For instance, afferent nerve fibres in the periphery transmit cytokine signals to specific nuclei in the brain and peripheral cytokines circulating in the blood during a systemic inflammation are thus able to communicate directly with the brain [12]. In this thesis, lumbar puncture was not conducted due to practical and patient safety reasons. Instead circulating cytokines as well as IgE, EDN and ECP were measured in peripheral blood based on the idea that these markers would act as proxies having a putative impact on behaviour although these peripheral markers would not be a perfect reflection of the situation inside the blood-brain barrier. The approach measuring inflammatory markers off-site from the region of interest has gained support in many studies and proven to be adequate in several settings such as in paper I and other studies where strong associations have been shown between circulating cytokines and behavioural changes due to injected endotoxin [48, 158, 159].

8.3 STRENGTHS AND LIMITATIONS

This thesis has several strengths. The main strengths include that the patient samples in the SIA and NOAK studies were, by and large, representative for patients treated in primary care regarding study group characteristics (paper II,III, IV), reasons for seeking medical care (paper II) and degree of asthma severity i.e. mild to moderate asthma (paper III, IV) [83]. In addition, both experimental and longitudinal designs were applied. The experimental approach used in paper I belongs to a fast-growing tradition, where stimuli like LPS (as in the endotoxin study in paper I), or, among other examples, typhoid vaccines [35] are commonly used. Studies of health trajectories in relation to inflammatory markers are still scarce, and hopefully, this this thesis can contribute to the knowledge base within this part of psychoneuroimmunology.

One limitation, as mentioned above, is the fact that the SicknessQ-scale was not developed at the starting point of the NOAK study (paper III, IV) and could therefore not be used consistently through this thesis. Another limitation is that analyses needed to be cross-sectional in study I, why studies of temporal relations, could not be studied. Finally, on average the participants in the included studies rated their initial health between “rather good” and “neither good nor poor”, thus somewhat limiting the range of variability in the investigated parameters. Despite the fact that a limited variability in self-rated health narrows the possibility to report eye-raising results, as a clinician you cannot really be unhappy about the fact that the majority of your patients actually rate their health on the positive half of the scale.

8.4 FUTURE CLINICAL PERSPECTIVE

In this thesis the associations between inflammatory markers and self-rated health show mixed results and the importance of inflammatory markers for self-rated health, sickness behaviour and other PROMs needs further investigation. However, we know from previous research that poor self-rated health is often the best and most robust predictor of future morbidity and mortality [3, 4, 62]. Still, during regular clinical practice, most Swedish GPs find themselves routinely ordering blood samples and tests, but seldom routinely ask the patient the simple question "How do you rate your health status?" or use other PROMs during the consultation. More and more evidence highlights the importance of incorporating the patient's subjective health during the clinical consultation. Personally, I believe that incorporating the patient's view of their health should be a matter of course. Not only should it be viewed as a valuable complement during the consultation, but as a way of getting information that cannot be obtained by objective clinical measures.

8.5 CONCLUSION

This thesis adds new knowledge about the role of sickness behaviour in the relation between inflammation and subjective health perception. The questionnaire SicknessQ was developed and validated as a measure of human sickness behaviour. Sickness behaviour was shown to be associated with inflammatory markers both in experimental and cross sectional settings. Health anxiety is suggested as a relevant factor to be considered in the study of determinants of self-rated health. Furthermore, the results suggest that both sickness behaviour and asthma-related quality of life are determinants of self-rated health by showing that these factors co-vary over a one-year period in patients with allergic asthma. However, sickness behaviour was not found to be a mediator in the association between inflammatory markers and self-rated health. High levels of IL-6 were associated with poor self-rated health in men, both in primary care center patients and patients with asthma, otherwise the association between inflammatory cytokines and self-rated health showed mixed results. The importance of IgE for perceived health in patients with asthma needs further investigation.

8.6 CONCLUDING REMARKS

As a note of caution, it is important to be aware of the limitations in trying to condense the highly complex processes intertwining human sickness behaviour, subjective health and inflammation into a few statistical calculations and assumptions, notably based on only a few sampling occasions for each participant. In light of scientific history, I think it is important to keep an open mind and remember that the current view will probably be substantially modified and differentiated during the years to come. Even though many great (and some less significant) discoveries have been made so far in psychoneuroimmunology, we have probably only started to scratch the surface of this interesting field.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Ospecifika diffusa sjukdomssymtom som trötthet, sjukdomskänsla och energibrist är vanliga orsaker till att patienter uppsöker primärvården. Dessa symtom orsakar ofta patienten stort lidande och är på grund av sin ospecifika natur ibland svåra för läkaren att tolka och behandla. Flera av dessa ospecifika symtom förknippas med låg självskattad hälsa. Självskattad hälsa är patientens egna svar på frågan ”Hur bedömer du ditt allmänna hälsotillstånd?” där läkarens tolkning inte ingår. Faktum är att det som allra bäst visat sig förutsäga patients framtida sjuklighet och död i många fall varit patients egen självskattade hälsa. Patienters subjektiva hälsobedömningar av olika tillstånd och symtom brukar med ett sammanfattande begrepp kallas för patientrapporterade utfallsmått och förkortas på engelska till PROMs. Man har i flera studier visat att flera PROMs, och däribland särskilt självskattad hälsa, till och med ger bättre mått på framtida sjuklighet och död än vad läkarens bedömning, provsvar och undersökningar kan ge. Man känner dock inte till den biologiska bakgrunden till detta samband.

Inflammatoriska processer ingår som en del i många sjukdomar som t.ex. infektioner, cancer, depression, fetma och astma. Under de senaste åren har mycket forskning inriktats på att förstå hur hjärnan påverkas av inflammatoriska signaler. Man har funnit att en grupp inflammatoriska proteiner som kallas cytokiner kan signalera till hjärnan. När hjärnan får dessa signaler kan den starta ett särskilt program med beteendeförändringar som med ett gemensamt begrepp kallas för sjukdomsbeteende. I sjukdomsbeteendet ingår att vi helst vill ligga stilla och vila, vi känner oss trötta och sjuka, får en ökad smärtkänslighet och vill inte roa oss eller äta. Genom att bete oss på detta sätt när vi har förhöjd inflammatorisk aktivitet undviker vi energikrävande beteenden och istället skapas förutsättningar för vila. Vi omdirigerar därigenom vår energi till det energikrävande immunsystemet så att det kan arbeta för att göra kroppen frisk igen. Sjukdomsbeteende fyller alltså en funktion vid akuta inflammatoriska sjukdomstillstånd, men tros kunna vara till skada om sjukdomsbeteendet fortsätter när den akuta inflammationen har läkt ut eller om inflammationen blir kronisk som t.ex. vid astma. Intressant nog påminner de symtom som ingår i sjukdomsbeteendet mycket starkt om de faktorer som gör att vi skattar vår hälsa som dålig. Genom att undersöka de biologiska och beteendemässiga effekterna av sjukdomsbeteende skulle man kunna få ökad kunskap om diffusa och ospecifika sjukdomssymtom och på sikt förhoppningsvis kunna ge patienter med dessa symtom bättre vård och behandling.

Syftet med denna avhandling var dels att undersöka hur sjukdomsbeteende påverkar den subjektiva hälsouppfattningen och dels att ta reda på om ett samband mellan inflammation och subjektiv hälsouppfattning förmedlas av sjukdomsbeteende (d.v.s. att inflammation ger upphov till sjukdomsbeteende som i sin tur påverkar hur man skattar sin hälsa) hos patienter i primärvården och hos patienter med en kronisk inflammatorisk sjukdom som astma.

I artikel I utvecklade och validerade vi ett frågeformulär för att mäta sjukdomsbeteende. Först fick friska försökspersoner en injektion med lågdos av bakteriedelar (s.k. lipopolysaccarid, LPS) eller placebo för att inducera ett kortvarigt experimentellt sjukdomsbeteende. Därefter

fick de lämna blodprov för inflammatoriska cytokiner och fylla i frågeformulär med frågor om sjukdomsbeteende och symtom. Vissa frågor och påståenden, som t.ex. ”jag känner mig trött” besvarades olika beroende på om deltagaren tillhörde gruppen som fått bakteriedelar eller placebo vid experimentet. De frågor där svaren skiljde sig åt mellan grupperna valdes ut och testades sedan på 179 patienter som sökt akut på vårdcentralens drop-in mottagning. Efter flera statistiska test av frågornas egenskaper valdes 10 frågor ut till frågeformuläret. Slutresultatet blev ett konstruerat och validerat frågeformulär, SicknessQ, som visade sig vara ett adekvat formulär för att mäta sjukdomsbeteende.

I artikel II undersöktes sambandet mellan inflammatoriska markörer (utandad kvävemonoxid och cytokinerna IL-6, IL-8 och TNF-α), hälsoångest, sjukdomsbeteende och självskattad hälsa hos 311 primärvårdspatienter. Dessutom undersöktes om ett eventuellt samband mellan inflammatoriska markörer och självskattad hälsa förmedlades av sjukdomsbeteende. Resultaten visade att låg självskattad hälsa hade ett samband med ökat sjukdomsbeteende och mer hälsoångest. Hos männen, men inte hos kvinnorna, fann vi ett samband mellan höga nivåer av IL-6 och låg självskattad hälsa. Sambandet mellan IL-6 och låg självskattad hälsa hos män förmedlades inte av sjukdomsbeteende.

I artikel III och IV undersöktes sambandet mellan självskattad hälsa, sjukdomsbeteende, astma-relaterad livskvalitet, inflammatoriska markörer och lungfunktion under ett års tid hos 181 patienter med allergisk astma.

Resultaten i artikel III visade att låg självskattad hälsa var kopplat till höga nivåer av IgE-antikroppar mot säsongsbundna allergen (pollen från björk, timotej och gråbo) och mat-allergen. Dock fanns ingen koppling till totalt IgE eller andra inflammatoriska markörer som EDN och ECP eller fraktionen utandad kvävemonoxid (F_ENO). När vi tittade på förändringen över tid kunde vi se att en ökning under året av perent IgE (allergen från hund, katt, häst och mögel) gav en försämring av den självskattade hälsan samt att en ökning av sjukdomsbeteende gav en sämre självskattad hälsa. Dessa fynd visade på sjukdomsbeteendets roll för hur man skattar sin hälsa och visade också att självskattad hälsa och sjukdomsbeteende samvarierar under en ett-års period hos patienter med allergisk astma.

I artikel IV fann vi att ökat sjukdomsbeteende var kopplat till lägre astma-relaterad livskvalitet. Hos män kunde vi se ett u-format samband där både låga och höga nivåer av cytokinen IL-6 var kopplade till ökat sjukdomsbeteende. Dessutom var dålig lungfunktion kopplat till låg självskattad hälsa. När vi tittade på förändringarna över tid kunde vi se att en förbättring under året av den astma-relaterade livskvaliten också gav en förbättrad självskattad hälsa. Hos män såg vi att en förbättring i lungfunktionen var kopplad till en ökning av IL-6, bättre självskattad hälsa och bättre astma-relaterad livskvalitet.

Sammanfattningsvis har denna avhandling gett ny kunskap om sjukdomsbeteendets roll i förhållande till subjektiv hälsouppfattning och inflammation. I avhandlingsarbetet ingick att utveckla och validera SicknessQ-skalan som mäter sjukdomsbeteende. Sjukdomsbeteende och astma-relaterad livskvalitet (hos patienter med astma) har betydelse för hur man skattar

sin hälsa. Sambanden mellan inflammatoriska markörer och självskattad hälsa, liksom betydelsen av IgE för den subjektiva hälsoupplevelsen vid IgE-relaterade ohälsotillstånd, behöver studeras mer i fortsatta forskningsstudier. Det viktigaste fyndet i denna avhandling var att sjukdomsbeteende och astmarelaterad livskvalitet visade sig vara viktiga faktorer för hur man skattar sin hälsa och att dessa samvarierar under en ett-års period hos patienter med allergisk astma.

10 ACKNOWLEDGEMENTS

I want to express my deep gratitude to all patients, healthy volunteers, co-workers, friends and organisations that have supported me and made this research possible. In particular, I want to thank:

Anna Andreasson, my main supervisor, for generously sharing your knowledge of and enthusiasm for psychoneuroimmunology with me. Special thanks for invaluable help in unravelling the mysteries of STATA. I am also very thankful for your efforts in trying to help me advance in my PhD-studies even when circumstances forced me to work from a distance.

Mats Lekander, my co-supervisor, for sharing your great scientific knowledge and always giving me good and honest advice.

Predrag Petrovic, my second co-supervisor, for support and interesting discussions.

Åsa Nilsonne, my mentor, for always changing my perspectives, giving me honest advice and having me turn my ideas around and upside-down. You have been an invaluable support during the last fourteen years.

Hildred Crill, for teaching me scientific writing. Your support has been outstanding and your linguistic advice excellent.

Jörgen Syk and Kjell Alving, my co-authors, for good advice and support.

The participants in the research school in general medicine, all members in Mats Lekander's research group, colleagues at Centre for Family Medicine and Primary care and Akademiskt primärvårdscentrum and all other research friends, colleagues and co-authors for good advice and interesting discussions.

All past and present co-workers at Älvsjö Vårdcentral. A special thanks to Aghdas Taghavi, Lisa Andersson, Kristina Rooth, Inger Dässman and Gunilla Andersson at the laboratory who were irreplaceable during the data collection of the SIA material. My clinical supervisors Christina Gustafsson, Eva Ygge and my director of studies Tova de Ruvo Lohmann for continuous support, advice and friendship. Karin Bringsarve and Kerstin Agdahl for practical help and cheering me on during the entire PhD process. My students Sara Kennerhed, Mikael Bergström and Philip Holmström for help during the data collection periods at Älvsjö VC.

All past and present co-workers at Stressmottagningen for inspiration, friendship and support.

Alasdair Skelton, Elin Tollefssen, Gabrielle Stockmann and Krister Junghahn for adding great adventures, fun and a geological time perspective to my life.

All my friends not otherwise mentioned.

Finally, my family and Mårten, for unconditional and unprecedented support and love.

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