Evidence-based curative health promotion

A systems biology-orientated treatment of seasonal allergic rhinitis with Citrus/Cydonia comp.

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Chapter 1

General introduction

Modern Western medicine has achieved enormous success in the last 150 years with the fighting disease approach as its most important concept. In summary, this entails fighting disease-related organisms, cells and functions in the body, reducing the symptoms of disease, and the manipulation and/or substitution of non-functioning or dysfunctional parts and processes of the body by means of *external* therapeutic resources.

Scientists, pharmaceutical industries, doctors and therapists have developed many, often successful, strategies to fight diseases and reduce symptoms. The development of all kinds of "anti-drugs," for example, antibiotics, antiviral drugs, anti-inflammatory drugs, and chemotherapeutics have saved many lives by fighting the presence of disease-causing bacteria or viruses in the human organism or by fighting the abnormal cell growth of cancers with great success. The development of numerous types of antidepressants, anxiolytics, and antipsychotics has resulted in a major improvement of the quality of life of psychiatric patients by reducing their psychological symptoms. In addition, healthcare is increasingly more able to replace body parts (e.g. new hips and knees) and/ or body functions (e.g. insulin and thyroxin treatment, or the heart-lung machine during surgical procedures) by means of manipulation and/or substitution. It is expected that the development of this approach will continue, and that it will result in important medical innovations.

However, in recent decades, there has been a growing interest in the additional approach of health promotion in both healthcare and society. One of the definitions of this approach is:

> *Health promotion is the process of enabling individuals, groups or societies to increase control over, and to improve their physical, mental, social and spiritual health. This could be reached by creating environments and societies characterized by clear structures and empowering environments where people are able to identify their internal and external resources, use and reuse them to realize aspirations, to satisfy needs, to perceive meaningfulness and to change or cope with the environment in a health promoting manner* [1].

Within the health promotion approach, one can distinguish between preventive health promotion, in which people aim to become healthier and remain healthy in order to prevent disease, and curative health promotion, which aims to cure disease by actively establishing or permanently restoring a healthy physiological and mental status.

In 1986, the first international conference on health promotion was organized in Ottawa [2],

and the first peer-reviewed journal of health promotion, the *American Journal of Health Promotion*, was launched. Nowadays, health promotion programs have been established in businesses, communities, clinics, commercial fitness centers, and schools. In society in general, the interest in lifestyle factors such as physical activities, nutrition, smoking, alcohol and drugs, and sexual behavior patterns, which can influence health status, is still growing. In 2000, the Consortium of Academic Health Centers for Integrative Medicine was established to support academic medical practice by focusing on, among other things, achieving optimal health and healing for patients. Since 2000, the number of universities that are members of this consortium has increased from 11 to 44 in North America, demonstrating the increasing interest in health promotion in the academic world [3]. Nowadays, health promotion is taught in academia in the areas of the social sciences and public health.

Although a great deal of effort has been invested worldwide, a large amount of scientific and practical work still has to be done in order to develop the health promotion approach to the same level of professionalism as the fighting disease approach.

This Chapter

This chapter is an introduction to some specific aspects of preventive and curative health promotion and the contribution of this thesis to the further development of the health promotion approach and its integration with the fighting disease approach in healthcare. Five questions will guide this chapter:

1. Is there a current need for professional health promotion?
2. What are the features of integrative medicine (IM), and can IM provide a contribution to professional health promotion?
3. What are the features and the burden of hay fever, and what are conventional and health-promoting anthroposophic medicine (AM) hay fever treatments?
4. Is the holistic concept of health promotion in accordance with current thinking in the fundamental holism-reductionism debate?
5. What is the contribution of this thesis to the development of professional health promotion and its integration with the fighting disease approach in healthcare?

Answers to these questions will be provided by first describing two important reasons for the further development of professional preventive and curative health promotion (see Section 1.1). Then the fields of integrative medicine (IM), complementary and alternative medicine (CAM), and anthroposophic medicine (AM) are introduced (see Section 1.2). These types of medicine often have a long history in clinical practice, and have their core orientation towards preventive and curative health promotion as an additional approach to the more conventional fighting disease approach. Therefore, they are hypothesized to involve much tacit knowledge on health promotion. This tacit knowledge can be converted into explicit knowledge and must then be systematized into

healthcare programs, guidelines and/or protocols, and subsequently tested on quality, safety, efficacy, effectiveness and cost-effectiveness in order to become 'evidence-based'.

Since a large part of this thesis focuses on the conversion of the tacit knowledge and the subsequent testing on the quality, safety, efficacy and effectiveness of the curative health promotion treatment with the anthroposophic drug Citrus/Cydonia compositum (comp.) for hay fever, a short introduction to hay fever and the conventional and anthroposophic treatments is presented (see Section 1.3).

The presented types of health promotion often have a formal (ontological, epistemological, and methodological) holistic character, which is not in accordance with the current reductionist approaches in medicine. This is one of the reasons (besides the lack of evidence on safety, efficacy and effectiveness) that practitioners of conventional medicine do not easily accept CAM approaches. Therefore, a short introduction to the holism versus reductionism debate in relationship to the fighting disease versus the health promotion approach is given. Some of the current developments demonstrating a shift from a reductionist towards a more holistic approach are described. They provide a new scientific opening for the acceptance of holistic approaches such as anthroposophic medicine in medicine (see Section 1.4).

This chapter ends with the objectives and outline of the thesis (see Section 1.5).

1.1 Fertile ground for the further development of professional preventive and curative health promotion

Demographic developments in the Netherlands and developments in the role of patients in healthcare provide fertile ground for the further development of professional preventive and curative health promotion.

1.1.1 Prognoses regarding growth, population composition, life expectancy and chronic diseases in the Dutch population

The prognosis for the coming decades is that the Dutch population will continue to grow and grow older, and that more than 25 percent of the population will be chronically ill. On June 2011, the population was 16.68 million [4]. In 2008, the prognosis was that the population would grow to 17.5 million by 2038. Then, due to a positive migration balance and negative birth growth, the population would supposedly shrink back to 16.8 million by 2050 [5].

The percentage of people who are older than 65 is expected to increase from 15 percent or 2.5 million people in 2009 to 25 percent or 4.2 million people in 2050, with an increasing proportion of individuals older than 80. The latest calculations for the Netherlands once again indicate that the

life expectancy of both men and women will increase in the near future. For men, their life expectancy at birth is expected to rise from 78.4 in 2008 to 81.1 in 2030. The life expectancy for women is expected to rise during this period from 82.5 to 85.3 [6].

One of the problems that arise as a consequence of these higher life expectancies is an increase in the prevalence of chronic diseases. Old age is often related to chronic diseases, which increases the need for social and health support and medical care. During the period of 2003 to 2007, more than 25 percent of the Dutch population suffered from a chronic disease, and 8 percent had more than one chronic disease. Of those who were 65 to 74 years old, fifty percent had a chronic disease, as did 57.5 percent of the population aged 75 and older. Older individuals are at an increased risk of having a chronic disease, which is associated with a lower quality of life. For society, this will result in an increase in healthcare-related costs. Therefore, for both society and the individual, it is very important to remain as healthy as possible into old age [6-8]. Further development and implementation of health promotion strategies might contribute to reducing healthcare-related costs.

1.1.2 Developments in the role and relationships of patients in healthcare

In healthcare practice, patients are increasingly assuming the role of competent patients who want to take responsibility for their own therapeutic processes. They use healthcare as part of their strategy for resolving a health and disease-related problem [9]. Healthcare professionals contribute to this process. Expressions of this development include, among other things, an increase in the use of certain terms in the healthcare sector, such as empowerment, self-management, shared decision making, the patient's own responsibility, and the autonomy of patients. Bopp et al. [10] describe the history of this development, in which patients have changed – since the 1960s to the year 2000 – from uninformed and incompetent (1960), informed (1970), empowered (1980), autonomous (1990) patients into competent patients. During the first period, the patient was hardly informed or not informed at all about the seriousness of his/her state of health, because he/she was believed to be unable to cope with this information. The doctor completely determined the 'what' and 'how' of the diagnosis and treatment. At the beginning of the 1970s, the concept of informed consent came over from the United States; it was generally accepted that patients were increasingly being informed in greater detail about the nature, extent and consequences of their disease and the necessary medical treatment. From the 1980s onwards, self-help groups appeared and patients became increasingly aware of their rights. Lawyers began to work on the area of patient rights. In the 1990s, patients became increasingly autonomous and models such as 'shared decision-making' were developed. This demonstrated that patients were becoming increasingly involved as partners of the doctor or therapist.

The development of the patient's role from the 1960s to the end of the 1990s, primarily concerns the relationship between the patient and other individuals: the doctor, representatives of the law, politicians, and insurers. At the end of the 20th century, the relationship between the patient and himself/herself is increasingly at the center. This includes such questions as: What is the role

I would like to play in dealing with my disease? What is my personal contribution to changing the status of my disease, and what does it look like? What is the doctor or therapist responsible for, and what am I responsible for? This is not necessarily an issue of patient rights, but rather concerns the sharing of responsibility. Over the past few years, the concept of patient competence has developed. This concerns the patient's ability to comply with the tasks that the disease brings, to reflect on his/her own and other resources for changing his/her own health and condition, to take advantage of these resources, and, at the same time, to take into consideration his/her personal needs, goals and autonomy [10].

The changing role of patients has made it necessary to place more emphasis in the field of healthcare on developing valid, evidence-based strategies that support and enable patients to actively contribute to their own health and disease status.

1.2 Integrative medicine: Integrating fighting disease and health promotion

1.2.1 The increasing demand for integrative medicine

Patients around the globe are increasingly embracing complementary and alternative medicine (CAM) as a contributor to health in addition to conventional medicine [11]. 'Out-of-pocket expenditure' on CAM is estimated in Australia to be as high as AU$4.13 billion (US$3.13 billion) per year [12], and in England, GB£450 million per year [13]. In the United States, CAM costs are estimated to be between US$27.0 billion and US$34.4 billion per year [14], and in 2007, they comprised 11.2 percent of the total out-of-pocket expenditures on healthcare in the United States [15].

Defining CAM is difficult because the field is very broad and is constantly changing. According to the National Center for Complementary and Alternative Medicine (NCCAM) in the United States, CAM is a group of diverse medical and healthcare systems, practices and products that are not generally considered to be part of conventional medicine [16]. The Cochrane Collaboration's definition of complementary medicine is that it includes all such practices and ideas that are outside of the domain of conventional medicine in several countries, and which users define as preventing or treating illness or promoting health and well-being. These practices complement mainstream medicine by satisfying a demand, which is not met by conventional practices, and they diversify the conceptual framework of medicine [17]. Two of the core features of CAM are its orientation towards preventive and curative health promotion as an additional approach to the more conventional fighting disease approach, and its holistic (compared to reductionist) and personalized approach to health and disease management.

Complementary medicine refers to the use of CAM *together with* conventional medicine, for example, using acupuncture in addition to the normal treatments that are used to help lessen pain. Alternative medicine refers to the use of CAM *in place of* conventional medicine. Integrative medicine refers to a practice that combines both conventional and CAM treatments for which evidence exists of their safety, efficacy and effectiveness.

A recent study by the United States National Institute of Health revealed that four out of 10 Americans used some form of CAM in 2007 [18]. Another study in Switzerland reported that approximately 11 percent of the population had used one of five CAM streams (anthroposophic medicine, homeopathy, neural therapy, phytotherapy, and traditional Chinese medicine) in 2002. In the Western world and industrialized countries, more than 50 percent of the population has used CAM at least one time in their life [19]. In the Netherlands, according to the results of the second Dutch national survey in general practice (DNSGP-2) [20], 6.5 percent of mainstream general practitioner patients had visited a CAM professional in the last year. In addition, there is an increase of doctors who specialize in CAM [21].

A review on the literature demonstrates that individuals who use CAM tend to be female, middle age, be more educated, and tend to have more than one medical condition [22]. The CAM doctors in a Swiss study treated patients that tended to be younger, female and better educated. These patients also tended to have a favorable attitude towards complementary medicine and exhibited chronic and more severe forms of disease. The majority of alternative medicine users appear to have chosen CAM mainly because they wished to undergo a certain procedure; additional reasons include the desire for more comprehensive treatment and the expectation of fewer side effects [23]. Van Dulmen [11] concluded in a Dutch study comparing patients visiting conventional general practitioners (GPs) and three types of CAM GPs (homeopathy, acupuncture, and naturopathy), that, contrary to expectations, patients do not consult a CAM physician because they are disappointed with mainstream GP care. CAM patients primarily appear to be seeking a physician who takes the time to talk with them and who will treat their complaints from a holistic viewpoint. Ernst and Hung [24] described the published evidence on the expectations of CAM users (in order of prevalence): hope to influence the natural history of the disease; disease prevention and health/general well-being promotion; fewer side effects; being in control over one's health; symptom relief; boosting the immune system; emotional support; holistic care; improving quality of life; relief of side effects of conventional medicine; positive therapeutic relationship; obtaining information; coping better with illness; supporting the natural healing process; and the availability of treatment.

In a referendum among the population in Switzerland in 2009, two-thirds of the voters were in favor of a wider coverage of CAM by public health insurance. In January 2011, based on the positive outcome of a national referendum, the Swiss authorities decided that five main streams of CAM (anthroposophic medicine, homeopathy, neural therapy, phytotherapy, and traditional Chinese medicine) will be covered by the mandatory health insurance for a period of six years (2012-2017) [25].

In the Netherlands the large number of patients attending CAM doctors and the increasing numbers of doctors specializing in CAM suggest that CAM will, in time, integrate with conventio-

nal medicine [11]. However, the current debate within the Dutch medical association KNMG (Koninklijke Nederlandse Maatschappij tot bevordering van de geneeskunst) focuses on the absence of evidence on the safety, efficacy, effectiveness and cost-effectiveness of CAM treatments. CAM treatments can only be prescribed to patients if sufficient evidence is available on their safety, efficacy, effectiveness and cost-effectiveness, and they are in accordance with the wishes, experiences and expectations of patients and other preconditions [26].

1.2.2 The efficacy, effectiveness and cost-effectiveness of CAM

In many cases, the efficacy and effectiveness of CAM has not been proven in clinical trials [27]. However, a lack of proof regarding its efficacy and effectiveness is obviously not the same as proof of its ineffectiveness. Clearly, the status of a treatment could change CAM into conventional medicine once scientific evidence of its efficacy and effectiveness becomes available. Three examples of CAM treatments that have become more widely accepted by conventional medicine are St. John's wort, acupuncture for specific complaints, and mindfulness. St. John's wort has been used for more than 90 years in anthroposophic medicine and has become part of the conventional guidelines for the treatment of depression, based on scientific evidence from randomized controlled trials [28]. Hopton and McPherson [29], on the basis of a systematic review of pooled data from meta-analyses, concluded that acupuncture is more than a placebo for commonly-occurring chronic pain conditions. In addition, in her thesis, van den Berg [30] recently demonstrated the positive effects of acupuncture on obstetric health problems (breech presentation). Currently, mindfulness treatment, which has its origins in Eastern Buddhism, is rapidly finding its way into healthcare. It has been demonstrated to be effective in, for example, supporting stress reduction [31], treating anxiety and mood problems, buffering the reduction of CD4+ T lymphocyte counts in HIV patients [32], decreasing blood pressure [33], improving the acceptance of chronic pain [34], and enhancing the general features of coping with distress and disability in everyday life, as well as under more extraordinary conditions such as serious disorders or stress in clinical populations [35].

In their review, Herman et al. [36] report that some studies indicate that CAM therapies may be considered to be cost-effective compared with the usual care for various conditions: acupuncture for migraines, manual therapy for neck pain, spa therapy for Parkinson's disease, self-administered stress management for cancer patients undergoing chemotherapy, pre- and post-operative oral nutritional supplementation for lower gastrointestinal tract surgery, biofeedback for patients with 'functional' disorders such as irritable bowel syndrome, and guided imagery, relaxation therapy and a potassium-rich diet for cardiac patients. A systematic review of randomized clinical trials (RCTs) on the use of so-called natural health products revealed evidence of the cost-effectiveness of these products in relation to post-operative surgery, but not regarding the other conditions that were assessed [37]. Studer and Busato [38] demonstrated that GPs who had completed certified additional training in CAM after obtaining their conventional medical degree (CAM GPs, n = 257) and GPs who had not (n = 174) had equal annual costs per patient, but significantly lower annual

costs per doctor (by 29%), although the CAM GPs took more time per patient.

These findings highlight the fact that the health-promoting methods that are considered CAM today could be effective and may have significant cost-saving potential.

1.2.3 From practice-based evidence to evidence-based practice

Several CAM streams have a long history in clinical practice, and have developed a lot of expert (tacit) knowledge, but they lack sufficient scientific evidence of quality, safety, efficacy, effectiveness and cost-effectiveness. In order to provide sufficient evidence, this tacit knowledge can be converted into explicit knowledge and then systematized into healthcare programs, guidelines and/or protocols and subsequently tested on the quality, safety, efficacy, effectiveness and cost-effectiveness in order to become 'evidence-based' [39]. This programmatic development 'from practice-based evidence to evidence-based practice' has only started in the last decades to varying degrees in the several CAM streams. Currently, several studies demonstrate the low quality of methodology and consequently low quality of evidence [26]. This situation is comparable to the situation of conventional medicine some decades ago [40]. Like what happened in conventional medicine in the last decades, investment in qualitatively solid research is necessary to improve the quality of the evidence. Based on this evidence, evidence-based CAM or IM 'products' for specific indications can be recommended to the several stakeholders, including patients, doctors, and policy makers.

1.2.4 Anthroposophic medicine: Integration of fighting disease and health promotion

As the anthroposophic drug Citrus/Cydonia comp. is being examined in this thesis as an example of curative health promotion (treatment of hay fever), this section will briefly describe the main features of anthroposophic medicine.

Anthroposophic medicine is an integrative diagnosis and therapy concept, an example of the integration of a holistic approach with a conventional approach, which has been developing since 1921 and is currently practiced in over 60 countries. It combines mainstream scientific medicine with Rudolf Steiner's anthroposophy. Anthroposophic medicine considers a human being as a whole entity – body, mind/soul and individuality. It aims to stimulate the self-healing forces of the body, restoring the balance of bodily functions and strengthening the immune system, rather than primarily relieving the symptoms of disease. Specific anthroposophic approaches include, among others, anthroposophic medicinal products, eurythmy therapy, massage therapy, art and music therapy, and speech [41].

A recent update of a systematic review demonstrated that, in recent decades, 256 studies on the effects of anthroposophic medicine have been performed [42, 43], seventy of them in the last six

years. Based on this systematic review, the authors concluded that the research on anthroposophic medicine for a wide range of diseases had revealed predominantly positive results with few side effects, a high degree of customer satisfaction, and indications of cost-effectiveness compared with conventional treatment [1, 42, 43].

Within the context of the reductionism-holism debate in science and medicine (see Section 1.4), the formal position of anthroposophic medicine is:

- There are non-material organizing principles in nature in addition to the material elements of reality (nature) (ontological aspect)
- There are higher, more complex levels of organization in organisms, which are responsible for the integrity and organizing of material elements in time, space and function (epistemological aspect)
- These higher levels of organization in organisms can be examined by means of qualitative-subjective and statistical pattern exploration and pattern recognition methodologies (methodological aspect)
- Health and disease can be diagnosed by integrating knowledge from the lower and higher levels of the organization of the system (methodological aspect)
- The anthroposophic treatment of disease must often be systems-oriented (e.g. several substances or therapies) and aimed at:
  - Influencing different essential aspects of the relevant organizing system level in health and disease at the same time or in phases (poly-target-treatment)
  - Restoring balance and wholeness within the system by stimulating the higher levels of organization in order to regulate the lower levels (health promotion) (methodological aspect)
- The anthroposophic treatment of disease requires self-activity of the organism and the patient towards an optimal functioning of the higher levels of organizing among each other and in the regulation of the lower levels. Therefore, it is a health promotion strategy, one that supports and enables patients to actively contribute to their own health and disease status (methodological aspect).

1.3 Hay fever or seasonal allergic rhinitis: The burden of disease, conventional and anthroposophic treatment

In a large part of this thesis, the chronic disease hay fever or seasonal allergic rhinitis (SAR) is the centre of attention. SAR is a type I immediate hypersensitivity reaction that is mediated by the formation of specific Immunoglobulin (Ig)E antibodies to a seasonal allergen, leading to mucosal inflammation, which is characterized by sneezing, itching, rhinorrhoea, and nasal blockage. Penetrating pollen allergens induce the activation of dendritic cells in the mucosal tissue,

which migrate to draining lymph nodes and stimulate $CD4^+$ helper T(h)-cells by the presentation of allergen-derived peptides. Due to the presence of high amounts of cytokines like Interleukin (IL)-4 in the lymph nodes of genetically predisposed allergic individuals, the activated T-cells differentiate into Th2 cells that produce large amounts of cytokines like IL-4, IL-5 and IL-13. Together, these cytokines induce the characteristic formation of allergen-specific IgE antibodies that selectively bind to high-affinity receptors expressed on mast cells in the tissue and basophilic granulocytes in the blood. The individual is now allergically sensitized, and upon a new encounter with the relevant pollen allergen, these molecules can cross-link the IgE antibodies and induce mediator release from the mast cells and basophils. The major mediator is histamine, which induces the characteristic set of SAR symptoms, including rhinoconjunctivitis, red and watery eyes, and respiratory complaints in the upper airways. Other characteristics in the allergically inflamed mucosal tissue like hyperplasia and the activation of mast cells (IL-3 and IL-4) and eosinophils (IL-5) are also a consequence of the Th2-derived cytokines [44].

Under normal conditions in healthy individuals, the balance in the T-cell system between Th1 and Th2 cells is maintained by the activity of regulatory T-cell populations, which are characterized by their production of immunosuppressive cytokines like IL-10 and Transforming Growth Factor Beta (TGF-β). For allergies, it is now actively researched whether abnormalities in the numbers or functioning of such Treg populations are responsible for the aberrant and overactive Th2 activity [45]. An overactive population of allergen-specific Th2-type cells is generally detected in the affected organs – the nose, eyes and upper respiratory system in SAR, but also in the peripheral blood mononuclear cell fraction (PBMC). Pollen from wind-pollinated grasses, trees and weeds and spores from fungi are the most common aeroallergens. Grass pollen is the most common cause of SAR. The highest levels of pollen in the atmosphere in the Netherlands are found in May, June and July [46].

The estimated prevalence of SAR in adults in several Western countries is 8 to 30.2 percent [47, 48]. It is expected that both the prevalence and duration of SAR will increase, due to the current presence of more SAR-related tropical plants like Ambrosia in the Netherlands and longer periods with higher temperatures (which will result in longer and more intense blooming periods) due to the supposed climate changes.

In 2008, an estimated 310,000 to 350,000 patients visiting a GP's practice in the Netherlands were known to have SAR, which is less than 2 percent of the entire Dutch population. The prevalence of SAR patients is estimated to be much higher (4-15 times), as many SAR patients do not visit a GP or receive medication from their doctor. The estimated total yearly cost (of visits to doctors, prescriptions and visits to specialists) for all of the patients who did visit their GP in the Netherlands in 2008 was 7.16 million euro. The mean annual number of days that people could not work due to SAR symptoms is 1.88, which costs the economy an estimated 540 million euro per year [49].

The conventional treatment of choice is symptomatic treatment with antihistamines and/or local corticosteroids. Immunotherapy is prescribed for a limited subpopulation of patients for whom treatments with antihistamines and/or local corticosteroids are insufficient and for whom immuno-

therapy is useful [50].

Citrus/Cydonia compositum (comp.) is an anthroposophic medicine, which contains extracts of lemon (Citrus lemon) and quince (Cydonia oblongata) [51]. For more than eighty years, Citrus/Cydonia comp. has been prescribed, especially as a subcutaneous injection or a nasal spray for SAR patients. If the effect of this treatment in counteracting the clinical symptoms of SAR is demonstrated in intervention studies, an immunological mechanism of action should also be provided.

1.4 The holism versus reductionism debate in relation to the fighting disease versus the health promotion approach

In order to become scientifically accepted, it is necessary that, apart from providing evidence of the safety, efficacy, effectiveness and cost-effectiveness of specific treatment approaches, the holistic concept and method of CAM is in accordance with current scientific theories. 'One of the fundamental theoretical debates in science is the holism-reductionism debate that describes the fundamental approach to nature.

This section summarizes the essence and current developments of the holism-reductionism debate, which are relevant for the acceptance of health promotion on conceptual grounds.

1.4.1 The essence of the holism-reductionism debate

A for this thesis relevant opposition from the philosophy of science, is that reductionism is usually set against holism or organicism. The statement 'An organism is essentially nothing but a complex set of atoms and molecules' lies at the heart of reductionism. At the opposite end of the scale, statements such as 'You cannot just simply reduce an organism to a sack of molecules' and 'The whole is greater than the sum of its composite parts' essentially characterize holism [52]. The reductionist model assumes that all traits and other characteristics that are demonstrated by living organisms – their morphology, physiology, behavior, and ecology – can ultimately be fully and exclusively explained in terms of the physical and chemical molecules (DNA, proteins, etc.) of which the organisms are composed. Reductionism assumes that the laws, concepts and theories that are formulated for a higher level of organization in biology (for example, the level of the organism) can be explained by theories developed for a lower level of organization, such as the level of organs, tissue and cells. Ultimately, this means that all biological concepts, laws and theories can be reduced to physics and chemistry [52].

Three aspects must be distinguished in the reductionism versus holism debate: (1) ontological,

(2) epistemological, and (3) methodological aspects [52]. Ontological aspects concern the entities, things or substances that are assumed to make up reality (nature), what characteristics are attributed to these things or entities, and what relationships and functions can be assumed to exist between them. Epistemological aspects refer to one's knowledge of reality, the way in which this knowledge is expressed in theories (amongst other things), and the logical connections between theories. In particular, these aspects deal with the links between theories, which are developed for different areas of reality or for different levels of organization. Methodological aspects concern the way in which knowledge is obtained and the basic principles, laws and strategies used in this process. This area is particularly related to the question of whether – in order to arrive at 'correct' knowledge or a true understanding of a certain level of organization (the level of the whole) – one should study the underlying lower levels of organizations (the composite parts and their interactions) or the higher level itself, or perhaps its relationship with other, even higher levels [52, 53].

1.4.1 The ontological level

On the ontological level, the atomistic reductionist scientific reasoning remains practically unchanged. Several authors state that although science should pass beyond reductionism, there is no need for non-materialistic theories [54, 55]. This is one of the reasons (besides a lack of evidence on the safety and effectiveness/efficacy of treatment) that some of the core conceptual aspects of some integrative medicine streams (for example, the concepts of 'Chi' in Traditional Chinese Medicine or the 'etheric level or organization' in anthroposophic medicine) are not accepted. In those instances where solid evidence of the effectiveness and efficacy of IM treatments exists, reductionist hypotheses are presented to explain the effects. For example, acupuncture effects are hypothesized to be partly placebo effects and/or effects of the mechanical manipulation of the extracellular matrix [56, 57].

For those health-promoting IM streams that have a strong holistic ontological position, the current reductionist ontological position of science and conventional medicine is a major obstacle for acceptance.

1.4.2 The epistemological level

As many of the molecular biologists in the 1950s came from physics, it is not surprising that they extended its classical approach to the study of living organisms. Molecular biology, with some exceptions [58], has largely adopted a reductionist view to explain biological systems according to the physical and chemical properties of their individual components. As Francis Crick (1916–2004) stated, "The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry" [59]. In due course, reductionism proved to be an extremely powerful analytical methodology, and it enabled scientists to analyze many basic molecular and

cellular processes. Complex systems exist at different levels of organization, which range from the subatomic realm to individual organisms to whole populations and beyond.

Nonetheless, biologists might be reaching the limits of this approach. Despite their best efforts, scientists are far from winning the war on cancer, owing largely to the complex nature of both the disease and the human organism. The human brain is a complex, nonlinear system that defies all reductionist and deterministic attempts to understand it [60, 61].

Throughout different fields of research, scientists increasingly question pure reductionist theories to describe and explain the complexity of biological organizations [62-64]. Therefore, new theories originating from both the research fields of the biological complexity in organisms and the genome project demonstrate a shift from reductionist towards more holistic concepts. Three important holistic concepts are shortly described: systems biology, emergence, and epigenetics.

Systems biology

Before the year 2000, only two articles contained the term 'systems biology' in either the title or the abstract in PubMed. In 2010, more than 2,500 entries had appeared with more than 90 percent in the last five years, demonstrating the increasing interest of scientists in this topic [65]. "Its recent rapid resurgence at the turn of the century reflects the problems encountered in interpreting the sequencing of the genome and the failure of that immense achievement to provide rapid and direct solutions to major multi-factorial diseases" [66, p. 9]. Different than the genetic and microbiological approach, systems biology aims to describe, understand and explain from the complex biological systems that are studied: all levels of structural and functional complexity, explicitly including the supracellular domain [65]; their systems behavior or phenotypes; their networks with relationships that interact with the genome, the environment and the phenotype [66, 67]; their multifactorial processes involved in maintaining homeostasis and the breakdown of homeostasis within the system [68].

Epigenetics

Recent insights into the mechanisms and molecular basis of gene regulation have led to the notion that organisms increase their complexity by altering the level of gene transcription without affecting their DNA base-pair sequence. This field of expertise is called epigenetics, and it refers to the fact that although all of the cells of an individual contain the same amount of DNA and the same sequences in the genome, different locations in the body instruct cells to perform different functions and even to react and modify their genetic potential as a way of reacting to their exposure to environmental factors [69]. Three main mechanisms are supposed to be responsible for epigenetic changes: DNA methylation, histone tail modifications, and noncoding RNAs [70]. These induced changes are heritable and can exert their influence over several subsequent generations through perinatal programming, and can therefore explain why certain diseases run in families [71]. In addition, epigenetic mechanisms contribute to the explanation for neurophysiologic and psychological functioning. Therefore, they are hypothesized to provide a physiological basis for the perceptions of disease, health and welfare [72].

Emergence

The features of organs are not found in the cells and consciousness is not found in organs. In nature, higher organization levels 'spontaneously' arise from lower levels and, at the same time, influence these lower levels. For example, a cell that is transplanted from one tissue to another often changes its functions according to the new tissue [73]. "Emergent phenomena are said to arise out of and be sustained by more basic phenomena, while at the same time exerting a 'top-down' control, constraint or some other sort of influence upon those very sustaining processes" [74, p xi]. Throughout several concepts, emergent phenomena are usually characterized as irreducible, unpredictable or unexplainable, to require new concepts and to be holistic [75].

The concept of emergence has received renewed attention in science in the past decades due to developments in a number of research programs within complexity theory, artificial life, physics, psychology, sociology, biology, philosophy of science and philosophy of mind [75].

1.4.3 The methodological level

The shift in conceptualization in biology and medicine toward a more holistic approach also affects the methodologies that are used to study the effects on the parts and organization levels of organisms.

Systems organize themselves upwards towards larger wholes and downwards towards ever-smaller parts. They include, for example, molecules, cells, organisms, populations (societies), and ecosystems. The upwards view describes holism or emergence (the complexity science view from different perspectives) [76], while the downwards view describes reductionism or analysis. Both views provide valid and valuable information, one being contextual, the other specific. Complex systems exist at different levels of self-organization that range from the subatomic realm to individual organisms to whole populations and beyond. Despite their differences, they all share common features such as emergent properties, and randomness and order, which are both relevant to the behavior of overall systems. Complex systems exist on the edge of chaos, as they might exhibit regular and predictable behaviors [77]. However, they can also undergo sudden massive and stochastic changes in response to what seem like minor modifications. The behavior of a cell, for example, is controlled both by the properties of its macromolecules and by the properties of the organ of which it is a part, as well as the surrounding extracellular matrix. The whole is not only more than the sum of its parts, but also less than the sum of its parts, because some properties of the parts can be inhibited by the organization of the whole. From an epistemological point of view, this means that it is not sufficient to analyze each individual part (reductionism), nor is it sufficient to analyze the system as a whole (holism). At the molecular level, several diseases have a common genetic or functional origin. Hence, a cooperative and collaborative approach to designing health interventions is required.

Three important shifts to a more holistic methodological approach can be distinguished. First of all, there is a shift from the emphasis on the methodological analysis of the parts only to me-

thodologies that can study the spatiotemporal processes and the patterns between the parts, the processes (e.g. transcriptomics, metabolomics, genomics), the several levels of organization, and the epigenetic influences [78]. Secondly, there is increased methodological interest in a more personalized approach regarding diagnostics and treatment choices based on the unique phenotype of a specific organism [67]. Thirdly, there is growing interest in the integration on several levels: for example, the integration of data from several adjacent research fields (e.g. lifestyle research and genomics); the integration of methodological approaches (e.g. epidemiological studies and qualitative studies); and the integration of large amounts of data by means of new computational and mathematical tools [79].

In conclusion, it is evident that in the holism-reductionism debate in science, there is a shift from a reductionist to more holistic approaches on the epistemological and the methodological levels, but not on the ontological level.

1.5 This thesis

This thesis focuses on some of the conceptual, methodological and empirical issues involved in further developing professional preventive and curative health promotion as a valid contribution to the continuation of innovation and cost reduction in medicine and healthcare.

1.5.1 Objectives and outline of the thesis

Objectives

The objectives of this thesis are:

1. To contribute to the development of a valid definition of health that can serve as an evidence-based theoretical fundament for the preventive and curative approaches to health promotion in medicine and healthcare
2. To contribute to the development of systems biology-orientated measuring instruments that are able to monitor health-related changes (in, for example, immunology) on the system level
3. To provide scientific evidence for the curative health-promoting effects and safety of the anthroposophic drug Citrus/Cydonia comp. in the treatment of SAR
4. To provide scientific evidence for the cost-effectiveness of integrative medicine in the Netherlands

Outline

This thesis contains three parts. The first part is theoretical, in which both the definition of

health and the fundamental concepts of holism and reductionism are examined in order to provide a scientific basis for a holistic, preventive and curative health promotion approach in medicine and healthcare. The second part presents the methodology that will be used to examine the relevant immunological parameters of patients with SAR in a more systems-biologic (holistic) manner. The third part describes the acquired evidence based on the results of a series of empirical outcome studies on the clinical and immunological effects and safety of the anthroposophic (curative) health-promoting treatment with Citrus/Cydonia comp. for SAR.

Part 1. Epistemology of health promotion

In Chapter 2, the literature on the definitions of health and health promotion is reviewed. It is hypothesized that health can be understood as the continuous activity of the inborn and/or acquired self-regulation skills of humans on the physical/physiological, mental and social levels, that is responsible for maintaining the integrity and organizing of material and cognitive elements in time, space and function. Health promotion supports and enables patients to actively contribute to their own health and disease status towards an optimal functioning of the higher levels of organizing among each other and in the regulation of the lower levels. In Chapter 3, the fundamental scientific discussion of holism versus reductionism is summarized. In this chapter, several scientific arguments are presented against the current reductionist point of view. This provides a scientific opening for a more holistic or systems biology-orientated concept of health. The Goethean phenomenologic method is introduced as a qualitative methodology that enables researchers to attain knowledge of the structures or patterns at higher levels of organization in organisms.

Part 2. Methodology of measuring health promotion

Chapter 4 describes a new systems biology-orientated methodological approach for developing immunological biomarkers in SAR (hay fever) research. These biomarkers are pattern variables that are computed by means of permuted stepwise regression analyses and based on the interactions between the relevant immunological parts of the subsystem of the immune system that is involved in SAR. It is argued that the computed pattern variables will demonstrate larger correct values (CVs) than the separate cytokines regarding the classification of cytokine samples in baseline and post-baseline.

Part 3. Empirical evidence of the efficacy, effectiveness, cost-effectiveness and safety of curative health promotion

Chapter 5 describes the results of a survey of the experiences of Dutch doctors with the anthroposophic health-promoting treatment for SAR. In Chapters 6 and 7, two *in vitro* studies on the effect of the anthroposophic drug Citrus/Cydonia comp. on the relevant peripheral blood monocyte cells and functions of SAR patients and healthy persons are described. Chapters 8 and 9 describe the results of two intervention studies on the health-promoting effects of the anthroposophic drug Citrus/Cydonia comp. The first (pilot) study is a small cohort study in which the effect of Citrus/Cydonia comp. on the severity of SAR symptoms is studied. The second study describes

the immunological and clinical results of a RCT comparing two routes of administration of Citrus/Cydonia comp., the nasal spray versus the subcutaneous injection route of administration. Chapter 10 presents the results of a systematic evaluation of the reported adverse drug reactions (ADRs) recorded in the pharmacovigilance databases of eight German anthroposophic and homeopathic manufacturers covering the period of 2000 to 2009, providing relevant data for this thesis on the safety of Citrus/Cydonia comp. subcutaneous injections.

General Discussion

The final chapter of this thesis (Chapter 11, General Discussion), after presenting the main findings, discusses the importance of the author's findings regarding the definition of health promotion and the future of professional health promotion practices in the field of healthcare. It reflects on the presented systems biology-orientated methodologies, and discusses the implications of the empirical and evidence-based foundations of the effective treatment of hay fever with Citrus/Cydonia comp. In addition, the results of a cost-effectiveness study (Appendix) are described, demonstrating the possible contribution of integrative medicine to promoting health and reducing healthcare costs. Finally, a reflection on the scientific status of anthroposophic medicine in light of current developments in the areas of holistic concepts, holistic methodologies and health promotion is presented. Furthermore, future perspectives are described concerning the implementation of new research lines, the further development of professional preventive and curative health promotion as a contribution to the further innovation of medicine and healthcare, and the investment in CAM/IM/AM research.

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Part 1

Theoretical aspects of curative health promotion

Chapter 2

Health by Self-Regulation: Towards Evidence-based Definitions of Health and Curative Health Promotion

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Abstract

The last decades the interest in health promotion in society and healthcare has increased enormously. Health promotion supplements the main (western) biomedical fighting disease approach. To further professionalize the health promotion approach, definitions of health and health promotion as well as measurement instruments must be validated, and preventive and curative health promotion interventions should be tested for quality, efficacy, effectiveness and cost-effectiveness. In this article the WHO definition of health (1948) is criticized, since it is primarily oriented at end states and does not clarify the mechanisms responsible for reaching and maintaining these states. Therefore, it does not provide sufficient conceptual tools to innovate and professionalize health promotion activities. In order to overcome these shortcomings, the WHO and current health definitions are analyzed and revised into the definition of 'health by self-regulation'. The definition is preliminarily validated by means of analyzing (1) its internal consistency, (2) its accordance with other scientific definitions (e.g., of epigenetics, systems biology, emergence and self-organisation) and (3) its accordance with empirical facts. Key features of the definition are the central position of dynamic and continuous self-regulation as a key organismal function and the holistic, system biological approach. We discuss theoretical and practical implications of the revised definition.

Keywords: definition, health, health promotion, self-regulation, holism

2.1 Introduction

All approximately 10^{27} molecules of the human body are renewed in a period of two weeks. In one year, approximately 97 percent of all cells of the human body have been replaced and, in most instances, perfectly (Bos, 2008). Every day, smaller and larger physical injuries and wounds in the human body are 'spontaneously' healed. Recent research has demonstrated an inverse association between childhood measles and allergic symptoms and diagnoses later in life (Rosenlund et al., 2009). Some cancer patients experience spontaneous tumor regression, sometimes for a few years, and sometimes for (almost) the rest of their lives (Rijke, 2001; Turner 2010). Others are cured or improve after severe feverish infections (Hobohm, 2005; Turner 2010). Our immune system has evolved to counteract - in an often-unnoticed way - assaults on the body by non-self infectious organisms or altered cancerous cells, either of which may compromise an individual's health. Normally these assaults are successfully repelled and disease is prevented, and this resistance is due to both basic and sophisticated immune responses. Throughout history people have encountered terrible situations like war, rape, and torture and some persons were able to remain healthy (Antonovsky, 1979, 1986). What makes the difference? Or more precisely: What is health, how does the organism keep people continuously healthy, and what is a healing process? How do disease and health relate to each other? If an answer to these questions is found: How can one use (the knowledge of) these sources to promote health in a professional way, both preventive and curative?

A fighting disease approach

Modern Western medicine, with the 'fighting disease' approach as its most important concept, has achieved enormous success in the last 150 years. In summary, the 'fighting disease' approach involves the fighting of disease-related organisms, cells and functions in the body; the reduction of the symptoms of disease; and the manipulation and/or substitution of non-functioning or dysfunctional parts and processes of the body by means of *external* therapeutic resources.

Scientists, pharmaceutical industries, doctors and therapists have developed many, often successful, strategies to fight diseases and diminish symptoms. The development of all kinds of "anti-drugs," e.g. antibiotics, antivirals, anti-inflammatories, and chemotherapeutics, has saved many lives by fighting the presence of disease-causing bacteria or viruses in the human organism or by fighting the abnormal cell growth of cancers with great success. The development of numerous types of antidepressants, anxiolytics, and antipsychotics has resulted in a major improvement of the quality of life of psychiatric patients by reducing their psychological symptoms. In addition, healthcare is increasingly able to replace body parts (e.g., hips or knees) and/ or body functions (e.g., insulin and thyroxin function, or the heart-lung function during surgical procedures) by means of manipulation and/or substitution. It is expected that the development of this approach will continue, and that it will continue to result in important medical innovations.

A growing interest in health promotion

In the last decades there has also been a growing interest in the supplemental approach of *health promotion*, in both healthcare and society more broadly. Here is a definition:

> *Health promotion is the process of enabling individuals, groups or societies to increase control over, and to improve, their physical, mental, social and spiritual health. This could be reached by creating environments and societies characterized of clear structures and empowering environments where people are able to identify their internal and external resources, use and reuse them to realize aspirations, to satisfy needs, to perceive meaningfulness and to change or cope with the environment in a health promoting manner* (Eriksson & Lindström, 2007).

The growing interest in health promotion is reflected in the increased use of terms such as 'empowerment,' 'self-reliance,' 'responsibility,' 'autonomy of patients,' and 'shared decision-making'. Patients increasingly assume the role of the *competent patient* who wants to steer his own therapy process (Bopp et al., 2005). He uses healthcare as part of his solution strategy (Van der Laan, 2006). Although in the last decades much progress has been achieved, health promotion has a long way to go if it is to reach the level of development achieved by the fighting disease approach. Central questions that have to be answered are: What are valid definitions of health and health promotion that provide a solid fundament for health promotion practice?; What are valid methods and measuring instruments to monitor changes in health status?; What is the quality, efficacy, effectiveness and cost-effectiveness of preventative and curative health promotion for specific indications?; What is the surplus value of health promotion compared to fighting disease?; How does one optimally integrate a fighting disease and health promotion approach in specific indications?

In this article we focus on the first question; we revise the 1948 WHO definition of health into a more valid definition of health that is more in line with current conceptualizations of health aspects and that is better able to support preventive and curative health promotion activities. We argue that the WHO definition demonstrates several shortcomings: (1) it lacks clarification of the relationships between its positive subdomains (physical, mental and social health); (2) it lacks clarification of the relationships between its positive and negative subdomains (health and disease); and (3) it is not in accordance with the current empirical evidence. In addition, the WHO definition does not describe the (tested) mechanisms for remaining or becoming healthy, which are necessary for rational preventive and curative health promotion (Campaner, 2011). It is therefore an inadequate tool for supporting the innovation and professionalisation of health promotion activities.

This article contains four sections. The first section describes the 1948 WHO definition of health, as well its conceptual shortcomings. We also provide an overview of some of the most important health definitions that have been developed since then, and some of the most important philosophical positions towards a definition of health. The second section focuses on the clarification of the relationships between the positive subdomains of the WHO definition (physical, mental and social health). The third section attempts to clarify aspects of the relationships between the po-

sitive and negative subdomains (health and disease). The fourth and final section presents a revised health definition for the purpose of health promotion, based on the results described in the previous section. Central in this definition are the systems ability or skill of self-regulation, and the adaptive use and maintenance of this skill across changing personal and environmental conditions of spatial, temporal and/or functional structures on the physical, mental and social level. We provide both probabalistic and mechanistic evidence in support of this definition: we demonstrate that there is a strong association between high levels of self-regulation and health, and a strong association between low levels of self-regulation and disease (probabalistic evidence), and we both describe a model of the different components that interact in maintaining and becoming healthy, and provide data on criteria of causality (e.g. temporal relation, biological plausibility, dose-response relation) (mechanistic evidence). Finally we examine two examples of the health promotion approach that are in line with the revised health definition: the prevention and treatment of seasonal allergic rhinitis (hay fever) and the phase 1 treatment of chronic traumatization. In conclusion we discuss the value of the revised health definition, and make future research recommendation for its validation.

2.2 The WHO health definition of 1948, current health definitions and their main shortcomings

2.2.1 The WHO health definition of 1948

The World Health Organization (WHO) defined health in 1948 as ‘a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity’. In the past 63 years, it has become clear that the WHO definition is inadequate. There are four main problems with this health definition (Franke, 2008; Ten Have, 1987). First, it is unclear what the mutual relationships are between the positive domains of the definition. How do physical, mental and social well-being relate to each other, and what are their mutual relationships and dependencies? Second, the relationship between the positive domains (a state of complete physical, mental and social well-being) and the negative domain (the absence of disease or infirmity) of the definition is unclear: what is the relationship between health and disease according to this definition? Third, the WHO definition of 1948 is not in accordance with the newer definitions of health (aspects) that have been developed since then and empirical facts. The fourth and last point is that the definition is not internally consistent, since it the definition appears to be open for multiple interpretations. In addition the definition is inadequate for the purposes of this paper, because it does not provide conceptual tools for preventive and curative health promotion.

We attempt to achieve an improvement of the WHO health concept by examining newer con-

ceptual approaches to health, and by clarifying their possible contributions to solving the four categories of problems that the WHO concept presents. From this, a revised and improved health definition can be formulated as a basis for professional health promotion.

2.2.2 Current health and related disease definitions and their main shortcomings

There are many different definitions and accounts of health (and disease) in the literature, which vary widely in style and approach.

One type of approach is the 'per exclusionem' account, where health is the absence of disease (and vice versa). The first health definition can be logically deduced from a pragmatic definition of disease: disease as that which doctors and therapists treat. Health is then *the absence of that which doctors and therapists treat* (Glas, 2008). The next health definition can be deduced from the definition of disease as subjective suffering or a subjective complaint (Glas, 2008). The health definition is then *the absence of subjective suffering* or *the absence of a subjective complaint*. The biomedical notion of health is *the absence of disease, in terms of either the expression of the disease entity or the conditions of the diseased state*. The biomedical definition is a reductionist, mechanistic one. It represents a default material state of the body responsible for both physical and mental health. The related disease concept is disease as a lesion, a pathological or traumatic discontinuity of tissue or loss of function of a part. The fourth 'per exclusionem' definition is the *statistical* health definition, based on the conditions and functioning of the human body that is most common in a specific population. Boorse (1987, 1997), for example, defines health as a normal function of a part or process within members of the reference class. It is a statistically typical contribution by it to their individual survival and reproduction. Disease or dysfunction would then be the conditions and functioning of the human body that is least present in a specific population.

The first problem with these 'per exclusionem' definitions is that there are several examples where, according to the health definition, a formal healthy state is actually a diseased state. For example, patients who are afraid of doctors or therapists and therefore do not attend healthcare institutions would be healthy according to the first definition. Patients with some types of cancer in the first stage or a bipolar disorder would not suffer from complaints and therefore would be healthy according to the second definition. A second conceptual problem is that the 'per exclusionem' concepts are purely descriptive, and do not provide insights into the mechanisms of maintaining health. With regard to the statistical approach, examples such as superior length or superior intelligence demonstrate that not all statistically deviant conditions are disease states and adverse. In addition, Schwartz (2007) describes the problem of the common disease (a disease that would be common in a population would not be classified as disease) and the problem of healthy populations that arise in any case where a trait's lowest level of functioning in some reference class is not low enough to be dysfunctional. Kingma (2010) criticizes Boorse's account of normal species function, since it fails to distinguish between physiological and pathological responses that are due to a specific environmental factor.

Two biological or environmental definitions of health are *the good functioning of the body in its adaptation to the environment* and health as a *reserve* or *hygiogenetic capacity* (Heusser, 1999), the physical ability or the self-healing capacity of the organism to, for example, recover quickly from a strenuous exercise or operation. Disease is then the poor functioning of the body in its adaptation to the environment and the lack of ability to recover quickly. The main conceptual problem with these biological definitions is that they are, again, purely descriptive, not presenting any insight into the mechanisms of health. Secondly, not all diseases have demonstrated material lesions (e.g., back problems).

On the basis of their review, Westerhof and Bohlmeijer (2010) describe the *triad of mental health*: happiness (the experience of positive emotions and satisfaction with life that is experienced as emotional well-being), self-realization (activities that match the person's goals and desires and the person's skills and competencies that contribute to the experience of psychological well-being), and societal integration (that is experienced as social well-being). Regarding the *well-being* definition, Ryff and Singer (1998) describe four essential features of positive health: (a) leading a life of purpose, embodied by projects and pursuits that give potential; (b) having a quality connection to others, such as having warm, trusting and loving interpersonal relations and a sense of belongingness; (c) possessing self-regard, characterized by such qualities as self-acceptance and self-respect; and (d) experience mastery, such as feelings of efficiency and control (Marcum, 2008).

Salutogenesis ('the origin of health') and *resilience* are stress resource oriented definitions. Salutogenesis focuses on resources, and maintaining and improving the movement towards health. It gives the answer as to why people, despite stressful situations and hardships, remain well. It is the opposite of the pathogenic concept, where the focus is on the obstacles and deficits. Two core concepts are essential in the salutogenic theory (Antonovsky, 1979, 1987): the sense of coherence (SOC) and generalized resistance resources (GRRs). The ability to comprehend the whole of a stressful situation and the capacity to use the resources available is called sense of coherence (SOC). SOC reflects a person's view of life and his/her capacity to respond to stressful situations. It is a global orientation to view life as structured, manageable, and meaningful or coherent. It is a personal way of thinking, being, and acting, with an inner trust, which leads people to identify, benefit, use, and re-use the resources at their disposal. Three elements, comprehensibility, manageability, and meaningfulness, form the SOC. The other key factors are the resources available to make possible such a movement towards health. The GRRs can be found within people as resources bound to their person and capacity, but also to their immediate and distant environment as both material and non-material qualities from the person to the entire society. The key factor is not what is available, but to be able to use and re-use the resources for the intended purpose. The GRRs provide a person with sets of meaningful and coherent life experiences thanks to the resources at the person's disposal (http://www.salutogenesis.fi/eng/Salutogenesis.5.html). The concept of *resilience* is clearly related to the salutogenesis concept and stems from psychology (Luthar, 2006). It is a way of explaining how people can manage life and live well in spite of adverse situations. As a scientific concept, it was first developed for children and young people and has later been expanded into adulthood.

An often-used *sociological* definition (Parsons, 1981) is: health is the state of optimum capacity

of an individual for the effective performance of the roles and tasks for which he has been socialized. Social inequalities are related to health differences. Mortality and morbidity patterns are related to social factors such as gender, social class, race and age (Nettleton, 2006). In his review, Keyes (1998) developed a framework with *five dimensions of social well-being*: social acceptance (a positive attitude towards people), social actualization (participating and believing that society develops itself in a positive way, believing that society realizes possibilities), social contribution (having the feeling that one has to offer something worthwhile to society; thinking that daily activities are appreciated by society), social coherence (to see a social world that is understandable, logical and predictable; taking care of and being interested in society and the surroundings), and social integration (feeling a part of society; believing that you belong to, are supported by and share things with the society).

2.2.3 Main philosophical approaches to defining health and disease

Ereshefsky (2009) distinguishes three main philosophical approaches to health and disease: (1) naturalism, (2) normativism and (3) hybrid theories.

Naturalists base their definitions on scientific theories. They attempt to highlight what is biologically natural and normal for humans. With regard to health they focus on physiological and psychological states ('is an organ or system normal or properly functioning?') in terms of survival and reproduction. The term 'disease' is used to express a derangement of underlying physical mechanisms that can be scientifically investigated and clinically diagnosed. One can distinguish between the ontological, physiological, evolutionary and genetic disease conceptions. The ontological disease conception is concerned with the disease causing entities (e.g., viruses). The physiological disease conception is concerned with the deviations from the functional norms (e.g., hypertension). The evolutionary disease conception is concerned with maladaptation, a lack of or inadequate defense in response to a challenge. The genetic disease conception involves the explanation of disease in terms of mutation in or the absence of genetic material (e.g., sickle cell anemia) (Marcum, 2008).

Some of the main shortcomings of naturalism are that (1) specifications of normal states of organisms are not described in science (taxonomy, genetics, physiology) and the current (e.g. physiological) descriptions may not be the statistically normal ones; (2) humans have several goals in life and some of them have nothing to do with biological fitness (survival and reproduction); and (3) it can be challenged that biological fitness is *the* goal of organisms, taking into account the states that biologists describe that have nothing to with this (e.g., eating for the sake of eating, pleasurable sex) (Ereshefsky, 2009).

Normativists claim that our use of health and disease reflects our values towards desirable (health) and undesirables (disease) states. They focus on whether a psychological or physiological state is valued or disvalued.

Hybrid theorists consider both components from naturalists and normativists and regard disease a state that is both dysfunctional and disvalued. The main problem of normativism is that it does

not capture the common view that there is more to deciding whether a state is a disease than normative considerations.

Finally, within the humanistic approach the term 'illness' is used. It includes the patient's suffering and existential concerns as part of the illness experience. This is an additional part (next to the diseased state) that must also be the object of therapy.

2.2.4 Homeostasis, allostasis and heterostasis

Next to the presented differences, there is also overlap in aspects of the health definition. First of all, health as a *homeostatic* state is somewhat explicitly a part of all health definitions (Franke, 2008). This is one of the oldest and lasting definitions of health and refers to the stability of physiological systems that maintain life, used here to apply strictly to a limited number of systems such as pH, body temperature, glucose levels, and oxygen tension; they are truly essential for life and are therefore maintained within a range optimal for the current life history stage (Pal, 2008). Secondly is health as allostasis, which refers to maintaining stability through change, as a fundamental process through which organisms actively adjust to both predictable and unpredictable events (McEwan & Winfield, 2002). Thirdly, the definition of heterostasis or flexibility is a key element in several approaches. It refers to the situation that one is healthy when one is able to encounter a problem and actively overcome it. Whereas homeostasis refers to returning to the old situation or the old state of equilibirum, heterostasis refers to a further dynamic development of skills to overcome these types of health-related situations (Franke, 2008). Development of skills can be found on both the biological level (e.g., immunological learning and physical training processes) and the psychosocial level (e.g., the development of self-regulating skills such as emotion regulation or problem solving).

2.2.5 First step towards the integration of several health definitions

As a first step towards the integration of the described health definitions into one valid health definition, the several approaches are now clustered *content-wise* into domains and subdomains. The aim is to distinguish (like in the WHO health definition of 1948) between the physical, mental and social domains.

Regarding physical health, one can distinguish five subdomains. The *ability to adapt to external challenges* refers to the proper functioning of several functions of the organism in response to several external challenges (e.g., temperature changes, bacteria). The *ability to recover from inner and outer influences, stress, wounds, etcetera* refers to recovery in the range from executing a function (e.g., membrane recovery of the cell, tissue recovery after physical exercise or sleep after being tired) up to recovering from damaged parts of the organism (e.g., infections or wounds). *Subjectively feeling physically well* refers to the feelings of being fit, without complaints and having

energy. The *resources to become biological healthy* refer to the physical elements that are required to perform proper biological adaptation and recovery (e.g., having no gene dysfunctions). Finally, at this moment, since the exact relationship between health and disease is not clarified, the last subdomain is the *absence of disease*.

Concerning mental health, one can distinguish four subdomains. *Happiness* that is experienced by the individual as emotional well-being is the first subdomain. Happiness is the result of positive emotions and a high quality of life that is associated with positive life experiences. *Self-realization* that is experienced by the individual as psychological well-being is the second subdomain. It is the result of having a purpose in life (meaning in life), the presence of self-acceptance and self-respect, the feeling of mastery in life, and a sense of coherence. The *resources to become mentally healthy* refer to the psychosocial elements that are required to become and remain happy and to realize oneself. Finally, since the exact relationship between health and disease is not clarified at this moment, the fourth subdomain is the *absence of disease*.

Regarding social health, one can distinguish seven subdomains. Five subdomains represent a person with a positive view on other people and society, who feels at home in society and who participates in it: *social acceptance*, *social actualization*, *social contribution*, *social cohesion*, *social integration/ quality connections with others*. The sixth subdomain, *resources to become socially healthy* refers to the elements that are required to effectively perform the roles and tasks for which an individual has been socialized. The last subdomain, since the exact relationship between health and disease is currently not clear, is the *absence of disease*.

A final important feature of the health definition is that the abilities or skills on the physical (adaption and recovery), mental (e.g., self-regulating problem solving skills) and social level (e.g., social actualization skills) can be developed by learning from experiences (heterostasis) and are able to deal with unexpected, new challenges (allostasis). By doing so, individuals are able to take care of the maintenance of the spatial, temporal and functional structures of biological, mental and social functioning (homeostasis).

2.3 The first problem for the WHO health definition: the unclarity of the relationships between the positive domains

An improvement of the health definition can be achieved if one gains more insight into the relationship between the positive health domains. Therefore, the question to be answered in this paragraph is: What is the contribution of these newer approaches to understanding the relationship between the positive health domains? How do (the subdomains of) physical, mental and social health relate to each other?

2.3.1 The relationships between mental, emotional and physical health

Psycho-immunological research shows that there is a two-way dialogue between the brain and the immune system (Zachariae, 2009). Research of the last decades demonstrates that psychological stressors and negative emotions such as depression and anxiety result in an increased production of proinflammatory cytokines (Lutgendorf et al., 1999; Segerstrom & Miller, 2004). These cytokines play a central role in age-related or degenerative diseases such as cardiovascular disease (the leading cause of death), type II diabetes, arthritis, osteoporosis, Alzheimer's disease, periodontal disease, some cancers, and frailty and functional decline (Kiecolt -Glaser, McGuire, Robles, & Glaser, 2002). Additionally, stress and depression contribute to a greater risk of infection, prolonged infectious episodes, and delayed wound healing (Kiecolt-Glaser & Glaser, 2005). These finding are in line with the findings that increase in conscious activities in evolution associated with an increase of the breaking down of the vital, anabolic processes in the organism (Bos, 2008).

Idler and Benyamini (1997) concluded, based on a review of 27 studies, that there is a strong correlation between the subjective assessment of the person's own health and predicted mortality in the short and long term. Pressman and Cohen (2005) concluded, based on a review of the studies on the relationship between positive affect and physical health, that positive affects lead to physical health by two pathways: directly (by means of the connected neurophysiological responses) and via the indirect route (persons with more positive affects exhibit better health behaviors) (Westerhof & Bohlmeijer, 2010). Chida and Steptoe (2008) found in a retrospective study, controlling for negative affects, a relationship between emotional well-being and mortality.

The review of the SOC research shows that the relationship between SOC and physical health is complex and not as strong as the relationship between SOC and mental health (Eriksson, 2007). More research in this area is necessary. Nevertheless, several findings demonstrate a clear relationship between SOC and (aspects of) physical health. In a study of patients with rheumatoid arthritis (Callahan & Pincus, 1995), low but significant correlations between SOC and pain, and general health status and ADL status ($r = 0.10$ and $r = 0.37$) were found. Two studies found strong correlations between high SOC scores with low levels of HbA1c, demonstrating the stability of blood sugar over time (Sanden-Eriksson, 2000; Williams et al., 2004). Higher correlations (up to $r = 0.50$) were found in studies where psychosomatic characteristics were measured as sleep, appetite, headache, etc. (Gebert et al., 1997; Rena et al., 1996). Surtees et al. (2003) performed a cross-sectional study on a population of 20,579 people. A strong SOC was associated with a 30 percent reduction in mortality for all causes as a whole, cardiovascular disease, and cancer in men (not women), regardless of social class, age, sex and the presence of chronic diseases. Krause and Shaw (2002) also found a positive association between personal 'meaning' and better health in old age. Combining the results of these two review studies provides indirect evidence for a relationship between SOC, subjectively rated health and predicted mortality in the short and long term (Eriksson, 2007; Idler & Benyamini, 1997).

In the study of the relationship between psyche and cancer Boesen and Johansen (2008) conclude that the hypothesis that psychotherapy enhances survival should be abandoned in the light of the

latest replication studies, which show null results for improved survival after psychotherapy.

There is a growing amount of literature suggesting that exercise, physical activity and physical activity interventions have beneficial effects across several physical and mental health outcomes (Penedo & Dahn, 2005). The relationship between chronic somatic diseases and quality of life and mental health is substantially researched. Comorbid conditions are associated with a poorer health-related quality of life. Increased age is associated with poorer physical health and better mental health. There is evidence that while physical function could be severely and negatively affected by both chronic disease and advanced age, mental health remains relatively high and stable (Hopman et al., 2009).

Based on the described results, one can conclude that there are clear indications that the presence of mental and emotional well-being can have a positive effect on aspects of physical well-being, but not on cancer. Physical activity can have a positive effect on mental well-being, whereas chronic disease (together with advanced age) do not seem to affect mental health. It is clear, however, that complex relationships exist and that more research is needed. Nevertheless we conclude that the three positive domains are separate elements of health that seem to influence each other.

2.4 The second problem for the WHO health definition: The relationship between health and disease

The WHO definition and 'per exclusionem' definitions of health do not provide insights in the relationships between health and disease. An improvement of the health definition can be achieved if one gains more insight into the relationship between the health and disease domains. Therefore, the questions that need to be answered are: Is health the absence of disease or is it simply the opposite, and is disease the absence of health? What are the relationships between these two domains? This section explores the literature on this topic.

2.4.1 The relationship between mental health and disease

Focus on self-regulation

Throughout their life, people are in constant interaction with 'the world'. They think about the things they experience, emotions come and go, and they take action in response to their thoughts and emotions. Often, they ignore experiences and continue with what they are doing. In some cases, something happens such as finding a life partner or being attacked that has a major impact on the rest of a person's life. Since the disappearance of the 'teapot cultures' (e.g., the religious streams as Catholicism) that formed people's values and behaviors top-down, to a large extent, at the beginning of the 20th century (Kunneman, 1996), Western people increasingly rely

on self-determination of their own values and what significance gives them life, and self-regulation of their inner and outer life (Van Dijk, 2007). Developing self-regulatory skills to make sense of the things they experience (Klein Wassink, 1999) and to deal adequately with what is (from the outside and inside) coming at them (Boekaerts et al., 2000; Rijke, 1991) is an important task in life. The approach to psychosocial health as the dynamic result of the continued deployment of adequate self-regulatory (intellectual) skills (e.g., attention regulation, emotion regulation, social and problem-solving skills) implies that the absence or reduced presence of these skills in a challenging context in which the skills are needed to defend the organism or the person in response to a challenge might result in a form of disease. Other aetiologies (e.g., genetic hereditary disease as a result of insufficient genetic material) are naturally also conceivable.

Disease due to the lack or inadequacy of self-regulatory skills

Westerhof and Bohlmeijer (2010, p. 73, translated in English) concluded in their study on mental health: "The good life requires constant attention and awareness in making meaningful choices and whether these choices are consistent with intrinsic needs, goals and behavior regulation." People respond differently to threatening situations. Siebert (1994) examined the personality traits of people who remained healthy after confronting various types of very dangerous and potentially traumatizing circumstances. His study resulted in a cluster of characteristics of the so-called 'survivor personality', in which psycho-social self-regulation was central. In addition, Sheehy (1982) conducted a questionnaire survey among 60,000 Americans that examined the well-being of people according to the WHO definition of health. Then she extensively interviewed those who scored highest on the questionnaire and some of those who scored lowest, following these individuals for several years. The end result of the study was that there were no differences in the number of life events and shocking events between the two groups. However, what did differ between the two groups was that those who scored high began to handle trauma and crisis in a different way in the course of their lives, sometimes at a younger age and sometimes later in life. Certain qualities of these people who had a high well-being appeared to play an important role. Some of the qualities were courage, willingness to take risks, creativity, flexibility, capacity for intimacy and friendship, humor, energy, the belief that life has meaning, and the ability to be an example for people in crisis.

Conversely, there is the study of the personality characteristics that are risk factors for developing post-traumatic disorder, a psychiatric disorder caused by a potentially traumatic event. Younger age, higher levels of experienced threat, shyness, an inhibited personality, insecurity about one's identity, avoiding leadership roles, coping with emotional suppression and wishful thinking, and a greater external locus of control are the main personality characteristics that increase the risk of acute dissociative reactions and the subsequent development of post-traumatic stress disorder (PTSD) (Marmar et al., 1994). There is a correlation between avoidant coping styles and the level of PTSD symptomatology. Problem-focused coping gives less chance of developing PTSD (Sutker et al, 1995). Trait anxiety and emotion-focused coping were the variables most predictive of stress-related variables (Zeidner et al., 1993). McNally et al. (1995) found that the lower the IQ of a person, the more severe the PTSD symptoms, after adjusting for combat exposure. Beere (1995) also

found that people who dissociate rapidly are more rigid with respect to changes in the environment, have less daily regularity and less emotional reaction in response to errors or conflicts at work, as well as in response to conflicts, errors, or negative assessments in relationships with others. At the end of their review, Foa et al. (1992) describe that the most important conclusion from their study is that in order to trigger PTSD symptoms, a stressor must not only be experienced as a potential threat to life, but it must also be experienced as uncontrollable and unpredictable. In addition, a lack of self-regulatory skills in chronically traumatized patients is associated with a poor stabilization and symptom reduction-oriented treatment result (Baars et al., 2011). On the other hand, preparedness for torture (finding meaning), social and family support, and religious beliefs may all be protective against PTSD following war trauma and torture (Johnson & Thompson, 2006; Van Dijk, 1995).

The summarized literature is theoretically in accordance with the hypothesis that a form of psychological disease may be caused by a lack of skills to give meaning in life (MIL) and a lack of well-developed self-regulatory skills in the presence of specific stressors.

2.4.2 The relationship between physical health and disease

Before discussing the relationship between physical health and disease, this paper will explore some aspects of physical health that will provide important 'substance' for the analysis of this relationship.

Focus on self-regulation

One hardly ever realizes that one's organism is a dynamic system that, among other things, is characterized by constant degradation and (re)construction processes. For example, the epidermis is replaced every few weeks throughout one's life, the cells of the small intestine are replaced every three days, and every cell is constantly in a process of renovation, demolition and construction (Rijke, 2001). Within 14 days, all 10^{27} molecules of the human body are replaced. Within one year, ninety-seven percent of all human cells are renewed (Bos, 2008). When tissues are damaged, there is (in most instances) a quick degradation and reconstruction of molecules, cells and tissue. In addition, from conception to adulthood, the organism grows from of a fusion of two cells (sperm and ovum) up to 10^{14} cells. In this period, all the organs are built and grow until probably the 25th year of life, while the described processes of constant renewal, demolition and construction of cells continue. Taken together, it is evident that a form of highly complex, continuous self-organization must be present for the proper functioning of the body.

In the self-regulating maintenance of the physical organism amongst others two 'systems' are important: the immune system (supported or not by the febrile reaction), and the homeostasis/chronobiological rhythmic system.

The immune system

The immune system has the task of protecting the organism against harmful invaders

and detecting danger coming from both inside and outside of the body (Matzinger, 2001). It is a complex system in the organism, with an innate and acquired subsystem. Here, the effect of the so-called T-cells as part of the acquired subsystem of the immune system is investigated. T-cells are the main cells controlling and regulating the immune response, but they are also responsible for tissue damage and autoimmune disease if they are not properly regulated. Broadly, the ontogenetic development of T-cells is as follows. After the 'production' of stem cells in bone marrow, they travel to the thymus, where an antigen-specific selection and differentiation into CD4+ helper Th-cells and CD+ cytotoxic Tc-cells takes place. Both T cells that react too strongly to cells of the organism and those who do not respond are removed. In this way, a dynamic T-cell population remains that is able to properly respond to antigens. Given the dynamic properties, these T cells are also potentially capable of an autoimmune reaction. Various control mechanisms are present to prevent this (Jutel & Akdis, 2011). After contacting an internal or external antigen, a specific T-cell matures into a set of T cells that specifically recognizes this antigen by their specific receptor for that antigen. Likewise, antigen-specific B-cells will be activated to produce antigen-specific antibodies. These antigen-antibody complexes are then removed from the organism. At a subsequent meeting between the corresponding specific T- and B-cells and the corresponding antigen, the antigen will be recognized quickly because of the persistence of T-memory and B-memory cells after the first contact. They specifically remain for that antigen and provide a more rapid response after subsequent antigen contact. While antigen-specific B-cells last for months up to years, memory T-cells can survive for 65 years.

This process demonstrates that there is a learning curve in the direction of self-regulatory functional 'structure': a 'knowing what' in combination with a 'knowing how' based on a specialization of an initial dynamic, omnipotent power of the T-cells and B-cells to recognize foreign antigens.

Fever

Apart from a few exceptions, reptiles, amphibians, fish and many invertebrates often have fever in response to an infection. Cold-blooded animals instinctively go to warmer places in order to raise their body temperature. In several studies on humans, it has been demonstrated that the magnitude of the fever response to infection varies inversely with mortality from disease (mortality) and the occurrence of disease (morbidity). For blood poisoning (sepsis or bacteriaemie) or peritonitis, there is a positive correlation between the severity of fever and survival of the disease. Conversely, other studies seem to indicate that antipyretics should be associated with increased mortality and morbidity. For example, there is evidence that antibiotics or antivirals disease prolong the duration of diseases such as smallpox and viral infections, nasal extension (Doran et al., 1989; Graham et al., 1990; Kluger, 2002). Some of the mechanisms of the effect of fever are enhanced neutrophil migration, increased production of antibacterial substances by neutrophils, increased production of interferon, increased antiviral and antitumor activity of interferon, and increased T-cell proliferation (Gelfand et al., 1998). A small rise in body temperature inhibits bacterial and viral replication while at the same time increasing the activity and migratory capacity of neutrophils. Currently, the role of fever as 'nature's engine' and 'part of a beneficial host response'

is discussed again in medicine (Fowler, 2009; Dixon et al., 2010).

In conclusion, fever is not really a failure of the organism to maintain the temperature within limits. Rather, it is a very highly regulated response of the organism, involving many hormones and active substances that are active and almost never reach dangerously high temperatures (Kluger, 2002).

Homeostasis and Chronobiological Rhythms

In the 20th century, the research on chronobiology research increased enormously (Hildebrandt, Moser & Lehofer, 1998; Koukkari & Sothern, 2006). Many biochemical, physiological and behavioral manifestations of organisms appear to exhibit rhythmic fluctuations. Chronobiology is the study of these rhythms in plants, animals and humans (Koukkari & Sothern, 2006). Hildebrandt, Moser and Lehofer (1998) argue that all life processes are not only spatially organized, but are also a complex arrangement in time that tune the interaction of the various functions in a meaningful way. It has become clear that a rhythmic system refers to the ability of the human organism to show a response of adaptation in disorders, to achieve a normalized function enabling the return to its optimal frequency. One of the most well-known rhythms is the wake-sleep rhythm. Sleep is regarded as a process of recovery (Minot, 2008). Sleep deprivation leads to cognitive impairment, neuro-endocrine changes, mood disorders and depression. In rats, sleep deprivation is lethal after two to three weeks. The organism handles these rhythmic processes themselves (Heusser et al., 1999). The rhythm can therefore be regarded as a self-regulatory, health-creating activity of the organism to the polar active organ systems involved in balance. It is often used within a subsystem of mutually influencing pro ('excitation') and inhibitory ('inhibition') control (Perreau-Lenz et al, 2004; Talathi et al, 2008). Even homeostasis is conceptualized as the result of balance within a system. The body always tries to maintain a balance between this storage and utilization of energy by balancing the parasympathetic and sympathetic activities. This is known as sympathovagal balance. Maintenance of good health and prevention of diseases is hypothesized to depend on how effectively a person maintains his sympathovagal balance (Pal, 2008). Recent studies provide evidence that the different rhythms in the organism are interconnected, at least in healthy subjects, by phase coupling (Moser et al., 1995), synchronization (Challet et al., 2003; Cyzarz et al., 2004; Zhou et al., 2005), or mutual modulation (Hildebrandt et al., 1998; Hrushesky et al., 1984; Pedemonte et al., 2005). The several rhythms are orchestrated into one interconnected 'symphony of life' as a precondition for maintaining health (Moser et al., 2008).

Disease due to the lack or inadequacy of self-regulatory skills

When physical health is the result of an ongoing self-regulatory activity of the immune system, the fever response and the homeostasis/ chronobiological rhythms in response to external and internal influences, the hypothesis can be deduced that a physical illness/disease can be caused by the lack or inadequacy of the self-regulatory activity or through a lack of 'resources' to implement self-regulatory activity. Here are some illustrative examples that substantiate this theory.

The 'fighting disease' approach in medicine has resulted in the situation that people in the Wes-

tern world in recent decades have had increasingly less contact with pathogenic microorganisms, have become more vaccinated, and have increasingly been treated with antibiotics and antipyretics. It is now clear that this approach, apart from its positive effects, also has its negative effects.

For example, the so-called hygiene hypothesis, that children today are experiencing few infectious diseases so their immune system is not sufficiently practiced, yet stands firm (Douwes & Pearce, 2008). The increased preventative hygiene measures might be (partly) the cause of the increase in allergic diseases like eczema, hay fever and asthma in the past decades. For example, Rosenlund et al. (2009) found in a study of 15,000 children an inverse relationship between being ill from measles (and not in being vaccinated for measles) and the presence of any allergic symptom or any allergic diagnosis. Foliaki et al. (2009) also found in a study of 193,412 children that antibiotic use in the first year of life is associated with the occurrence of asthma, eczema and rhinoconjuctivitis in children between the age of 6 and 7 years. In addition, several epidemiological studies demonstrate the protective effect of growing up on a farm, which offers contact with higher numbers of microorganisms (Van Mutius, 2010).

Kienle (2003) describes how in the recent period of nearly 200 years, the conceptualization of cancer has changed from cancer as a cellular problem to a problem of the hierarchical organization of the organism as a whole. Sporn (1997) describes in his review that one must regard cancer as a disease of the whole organism. The author claims that although molecular and cell biology have enormous power as analytical tools, the ultimate understanding and control of the process of carcinogenesis will require a synthesis at the level or tissue, organ and organism.

The ultimate in cooperation and complex dependency within living beings is the organism, which is composed of multiple organ systems with organs working together to perform particular functions but also to ensure the survival of the organisms as a whole in the ever-changing environment. In a strongly and traditional reductionist view, the human body consists of seven hierarchal levels of organization that can all be affected in cancer development. These levels consist of atoms that combine to form molecules, which combine to form cells that work together to form tissues, which together form organs and subsequent organ systems that finally build up the organism. Therefore, the functioning of the organisms is dependent on the coordinated work of all elements into a cohesive unit.

Meanwhile, by examining the relationship between the self-regulating, organizing activity of fever and cancer has become increasingly evident. There is an inverse correlation between different types of febrile illnesses in childhood and the occurrence of cancer (Hobohm et al., 2008). In addition, there is increasing evidence of the positive effects of febrile illnesses in the spontaneous healing or improvement of cancer (Coley, 1893, 1906; Hobohm, 2008).

Menke et al. (2007) describe a wound-healing model. Within this model, the inflammatory profile of a non-healing wound is one in which the equilibrium between synthesis and degradation has shifted toward degradation. Reutelingsperger (2010) describes the role of apoptosis (programmed cell death) as an opposite function to ongoing cell division in organisms. Within this system, the balance between cell division and cell death may be disturbed, after which two types of diseases can occur as a result of the imbalance. When there is too little cell death over cell division, for example, mutations

in the gene-encoding proteins involved in the execution of cell death, diseases such as cancer and autoimmune diseases can occur. For example, cancer cells may have defective proteins that are not sufficiently capable of activating self-killing programs, while, on the other hand, they are able to perform cell division. The result is an increase in cancer cells. In that case, therapies aiming at stimulation of the suicide program can restore the balance. The balance swings the other way by excessive cell death. This affects, for example, the development of metabolic syndromes such as neurodegenerative Alzheimer's dementia and in the process leading to heart failure.

Finally, there is increasing empirical evidence of disturbances of chronobiological rhythms. The most common are disturbances of the day-night rhythm associated with impaired cognition, depression (Mignot, 2008), type 2 diabetes, obesity (Bass & Takahashi, 2010) and breast cancer (Moser et al., 2005, 2006). There are also indications that migraines are the result of excessive excitation or a failure of inhibition, in other words, the result of an imbalance of the relevant underlying chronobiological system (Talathi, 2008).

2.5 Towards a revised and more valid definition of health

2.5.1 Focus on self-regulation

In general, there is a tendency in biology and medicine to look for reductionist explanations in health- and disease-related problems. For example, scientists look for the gene, molecule or cell that is responsible for a particular physiological or psychological effect. However, accounts of health such as MIL, SOC, the triad of mental health, and three physiological systems (immune system, the fever response and the homeostasis/ rhythmic system) focus increasingly on various self-regulatory organization levels.

2.5.2 'Health by self-regulation'

By integrating the health definition of the WHO from 1948 with the developed (subdomains of the) definitions, relevant theories and empirical facts, a better health definition can be developed. Central to this is the change from thinking in end states (state of perfect health and absence from disease) into thinking in terms of the continuous activity of self-regulating processes that are responsible for reaching these states.

Health is first the proper functioning of the body (forms and functions) processes and psychosocial processes with the absence of disease. This, however, must be considered a dynamic end situation in terms of 'material elements,' since the molecules and cells in the body are constantly

replaced throughout life. Second, human health is the self-regulating ability to constantly manifest itself in and adapt to the environment, both physically/physiologically, mentally and socially. Third, health concerns the self-regulatory ability to recover from physical, mental and social endeavors, wounds, injuries, stress, traumatic events, etcetera. To this end, internal (e.g., certain active genes and proteins in the inflammatory response or specific coping strategies) and external (e.g., plaster for a fracture or psychological help with a shocking event) resources are necessary. Some self-regulatory abilities have been sufficiently built at birth, but can develop further throughout life (e.g., the immune system). Others should be largely developed (psychological self-regulation: perception, processing, reaction; sense of coherence: comprehensibility, manageability, meaningfulness) or trained (e.g., physical endurance) throughout life. When a person at all three levels is able to function optimally, the subjective experience of this is that he/she feels emotionally and psychological well, physically fit and energetic, without complaints and suffering, and that he/she has a positive view on other people and society and feels at home in society.

The relationship between the positive domains is that a high mental and social functioning is related through two main pathways to better physical health (directly and indirectly through healthy neurophysiological behavior). Conversely, physical activity can have a positive effect on mental well-being. One of the connections between health and disease is that physical or mental diseases may be the result of a lack of or inadequate physical, mental or social self-regulatory skills or processes. A second relationship is that psychological stressors and negative emotions adversely affect physical health, whereas chronic disease (together with advanced age) do not seem to affect mental health.

2.5.3 Connecting the revised health definition to some related theories

Theories should be judged according to formal criteria (Oost, 1999; Oost et al., 2002), which state that existing theories may fail: (1) on internal conceptual grounds (logical inconsistency, use of ambiguous concepts); (2) on external conceptual grounds (a theory "does not fit in" with other theories); and (3) on empirical grounds (a theory "does not fit in" with reality).

The new concept is regarded as more internally consistent than the WHO concept of 1948; the former is broadened with conceptual information on the relationships between the positive domains and between health and disease, and the concept is logically consistent. Since the concept is based on (new) empirical evidence, it is also considered to be valid on empirical grounds. Therefore, only the last criterion is tested here. This leads to the question of whether the concept fits in with some of the current important related theories of systems biology, emergence, epigenetics, and the self-organization of complex systems.

Systems biology

Before the year 2000, only two articles contained the term 'systems biology' in either the title or the abstract in PubMed. In 2010, more than 2,500 entries had appeared with more than 90

percent in the last five years, demonstrating the increasing interest of scientists in this topic (Kohl et al., 2010). "Its recent rapid resurgence at the turn of the century reflects the problems encountered in interpreting the sequencing of the genome and the failure of that immense achievement to provide rapid and direct solutions to major multi-factorial diseases" (Noble, 2011, p. 9). Different than the genetic and microbiological approach, systems biology aims to describe, understand and explain from the complex biological systems that are studied: all levels of structural and functional complexity, explicitly including the supracellular domain (Kohl et al., 2010); their systems behavior or phenotypes; their networks with relationships that interact with the genome, the environment and the phenotype (Noble, 2011; Van der Greef et al., 2007); their multifactorial processes involved in maintaining homeostasis and the breakdown of homeostasis within the system (Van Ommen et al., 2009).

Epigenetics

Recent insights into the mechanisms and molecular basis of gene regulation have led to the notion that organisms increase their complexity by altering the level of gene transcription without affecting their DNA base-pair sequence. This field of expertise is called epigenetics, and it refers to the fact that although all of the cells of an individual contain the same amount of DNA and the same sequences in the genome, different locations in the body instruct cells to perform different functions and even to react and modify their genetic potential as a way of reacting to their exposure to environmental factors (Bernstein et al., 2007). Three main mechanisms are supposed to be responsible for epigenetic changes: DNA methylation, histone tail modifications, and noncoding RNAs (Huang & Fan, 2011). These induced changes are heritable and can exert their influence over several subsequent generations through perinatal programming, and can therefore explain why certain diseases run in families (Barker, 2007). In addition, epigenetic mechanisms contribute to the explanation for neurophysiologic and psychological functioning. Therefore, they are hypothesized to provide a physiological basis for the perceptions of disease, health and welfare (Graff & Mansuy, 2008).

Emergence

The features of organs are not found in the cells and consciousness is not found in organs. In nature, higher organization levels 'spontaneously' arise from lower levels and, at the same time, influence these lower levels. For example, a cell that is transplanted from one tissue to another often changes its functions according to the new tissue (Gurdon, 1986). "Emergent phenomena are said to arise out of and be sustained by more basic phenomena, while at the same time exerting a 'top-down' control, constraint or some other sort of influence upon those very sustaining processes" (Corradini & O'Connor, 2010, p. xi). Throughout several concepts, emergent phenomena are usually characterized as irreducible, unpredictable or unexplainable, to require new concepts and to be holistic (Bedau & Humphreys, 2008).

The concept of emergence has received renewed attention in science in the past decades due to developments in a number of research programs within complexity theory, artificial life, phy-

sics, psychology, sociology, biology, philosophy of science and philosophy of mind (Bedau & Humphreys, 2008).

Self-organisation

De Wolf and Holvoet (2005, p.7) propose the following work definition for self-organisation: "Self-organisation is a dynamical and adaptive process where systems acquire and maintain structure themselves, without external control." These structures can be spatial, temporal and/or functional. Four characteristics of self-organisation are, according to the authors, supposed to be essential: (1) *increase of order*: self-organisation needs to find sufficient order, that is a balance between no order and too much order, in order to reach a structure; (2) *autonomy*: the absence of external control; (3) *adaptability or robustness with regard to change*: adaptability in the presence of perturbations and change. A self-organising system is expected to cope with that change and to maintain its organisation autonomously; and (4) *dynamical i.e. far from equilibrium*: self-organization is a process. Over time, there is an increase in order, i.e. a dynamic towards more order. Changes influence the organised structure. In order to maintain that structure, there needs to be a constant dynamic that handles these changes.

We state here that the definition "health by self-regulation" is in accordance with theories of systems biology, emergence, epigenetics and self-organisation of complex systems.

2.5.4 The definition of health promotion

The fighting disease approach has its targets in eliminating disease-related organisms, cells and functions in the body; disease-related symptoms, and the manipulation and/ or substitution of non-functioning or dysfunctional parts of the body by means of *external* therapeutic resources. The health promotion approach is the process of enabling individuals, groups or societies to increase control over and improve their physical, mental, social and spiritual health (Eriksson & Lindstrom, 1997). Based on the results of this study, one can conclude that health promotion aims at improving the development and quality of the self-organizing skills on the physical, mental and social level, and at improving the integration of these higher organization levels with the lower levels and parts of the system.

An example of the integration of the fighting disease and curative health promotion approaches in hay fever treatment

Allergic rhinitis is a condition characterized by sneezing, watery nasal discharge, and nasal obstruction and itching. It is an increasingly prevalent condition, particularly in the Western world where it affects around 20% of the adult population. Allergic rhinitis is divided into seasonal allergic rhinitis (SAR) (hay fever), which is triggered by pollens and moulds, and perennial allergic rhinitis (PAR) in which house dust mites and pet dander are the predominant triggers. The

spectrum of severity is wide.

The increasing prevalence of allergic rhinitis in Western countries is associated with modern lifestyle. The (hygiene) hypothesis is that high living standards and hygienic conditions are correlated with an increased risk for the development of an allergic disease. Due to reduced exposure to microbial components, the proposed allergy-preventing potential of these factors is no more present in sufficient qualities and/or quantities, which leads to an imbalance of the immune system with a predisposition to the development of allergic disorders (Garn and Renz, 2007; Mas and Horner, 2008).

The treatment of choice of SAR is the treatment with local or oral antihistamines and/or local corticosteroids. Both types of treatment are typical *fighting disease* treatments, whereas these treatments (1) are manipulating disease-related dysfunctioning or pathophysiological processes in the body: respectively the overproduction of histamine (antihistamines) and the chronic inflammatory reaction as a result of a too active allergic Th2 pathway in the immune system (local corticosteroids); and (2) reduce the disease-related symptoms. However, these interventions do not change the diseases status or set the precondition for a healing process that changes the organism into a healthier status. Therefore, allergic rhinitis patients that are treated in this way (symptomatically), will have to be treated for the rest of their lives.

A second type of allergic rhinitis treatment is immunotherapy. It is indicated in a limited subpopulation of patients and includes a significant number of sufferers with severe symptoms that are resistant to treatment with usual pharmacotherapy (antihistamines and topical nasal corticosteroids) (Sachs et al., 2006). Optimal treatment effects are established in three years. During treatment patients receive, often on a weekly basis allergy shots. Each allergy shot contains a tiny amount of the specific substance or substances that trigger the allergic reactions. Allergy shots contain just enough allergens to stimulate the immune system — but not enough to cause a full-blown allergic reaction. Over time, the dose of allergens in each of the allergy shots increases. This helps to get the organism used to the allergens (become desensitized). The immune system builds up a tolerance to the allergens, and the allergy symptoms diminish over time. Immunotherapy is a typical *curative health promotion* therapy, since the effect of the therapy is a permanent change into a better health status that is based on an improvement of the organism's ability or in other words its organic skill to cope with specific allergens. The working mechanism of this type of health promotion is, analogue to psychotherapeutic phobia treatment, the increase of the exposure to a stimulus in order to desensitize the reaction to the stimulus.

A third type of allergic rhinitis treatment is treatment with Citrus/Cydonia comp. subcutaneous injections and/ or nasal spray. The experiences of prescribing GP's is that SAR patients are claiming to permanently suffer less from hay fever symptoms or even that they are free from complaints after the treatment with Citrus/Cydonia comp.. The effect is occurring within a period of two weeks, up to three months, after treatment onset. The effect is optimal after a treatment of several years (4). Positive effects, without side effects, were also observed in two cohort studies: a group of 13 patients suffering from grass pollen SAR treated with subcutaneous injections (5) and in a group of 140 patients, who were treated with nasal spray (6). In a randomized trial compa-

ring two routes of administration (nasal spray versus subcutaneous injections), immunological and clinical effects of both routes were demonstrated. In the immunological studies the effects of this treatment were the induction of regulatory (IL-10 producing) T-cell subsets and the suppression of the Th2 pathway cytokines IL-4 and IL-5. The working mechanism of this type of health promotion is, analogue to psychotherapeutic skills training (e.g. emotion regulation, problem solving) the improvement of skills that are able to inhibit unwanted reactions and restore the balance of the functional of the subsystem as a whole.

Combining the best of both worlds of fighting disease and (preventative and curative) health promotion in order to improve the health status of healthy or diseased persons is an important goal to strive towards. The integration of both approaches could result in:

Preventative health promotion recommendations for parents about handling hygiene during childhood of their children and the safe use of for example probiotics as a replacement for bacteria exposure in normal life situations.

Integration of fighting disease and curative health promotion in treating SAR. A quick reduction of SAR symptoms can be achieved by means of antihistamines and topical nasal corticosteroids treatment, and can be combined with either/or immunomodulation (especially in severe, pharmacotherapy resistant SAR) or treatment with Citrus/Cydonia comp..

An example of the integration of the fighting disease and curative health promotion approaches in phase 1 treatment of chronic traumatization

Prospective longitudinal and retrospective studies have demonstrated that chronic childhood abuse and neglect may have pervasive effects on adult function (Anda et al., 2006; MacMillan et al., 2001; Putnam, 2003; Springer, Sheridan, Kuo & Carnes, 2007; Teicher, Andersen, Polcari, Anderson & Navalta, 2002). Childhood abuse and neglect has also been found to be associated with borderline personality disorder (Herman, Perry & Van der Kolk, 1989; Ogata et al., 1990), somatization disorder (Saxe et al., 1994), eating disorders (Herzog, Staley, Carmody, Robbins & Van der Kolk, 1993), sexual disorders (Putnam, 2003), DSM-IV Axis I diagnoses of dissociative disorders (Boon & Draijer, 1993; Ross et al., 1991; Ross, Norton & Wozney, 1989), post-traumatic stress disorder (PTSD) (Bremner, 1993; Widom, 1999), substance abuse disorders (Putnam, 2003) and a range of persistent symptoms more complicated than those of PTSD, often called "complex PTSD" (Herman, 1992) or "disorders of extreme stress not otherwise specified" (DESNOS; Pelcovitz et al., 1997; Roth, Newman, Pelcovitz, Van der Kolk & Mandel, 1997). This paper refers to the DESNOS symptom clusters as the diagnosis of complex PTSD; however, it is important to note that 'complex PTSD' is subsumed under "Associated descriptive features and mental disorders" of PTSD in the DSM-IV classification (APA, 1994, p. 425).

The current clinical standard of care for complex trauma-related disorders is phase-oriented treatment (Brown, Scheflin & Hammond, 1998; Chu, 1998; Courtois, 1999; Herman, 1992; Van der Hart, Nijenhuis & Steele, 2006). In most of these models, there are typically three phases of treatment involving 1) stabilization and symptom reduction, 2) integration of traumatic memories, and 3) reintegration of the personality and rehabilitation. This treatment model does not imply that

the phases will occur strictly sequentially. Rather, phase 2 treatment will periodically alternate with phase 1 treatment, and later in the course of therapy, phase 2 and phase 1 work will alternate with phase 3 treatment (Courtois, 1999; Van der Hart, Brown & Van der Kolk, 1989). Not all patients are able to reach phase 2 (Boon, 1997).

Two central first phase treatment goals are: the reduction of symptoms (depression, anxiety, etcetera) and breaking maladaptive patterns (e.g., drug abuse, self-mutilation as a means to cope with stress) and subsequently developing adequate coping skills. Symptom reduction can generally be accomplished with psychiatric fighting disease drugs such as antidepressants and anxiolytica. Psycho-education and skills training (e.g., learning skills, management and modulation of distress, emotion regulation skills, critical thinking and judgment skills, problem-solving and social skills) are important health promotion treatments. Psycho-education and skills training help patients to develop self-regulation skills, which can be defined as the adaptive use of skills across changing personal and environmental conditions (Boekaerts et al., 2000).

The reduction of symptoms only is, in most instances, not sufficient to reach phase 2. On the other hand, the development of self-regulation skills can only be reached when patients are treated before and/or in addition to symptom reduction pharmaceutics (Baars et al., 2011). Consequently, the phase 1 treatment of chronic traumatization must be regarded as an integrated fighting disease and health promotion approach.

2.6 Discussion

Based on a conceptual analysis of historic and current health-related concepts and an analysis of the conceptual shortcomings of the WHO health concept of 1948, a revised health definition ‘health by self-regulation’ was constructed. The validity of the concept was preliminarily and successfully tested, since the internal consistency of the concept was improved compared to the WHO concept of 1948; the concept was based on empirical evidence, and it fit in with some of the current important relevant theories of systems biology, epigenetics, emergentism, and self-organization. Valid definitions of health (and disease) are very important, since they provide an entrance for preventive and therapeutic action (Canguilhem, 1991).

Compared to the WHO health definition we regard the presented definition ‘health by self-regulation’ an improvement since, contrary to the WHO definition, (1) it provides information on the relationships between the positive domains, (2) it provides information on the relationships between health and disease, (3) is based on both probabilistic and mechanistic evidence, and (4) it provides conceptual information for the further development and innovation of preventive and curative health promotion strategies in clinical practice.

The major limitation of the study is that only a limited amount of literature on the health promotion and fighting disease concepts and empirical evidence was analyzed. Therefore, further conceptual analysis, improvement and validation of the presented concept are required.

The presented concept is theoretically in line with the results of an invitational conference in the Netherlands where 38 international specialists discussed the health definition guided by a review of the literature (Huber et al., 2011). The result of this conference was that health should be conceptualized as the ability to adapt and to self manage.

The new definition provides a theoretical fundament that can be further explored and refined, based on the analysis of other theories and empirical evidence. It can also help the further conceptualization of the role of, for example, fever and inflammation in young age but also in the treatment of, for example, cancer, and the conceptualization of the development of (biological and psychosocial) health promotion skills. Furthermore, it provides a framework to study the role of chronobiological imbalances of subsystems in various diseases.

The definition can provide an evidence-based fundament for the development and validation of health promotion strategies in clinical practice. Three steps can be taken: (1) the designing of preventive and curative health promotion strategies based on the validated health definition; (2) the testing of quality, efficacy, effectiveness and cost-effectiveness of preventative and curative health promotion strategies for specific indications; and (3) the integration of fighting disease and health promotion strategies for specific indications.

Important future research topics are: (1) the exact relationship between meaning in life and psychosocial functioning and physical health; (2) the exploration of the surplus value of health promotion compared to fighting disease; (3) the development and testing of optimal integration of the fighting disease and health promotion approaches for specific indications; (4) further exploration of the exact health promotion mechanism and its interactions with the genome, environment, epigenetic mechanism, etcetera; and (5) the development and validation of the methods and measuring instruments for monitoring changes in health status in addition to disease status changes.

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Chapter 3

Towards a philosophical underpinning of the holistic concept of integrity of organisms within organic agriculture

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Abstract

The concept of naturalness can be used to characterize organic agriculture and to distinguish it from conventional agriculture, provided naturalness not only refers to the non-use of synthesized chemicals, but also to the ecological and systemic principles, and to a respect for the integrity of living organisms. Examples of the implicit use of the integrity concept in agriculture will be described to show its practical aspects and implications. The (non-atomistic) holistic concept of integrity of organisms has been the subject of severe scientific criticism – specially from in essence ontological reductionists. In their view, an organism is essentially no more than a complex set of atoms and molecules and its integrity a nonconcept. In order to reach scientific acceptance of the integrity concept and to support its use in organic agricultural practice, it needs further underpinning. In this article, based on a critical analysis of (a) ontological and methodological aspects of reductionism, and (b) expert knowledge and the process of pattern recognition and application, the validity of the holistic concept of integrity will be explored.

Additional keywords: holism-reductionism debate, wholeness of organisms

3.1 Introduction

During the last decades it has become evident that the concept of naturalness can be used to characterize organic agriculture and to distinguish it from conventional agriculture, provided naturalness refers to (1) abstaining from the use of artificially synthesized chemicals, (2) adopting the ecological principles, and (3) respecting the integrity of living organisms as a holistic concept of thinking (e.g. Lammerts Van Bueren et al., 2003; Verhoog et al., 2003; Lammerts Van Bueren & Struik, 2005).

In this paper we focus on the holistic concept of the integrity of organisms.

Starting at the beginning of the 1990s, the concept of integrity received increasing attention in bioethical literature. For example, in the biotechnology discussion the concept of 'genetic integrity' (Vorstenbosch, 1993) was used in connection with the genome of an individual animal. Lammerts Van Bueren *et al.* (2003) distinguished four levels of integrity in relation to plant breeding in organic farming: (1) integrity of life, (2) planttypic integrity, (3) genotypic integrity, and (4) phenotypic integrity. Similar kinds of levels were first developed in relation to the genetic engineering of animals. Expanding on the concept of ecosystem integrity, Thompson (1997) introduced the notion of functional integrity in the discussion on sustainability in livestock farming. We shall argue that, besides this explicit use of the concept of integrity, in agricultural practice there is also an implicit use of this concept.

In spite of this explicit and implicit use of the integrity concept, the concept of integrity has hardly been accepted in agricultural sciences. This is mainly due to the philosophical background of many scientists who are reductionists and therefore consider an organism in essence no more than a complex set of atoms and molecules. In their view, an organism is best studied in an experimental setting where man's purported subjectivity has been completely eliminated and where features of objects and (causal) relationships between the objects can be examined (Looijen, 1998). In this scientific approach there is no room for a holistic concept of integrity, a concept that refers to the existence of a real holistic being. The denial of the latter implies that organisms can be constructed in accordance with human desires and that there is no need for research methodologies at higher levels than the level of pure matter: mainly atoms and molecules.

In order to defend a holistic approach of integrity that not only is acceptable to agricultural practice but also to agricultural sciences, it is necessary to strengthen the philosophical basis of this new concept. This is done in this article by means of:

- a critical analysis of some ontological and methodological aspects of reductionism, and
- a reflection on the methodological role of expert knowledge expressed by practitioners.

3.2 The implicit use of the integrity concept in agricultural practice

In agricultural practice and in the way people treat domesticated and wild animals, the concept of integrity has already been used for many years, although often in an implicit rather than an explicit way. For example, animal breeder and geneticist Frederik Bakels integrated knowledge of the domestication process, the habits and living conditions of wild cows into his breeding goals and concepts. His central foci were the cow's needs as a ruminant and its perfect body shape based on wild cows rather than the cow as an efficient producer of proteins and fat (Bakels & Postler, 1986). Bakels' implicit awareness of animal integrity was transformed in his breeding concept based on 'longevity' and 'lifetime production'.

Animal ethologist Temple Grandin developed slaughter houses and improved walking ways for cattle, based on her empathic knowledge of and involvement with animal needs. In relation to her autistic personality, Grandin developed the ability to see the animal-specific fears during their walking into a new and strange environment and she was able to adapt this environment taking into account the animal's needs (Grandin & Johnson, 2005).

In the 1960s, animal ethologist Jane Goodall was criticized by the main-stream scientific community, because she gave names to the chimpanzees instead of 'objective' numbers. In those days animals were seen as unconscious machines. In her view, however, animals are sentient beings (Goodall & Bekoff, 2002). Her present work on chimpanzee protection and chimpanzee liberation is based on her empathic involvement with the animals in their natural environment. Her scientific methodologies have been adopted by animal ethologists observing other mammals (elephants, wolves, gorillas) (Turner & D'Silva, 2006). For other examples see Verhoog (2007) in this issue. The concept of integrity is also important for answering ethical questions within organic agriculture, such as whether specific treatments of organisms are 'acceptable'. In (organic) agriculture, the acceptance of the concept of integrity has three major consequences. First, there are consequences for our *basic attitude* towards living organisms. Instead of treating an animal as if it were 'a piece of meat' or 'an efficient milk factory', we encounter a real and true 'being' with a specific nature. This refers to the natural living approach to animal welfare in organic agriculture (Wagenaar & Langhout, 2007). On the basis of the notion of animal sentience, we have to treat the animal in a respectful way (Turner & D'Silva, 2006).

Secondly, we should develop a *personal relationship* with this 'being', like we do in the encounter with fellow human beings, in order to develop an insight into its wholeness. Instead of creating the ultimate professional gap between subject and object in the search for measurable, objective knowledge of the parts and their relationships, we have to 'catch' its identity in the way it expresses itself in the organization of the parts in time (e.g. physiological processes) and place (e.g. morphological processes) (e.g. Schad, 1971; Bortoft, 1996). This is exactly what experienced practitioners implicitly do and what the research topic is in experiential science (Baars & Baars, 2007). Goethean phenomenological science as defined below is an exact and explicit methodology

to acquire embedded insight into the observed. The method is currently used in several areas of agriculture, such as landscape development (Pedroli *et al.*, 2007), plant breeding and research into medicinal herbs. A third consequence directs our way of handling organisms to focus on the *creation of preconditions in such a manner that the being is able to express itself in an optimal way.*

3.3 An introduction to the holism-reductionism debate

In the foregoing we have explained that the holistic concept of integrity is an important concept in organic agriculture, either explicitly based on ethical reflection, or implicitlyby experienced practitioners. But a better philosophical foundation is needed. For this we have to join the holism–reductionism debate. A negative way of defending holism is showing the shortcomings of reductionism. A positive way is to refer to the importance of pattern recognition in science, which is based on a holistic perception.

3.3.1 Definitions and historical development

Definitions

In the philosophy of science, reductionism is usually set against holism or organicism. The statement: '*An organism is essentially nothing but a complex set of atoms and molecules*' lies at the heart of reductionism. At the opposite side of the scale, statements such as '*You cannot just simply reduce an organism to a sack of molecules*' and '*The whole is greater than the sum of its composing parts*' essentially characterize holism (Looijen, 1998). The reductionist model argues that all traits and other characteristics demonstrated by living organisms – their morphology, physiology, behaviour and ecology – can ultimately be fully and exclusively explained in terms of the physical and chemical molecules (DNA, proteins, etc.) of which they are composed. Reductionism assumes that the laws, concepts and theories formulated for a higher level of organization in biology (for example the level of the organism) can be explained by theories developed for a lower level of organization, such as the level of organs, tissue and cells. This means that ultimately all biological concepts, laws and theories can be reduced to the physical and chemical (Looyen, 1998).

Three aspects must be distinguished in the reductionism-versus-holism debate: (1) ontological, (2) pistemological, and (3) methodological aspects (Looyen, 1998). *Ontological* aspects concern the question what entities, things or substances are assumed to make up reality (nature); what characteristics are attributed to these things or entities, and what relationships and functions can be assumed to exist between them. *Epistemological logical* aspects are about our knowledge of reality; the way in which this knowledge is expressed in theories (amongst other things), and logical connections between theories. In particular, these aspects deal with links between theories deve-

loped for different areas of reality or for different levels of organization. *Methodological* aspects concern the way in which knowledge is obtained and the basic principles, laws and strategies used in the process. This is particularly related to the question whether – in order to arrive at 'correct' knowledge or understanding of a certain level of organization (the level of the whole) – we should study the underlying lower levels of organizations (the composing parts and their interactions) or the higher level itself, or perhaps its relationship with still higher levels.

The division of holism and reductionism into these three aspects has important implications for our understanding of the positions of the distinctive scientific approaches. At all three levels either holistic or reductionist choices can be made. At the ontological level one can theoretically distinguish between ontological reductionism (so-called atomism) and ontological holism (so-called vitalism, emergentism and organicism). *Atomism* implies that the entities of the 'lowest' level of organization (atoms, subatomic particles, quantum particles, etc.) are somehow 'fundamental': they are the 'building blocks' of nature. Ontological reductionism states that, for instance in the discussion on genetic engineering, there is nothing but only DNA or that in causal explanations of biological results there is nothing but laws of physics and chemistry. In contrast, *vitalism* claims that animate nature is different from inanimate nature in that there are non-material forces operative in living beings. Another interpretation of holistic ontology is found in *emergentism*. In this holistic view, at each higher level of organization new and irreducible properties appear that are not present at lower levels. These so-called 'emergent' properties are defined as properties of wholes that are not possessed by their composing parts. At the epistemological level one can distinguish between *provincialism* – the reductionist view that biology, like chemistry, is a special branch or province of physics – and *autonomism*, which defends biology's autonomy with respect to physics and chemistry.

At the methodological level, the reductionist strategy to obtain knowledge of higher levels of organization is to study lower level entities and interactions between them. The best way to understand phenomena at the level of the whole is to study causal mechanisms at the level of its constituent parts. Holists, on the other hand, claim that in order to obtain knowledge of a certain organization level in biology, one must not study (or at least not only) the lower levels of organization, but (also) the higher level itself as well as its relationships with still higher levels. Because of the emphasis put on the study of wholes at their own level of organization, holism is often associated with a descriptive or phenomenological method (Looijen, 1998).

Historical development

Reductionist and organicist or holistic descriptions and theories have alternated in science since the Middle Ages. Verhoog (1993) summarizes this trend as follows: "In the medieval notion of reading the Book of Nature, nature is seen as an organism, as the body of Mother Earth, a harmonious and self-regulating entity that is treated with respect. In the 16th century this concept of nature was replaced by the concept of 'fallen nature'. Nature is now seen as disorderly and chaotic; the 'blind' forces of nature must be controlled by human reason. So man is no longer seen as an intrinsic element in a nature created by God. The measurable parts of Nature are objectified and

materialistically reduced during the mainstream of scientific development of the 16th and 17th centuries. In a nature no longer guided by a divine providence, humans are free to manipulate and use nature as an instrument and for their own purpose. Experimental science provided the means to do this."

In addition to the development of reductionist scientific thinking, Gloy (1996) de-scribes the historical development of *holistic* thought as a development in stages: current ecological thinking was preceded by natural magic in the Renaissance (end 14th – end 16th century), by Leibniz' Monadology, by the natural philosophy of German Idealism and the Romantic Movement, and by vitalistic and holistic concepts at the start of the 20th century. Reductionism often dominates current social and scientific thinking.

3.3.2 A critical analysis of reductionism

There are three central statements related to the theory of reductionism that can be used as objects of criticism:

1. All traits and other characteristics demonstrated by living organisms – their morphology, physiology, behaviour and ecology – can ultimately be fully and exclusively explained in terms of the molecules (DNA, proteins, etc.) of which they are composed. There are no higher levels or non-atomistic organizing principles present (reductionist ontology).
2. The specific concepts, laws and theories used in the sciences of higher levels of organizations of organisms can eventually all be reduced, step by step, to the fundamental theories of physics. There is no need for non-reducible holistic theories about reality (reductionist epistemology).
3. The strategy to obtain knowledge at higher levels of organization is to study lower-level entities and interactions between them. The best way to understand phenomena at the level of the whole is to study causal mechanisms at the level of their constituent parts. There is no need for holistic methodologies that specifically obtain knowledge from the level of the whole (reductionist methodology).

In this article, we shall restrict ourselves mainly to the two central statements: 1 and 3.

So the leading research questions for this article are:

- What is the tenablity of reductionist ontology?
- What is the tenability of reductionist methodology?
- What is the philosophical basis for a non-atomistic holistic concept of integrity of organisms?

Although this article discusses thoughts on integrity in agriculture, several topics from other scientific fields will be used in the criticism of the reductionism theory. This is justified, since the reductionism theory is the general basis for reductionist scientists to criticize the holistic concept of integrity of organisms in all scientific fields, including agriculture.

Criticism of reductionist ontology in molecular biology

As stated above, ontological reductionism or atomism implies that the entities of the 'lowest' level of *organization* (atoms, sub-atomic particles, quantum particles, etc.) are somehow 'fundamental'. They are the indivisible 'building blocks' of nature, the 'cement of the universe'. However, Gloy (1995) states that since the atomism theory was introduced three fundamental problems with this theory have not been solved (sufficiently). First of all, in no version of the atomism theory, the fundamental opposition has been solved between the postulate of smallest indivisible parts and simultaneously the postulate of their spatial extension. Secondly, although necessary content-wise, within the atomism theory mechanistic and teleological thought remain unconnected. Thirdly, it still remains to be seen whether or not the elements of a mechanistic world view will ever be able to construct living nature. So far, this view does not seem to be able to do this. All 'new organisms produced by men' (e.g. cloned animals) have originated from already living organisms, and they have not been built from the fundamental 'building blocks of nature'.

The limitations of reductionist thinking are also demonstrated in genetic research. Original reductionist paradigms are (1) DNA – and in some viruses RNA – forms the genetic material, (2) the genetic stream of information goes from DNA to RNA to protein, and (3) the basic DNA sequence transforms, via the RNA, exactly into the amino acid sequence of the polypeptides. Furthermore, the assumption that a single gene encodes for a single protein is currently refuted in various manners. All options are possible: (1) a gene encodes for a protein, (2) a gene encodes for several proteins, (3) many genes encode for the same protein, and (4) many genes encode for many proteins (Ho, 1999). In the inheritance of genetic information between generations, there is more than only the inheritance of the DNA; frequently, so-called 'epigenetic inheritance' occurs (Russo *et al.*, 1996; Jablonka & Lamb, 2005). In several studies the determining importance of context in gene expression has been described (e.g. Gurdon, 1999; Ho, 1999). For example, the transplantation of DNA nucleids of highly specialized cells into other surroundings shows that both the function and the devel-opmental stage are adopted (Kienle & Kiene, 2003). Molecular biologists argue, however, that in many cases, reductionist thinking itself was able to explain and to incorporate all new data, by extending its theoretical fundament accordingly. In addition, they state that current and future unexplained phenomena would eventually be explained by experimentally tested reductionistic hypotheses.

Furthermore, genetically equal cells differentiate systematically into distinguishable cell types and distinguishable protein compositions and therefore distinguishable gene expressions. This leads to the question how the different genes are switched on and off when the genetic 'equipment', the 'genetic programme' is the same everywhere. This is the so-called logical dilemma of developmental biology (Hamburgh, 1971). The cause must therefore be sought outside of the DNA: 'epigenetically'. In people and animals extra cellular forms and patterns such as arms and legs occur in equal cell types (bone, skin cells, etc.); the extra cellular form, the spatial partitioning of the equal cells, is therefore independent of gene expression (Müller & Hassel, 2002).

Comparative evidence indicates substantial incongruities between genetic and morphological evolution, and demonstrates that the same genotypes do not necessarily correspond with identical

phenotypes (Lowe & Wray, 1997). On the one hand, genetic and developmental pathways can change over evolutionary time even when morphology remains constant (Felix *et al.*, 2000); on the other, similar gene expression patterns can be associated with different morphologies (Muller & Newman, 2003). Nijhout (1990) concludes that genes do not cause or control morphogenesis; they enable it to take place.

Summarizing we can conclude that ontogenesis and morphogenesis of organisms show that – in addition to DNA – other sources of information, organization and other heredity flows must be present (Kienle & Kiene, 2003; Jablonka & Lamb, 2005).

Criticism concerning an ontological reductionist approach of the organization of organisms

Ontological reductionism also states that there are no higher-level, non-atomistic organizing principles. However, research on topics linked by one common underlying theme, the organization of the elements of organisms in time and place (e.g. self-regulation, immunology, chronobiology, morphology, complexity), questions the tenability of the reductionist view. For example, Müller & Hassel (2003) describe with regard to the research on (the origin and the diversification of) organismal form that the nature of the determinants and rules for the organization of design elements constitutes one of the major unsolved problems in the scientific account of organismal form. The Neo-Darwinian paradigm that still represents the central explanatory framework of evolution can account for the phenomena it concentrates on, namely variation of traits in populations. However, it leaves aside a number of other aspects of evolution, such as the roles of developmental plasticity and epigenesis or of non-standard mechanisms such as assimilation. Most importantly, according to the authors, it completely avoids the origin of phenotypic traits and of organismal form. As to the organization of matter several authors conclude: "It is clear that what the parts (molecules and ions) are doing and the patterns they form are what they are *because* of their incorporation into the system-as-a-whole. In fact, these are patterns within the system in question." (Peacocke, 2003). "The parts would not be behaving as observed if they were not parts of that particular system (the 'whole'). The state of a system-as-a-whole is affecting (i.e., acting like a cause on) what the parts, the constituents, actually do. Many other examples of this kind could be taken from the literature on, for example, self-organizing and dissipative systems." (Peacocke, 2003); "The properties or the behaviour of the parts can be explained only in terms of their function in the whole: they contribute to the adequate functioning, the survival and reproduction of the whole. According to organicists (holists), such *functional explanations* are indispensable in biology. And because they do not occur in physics and chemistry, they form an important argument in favour of biology's autonomy with respect to these other sciences" (Looijen, 1998). On the basis of research in complexity of organisms, Service (1999) concludes that when we get to a certain network complexity, we completely fail to understand how it works.

Criticism concerning an ontological reductionist approach of the psycho-social aspects of man

In a strictly reductionist approach, the various aspects of the psycho-social aspects of man can ultimately be explained by reductionist physico-chemical theories. This so-called issue of physicalism is central to the philosophy of mind and a currently extensively debated topic. In a review Stoljar (2001) states that there are three main arguments against physicalism: (1) the notion of qualia or felt qualities of experience, (2) the problem of intentionality of mental states, and (3) methodological issues. According to many authors (e.g. Chalmers, 1997) the qualia or experience problem is *the* unsolved mystery and *the* argument against physicalism in the philosophy of mind. Being able to discuss this topic only briefly, we shall focus on this main argument: "The notion of qualiaraises puzzles of its own, puzzles having to do with its connection to other notions such as consciousness, introspection, epistemic access, acquaintance, the first-person perspective and so on." When we think and perceive, there is a whir of information processing, but there is also a subjective aspect. "Why is it that when our cognitive systems engage in visual and auditory information processing, we have visual or auditory experiences: the quality of deep blue, the sensation of middle C? How can we explain why there is something it is like to entertain a mental image, or to experience an emotion?" (Chalmers, 2004, p. 619). "But, for example, would purely objective research into colour vision ever have discovered that certain colour combinations are very pleasant, or that some colours appear warm, others cold, or that some people hear coloured sounds?" (Gordon 2004, p. 227). "It is widely agreed that experience arises from a physical basis, but there is no good explanation of why and how it so arises. Why should physical processing give rise to a rich inner life at all? It seems objectively unreasonable that it should, and yet it does" (Chalmers, 2004, p. 619).

Although the physicalism debate has not reached its finale, for the time being we can conclude that there are strong arguments against a strictly reductionist approach towards the various psycho-social aspects of man.

Criticism on reductionist methodology

The golden standard of methodology in reductionist science is the experiment. The experiment is a phase in the so-called empirical cycle in which a hypothesis is tested. The entire empirical cycle includes the phases of: (1) observation, (2) induction, (3) deduction, (4) testing (of the hypothesis by means of the experiment), and (5) evaluation (whether the tested hypothesis based on the experimental results can be rejected or not).

Several authors have criticized (aspects of) the phases of the empirical cycle (e.g. Hume, 1896; Chalmers, 1999; Gloy, 2003; Gordon, 2004) as well as the experiment specifically in its role as the golden standard (e.g. McComas, 1996; Chalmers, 1999; Kiene, 2001). In this article we focus on the problem that the empirical cycle does not fully cover the reality of the scientific process. This is especially true for the *generation of new hypotheses*. Neither induction nor deduction is able to produce new hypotheses (Broeders, 2003). Therefore, already Peirce (1878) introduced the term of abduction, next to induction and deduction. Abduction is to look for a

pattern in a phenomenon, and suggests a hypothesis.

More generally, the studies of for example the great discoveries in science (e.g. Van Der Bie, 2003), creative thinking (e.g. Robertson, 2001), and intuition (e.g. Davis-Floyd & Sven Arvidson, 1997) show that there is always a creative moment in 'producing new insight'. Even the toughest empirical scientist who tries to follow the empirical cycle rigorously must depend on intuitive moments that bring new insight, even if – as an empirical scientist – he is striving towards excluding metaphysical elements. Davidson (2003) describes on the basis of his review that so far no satisfactory explanation has been found for the phenomenon of new insights as 'the sudden realizations of a solution'. In the context of his study on the great discoveries in science, Van Der Bie (2003) describes this phenomenon of new insight as the creative moment in which a new pattern enters man's consciousness that subsequently causes a new ranking order of known facts and observations within the context of this pattern.

In other words, we can conclude that even in reductionist methodology there is a holistic moment in which holistic patterns arise in the researcher's mind that organize the already existing knowledge parts into a new whole.

3.4 A non-atomistic holistic approach

The critical analysis of several aspects of both the reductionist view on matter, its organization, its relationship to psycho-social aspects of man, and some aspects of reductionist methodology with regard to the generation of new hypotheses demonstrates some of the shortcomings of reductionism and opens the gate towards a non-atomistic holistic approach of nature. With this we mean an approach that is also holistic in an ontological sense, thus accepting the status of living organisms as whole beings. Every time we mention holism we mean with this term the non-atomistic interpretation of holism. The acceptance of the existence of whole entities at a specific ontological level implies that we should use a holistic methodology as well. This we can find in pattern detection and pattern recognition, which play an important role in hypothesis generation, creative thinking, and pattern application. This topic of cognitive handling of patterns is also central in another way of gaining and using knowledge – often neglected in the discussion on scientific methods: namely the methodological role of expertise. However, as the methodological use of expert knowledge serves as a holistic methodology that is able to obtain knowledge from the level of the whole, we shall explore the topic of expertise and the holistic methodological role of pattern recognition and pattern application in handling the level of the whole of organisms.

3.4.1 Reflection on expert knowledge, pattern recognition and pattern application

Adequately diagnosing and solving unique complex and context-specific problems can be performed by experts on the basis of so-called tacit knowledge, craftsmanship, the 'clinical look' or 'breeder's eye' (e.g. Snoek, 1993; Glas, 1997; Robertson, 2001). Experienced workers seem to have learned, consciously or unconsciously, to handle prevailing laws and situations, and in doing so have developed self-regulation skills based on valid and practical, useful knowledge ('appropriate conclusions and correct predictions'). Self-regulation can be defined as the adaptive use of skill across changing personal and environmental conditions (Boekaerts *et al.*, 2000). Expert knowledge is represented at an intermediate level of abstraction and is called the 'moderately abstract conceptual representation': a compromise between different abstractions like comparisons in the disciplines of physics and chemistry and concrete specific problems (Zeitz, 1997). The key element of expert information processing is the intuitive recognition and application of a pattern ('Gestalt') (e.g. Van Der Laan, 2006).
Although there are differences in approach, the various scientific explanation models agree that pattern recognition is a process of matching between (e.g. visual) stimuli and information from memory. There are three kinds of theories that try to understand the phenomenon of pattern recognition: (1) template matching theories, (2) feature detection theories, and (3) prototype theories (Lund, 2001). None of these theories, however, is able to explain all phenomena. This is true in particular for the influence that context, expectations and experience have, although many examples indicate that pattern recognition is influenced by these (Robertson, 2001). Lund (2001) describes that the influence of context, expectation and experience seems to be explained best by 'top-down' theories of perception, in which recognition is supposed to take place from the whole ('top') to the parts ('down').

3.4.2 What exactly matches what?

An important next question presenting itself is: What exactly matches what? Two examples will be presented to illustrate this. When somebody sings a melody and subsequently sings it five tones higher, a song is produced in which none of the notes of the first melody returns a second time. It is clear that in both melodies not a single element is the same. Nevertheless, we immediately recognize that exactly the same melody is sung in both cases. Another example concerns the fact that people are able to recognize patterns they learned in a specific domain in *another* domain, which is the case, for example, in many scientific discoveries (Van Der Bie, 2003). The explanation is that – in the recognition of the melody or a pattern in a new domain – what exists *between* the parts or in the specific relationship (e.g. in space and time) between the parts (e.g. the tones in the melody) is recognized. This theory is confirmed by research results concerning the development of self-regulatory skills by experts, in which, as was said earlier, pattern recognition is central.

There are various models describing the stages in the development of self-regulation skills in expertise (Schumacher & Czerwinski, 1992; Glaser, 1996; Boekaerts *et al.*, 2000). Taking together these three models, we can conclude that this development is closely related to the development of expertise, based on the internalization of a model. That model in turn is based on knowledge of systematic connections or relationships of the system. A match occurs between this internal knowledge (in the memory) on the one hand, and on the other hand any specific form in which these relationships appear. At the stage of '*self-regulation*', after all, we are dealing with an 'adaptive use of skill across *changing* personal and environmental conditions'. In other words, we are dealing with the possibility to recognize a universal pattern in whatever specific manifestation (during the knowledge acquisition process) and the application of a universal pattern in whatever specific manifestation (during the knowledge acquisition process).

In conclusion, we may state that both pattern recognition and pattern application require a match between the universal connection in itself on the one hand, and the specifically perceived connection between specifically perceived 'data points' on the other. In philosophical terms, a 'match' occurs between the universal and the specific, in which the universal appears and ensures the connection.

3.4.3 The holistic methodological role of pattern recognition and pattern application in handling the level of the whole of organisms

Goethean phenomenology (Seamon & Zajonc, 1998) is a methodology that studies both the underlying lower levels of organizations (the composing parts and their interactions) and the higher level itself with the aim to arrive at 'correct' knowledge or understanding of the level of the whole of organization. It is a methodology that fulfills the criteria of a scientific method (Baars, 2005). It is based on specifically trained judgement skills of the researcher. By using the phenomenological approach, the factual knowledge obtained through reductionism is placed in a larger perspective. Then, the researcher arrives at the level of wholeness that is responsible for the cohesive organization of the organism in time and place.

Several researchers successfully performed studies that demonstrated levels of wholeness in for example plants (Bockemühl, 1985; Bockemühl & Järvinen, 2005), mammals (Schad, 1971), physiology (Van Tellingen, 2003) and immunology (Van Der Bie, 2006). In all studies it was demonstrated that the level of wholeness determines the relationships between the parts at the underlying lower levels of organizations. By means of pattern recognition the researcher is able to recognize the universal level of wholeness as the organizing principle in whatever specific manifestation at the underlying lower levels of organizations. By means of pattern application the researcher can organize the conditions of lower levels of organizations in such a way that the level of wholeness can serve as the organizing principle of the composing parts in time and place.

3.5 Concluding remarks

A central focus within the thoughts on integrity within (organic) agriculture is formed by the (non-atomistic) holistic concept of the non-reducible wholeness of organisms and by the methodical role of expert knowledge and craftsmanship. The breeder's eye that enables the experienced breeder to catch this level of integrity in understanding and using it in his (breeding) activities, may serve as an example. The non-atomistic concept of integrity states that organisms are self-organizing entities in which an ontological level of wholeness is responsible for the interconnectedness and the balanced harmony of its parts, for its characteristic species-specific nature, for its functional and morphological wholeness, and finally for the balance struck with its species-specific environment. This ontological level of wholeness can be distinguished from the level of the 'parts' of the organism.

In this article our aim was to develop and present a philosophical underpinning of a non-atomistic, holistic concept of integrity of organisms. To that end we drew up critical analyses of several aspects of reductionist ontology and methodology, demonstrating some of the shortcomings of reductionism. For this we were inspired by the ideas of Oost (1999), who states that existing theories may fail:

- on internal conceptual grounds: logical inconsistency, use of ambiguous concepts;
- on external conceptual grounds: a theory "does not fit in" with other theories;
- on empirical grounds: a theory does not fit in with reality.

Reductionism 'does not fit in' with the different theories on the organization of matter but also does not match with several empirical facts concerning the organization of matter. Furthermore, the analyses at both the ontological and the methodological level demonstrated the need for the acceptance of the existence of a level of wholeness that can be distinguished from the level of the parts. Besides this negative evidence for the importance of holistic approaches, positive evidence can be found in what is known about pattern detection, recognition and application. This philosophical underpinning may serve as a first step towards a wider acceptance of this concept of integrity: not only in (organic) agricultural practice, but also in science.

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Part 2

Methodological aspects of curative health promotion

Chapter 4

Development of systems biology-orientated biomarkers by permuted stepwise regression for the monitoring of seasonal allergic rhinitis treatment effects

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Abstract

Background: The immune system, a complex set of integrated responses, often cannot be explained, predicted, or monitored by examining its separate components as biomarkers. Combining different components may therefore be a suitable approach to develop relevant biomarkers reflecting immune system functioning in an appropriate way.

Methods: Here we compute and test pattern variables that should reflect immune system functioning on the systems level. Computation was based on a dataset (from a randomized controlled trial comparing two routes of administration) of allergen-specifically induced expression levels of cytokines (IL-1β, IL-5, IL-10, IL-12, IL-13, IL-17, IFN-γ and TNF-α) and symptom severity scores from 22 seasonal allergic rhinitis (SAR) patients measured before and after six weeks of treatment with medicinal products containing Citrus and Cydonia. By means of stepwise regression analyses we explored and tested pattern variables of the immunological data using permuted stepwise regression (PStR) to distinguish optimally between (immunological) baseline and post-baseline data for the whole treatment group (22 patients) and the two separate treatment groups (11 patients in each group). The validity of the stepwise selection method for the computed pattern variables was tested by means of random permutation tests and evaluated with the cross-validated correct rate of classification (CV correct).

Results: For the total group a pattern variable was computed with three variables: IL-10 (day 7), TNF-α (day 1) and IL-10 (day 1) (CV correct: 0.91; $p < 0.001$; $R^2 = 0.66$), demonstrating a small improvement from the model with IL-10 (day 7) only (CV correct: 0.84; $p < 0.001$; $R^2 = 0.47$). For the subcutaneous injection group a pattern variable was computed with four variables: IL-10 (day 7), IL-10 (day 1), IL-17 (day 7) and IFN-γ (day 7) (CV correct: 0.90; $p < 0.01$; $R^2 = 0.78$), demonstrating a very small improvement from the model with IL-10 (day 7) only (CV correct: 0.86; $p < 0.01$; $R^2 = 0.58$). For the nasal spray group a pattern variable was computed with three variables: IL-10 (day 7), TNF-α (day 1) and IL-10 (day 1) (CV correct: 0.95; $p < 0.01$; $R^2 = 0.79$), demonstrating a moderate improvement from the model with IL-10 (day 7) only (CV correct: 0.79; $p < 0.05$; $R^2 = 0.37$).

Conclusion/discussion: In this study three robust systems biology-orientated biomarkers for the monitoring of SAR were computed that demonstrated small to moderate improvement compared to monitoring of a single cytokine (IL-10 (day 7)) (CV correct improvement: 0.07 (total group), 0.04 (subcutaneous injection group), 0.16 (nasal spray group)). Further computation and biomarker validation with larger datasets, including data from healthy persons and SAR patients, is indicated.

Keywords: biomarkers, cytokines, systems biology, permuted stepwise regression, seasonal allergic rhinitis, Citrus, Cydonia

4.1 Introduction

The immune system is increasingly found to participate in the development of several chronic illnesses including allergy, thus motivating the targeting of the immune system to sustain health at all ages and to modulate the risk of certain chronic illnesses. Biomarker examination is a means of monitoring and validating such immunomodulatory approaches. These biomarkers are generally associated with communicatory molecules including cytokines, chemokines, acute-phase proteins, heat-shock proteins and antibodies. However, consensus on the appropriate markers to monitor the maintenance of a healthy immune system or the improvement of an imbalanced immune system remains elusive, primarily because biological heterogeneity precludes readily identifiable thresholds for the prevention of chronic disease (Albers et al., 2005; Kant, 2010). However, the use of a constellation of markers and proteomics, of which cytokines and cytokine receptors would be a major part, represent potential novel avenues of exploration (Jacobs et al., 2005).

Since we expect no single marker to fully reflect immune status, since the analyses of multiple separate biomarkers can be difficult to interpret, and since most physiological systems are characterized by homeostatic control with large interconnectedness between the elements of the system, combining analyses of various physiologically related biomarkers may prove suitable and efficient. To achieve this goal, analysis methods are used that are generally based on prevailing immunological hypotheses about the role of environmental factors (including allergens) in maintaining health and preventing disease. Alternatively, one can focus on generated applications that provide selected clusters of biomarkers indicative of immune activity and permit objective and quantitative estimates of the effect of immune interventions and can predict associations with particular health outcomes. These (systems biology-orientated) methods usually combine feature selection with repeated sampling to investigate the main relations in the data, like for instance with permuted stepwise regression (PStR) (Andersson et al., 2009, 2011).

In this study we focus on biomarkers for seasonal allergic rhinitis (SAR) or “hay fever”, a type I immediate hypersensitivity reaction mediated by specific IgE antibodies to a seasonal allergen, leading to chronic mucosal inflammation characterized by sneezing, itching, rhinorrhoea, and nasal blockage. An overactive population of allergen-specific Th2-type cells is generally detected in the affected organs - the nose, eyes, and upper respiratory system in SAR, but also in the peripheral blood mononuclear cell fraction (PBMC). The diverse CD4+ helper T cell populations consist of Th0, Th1, Th2, Th3, Th17, and Th22 cells, as well as various subsets of regulatory T cells (Akdis et al., 2004; Akdis and Akdis, 2009; Bonilla and Oettgen, 2010). These T cell subsets are identified based on the production of signature cytokines after exposure to the specific allergen, such as interferon-gamma (IFN-γ) from Th1 cells, interleukin (IL)-5 and IL-13 from Th2 cells, and IL-10 from regulatory T cells (Treg). The differentiation of Th1 cells is induced after activation of monocytes as antigen-presenting cells, subsequently producing IL-12p70 as the active cytokine. The generation of Th2 cells is dependent upon other environmental factors and various cytokines, including IL-10 itself (Fujimura and Okamoto, 2010; Chaplin, 2010).

Cytokine genes are only expressed after activation and full differentiation of the T cells, and

this production is highly variable among humans, partly due to an extensive degree of polymorphism in the cytokine genes. Therefore, in order to establish the effects of interventions, it is our hypothesis that it is critical to compare sets or patterns of cytokines rather than absolute levels of cytokine production in stimulated, cultured PBMCs with full differentiation of T cell subsets.

Here we demonstrate the development and testing of a set of pattern variables for monitoring intervention effects in SAR patients. At first we explored and tested pattern variables of the immunological data using permuted stepwise regression (PStR) to distinguish optimally between (immunological) baseline and post-baseline data. PStR is a technique for building statistical models automatically, by selecting variables from a pre-defined set of candidate variables and testing the significance of the complete selection procedure with random permutation (Andersson et al., 2009, 2011). Computation was based on a dataset (from a randomized controlled trial comparing two routes of administration) of allergen-stimulated PBMC culture supernatant levels of cytokines (IL-1β, IL-5, IL-10, IL-12, IL-13, IL-17, IFN-γ, and TNF-α) from 22 seasonal allergic rhinitis (SAR) patients measured before and after six weeks of treatment with either Citrus/Cydonia comp.® (compositum) 1% solution for injection or Gencydo® nasal spray (Baars et al., 2011).

The use of systems biology-orientated pattern variables should improve the separate biomarker approach in monitoring health related changes as a result of the effect of immune interventions. Therefore the following hypotheses were tested in this study:

1. Even in a homogeneous group of SAR patients the individual production of cytokines is highly variable among humans, partly due to an extensive degree of polymorphism in the cytokine genes. This high variability will result in a broad range of cytokine production measurements and a non-normally distributed dataset.
2. The computed (data-driven) pattern variables demonstrate larger cross-validated correct rate of classification (CV correct) values (Hellgren et al., 2008) than the separate cytokines with regard to the classification of cytokine samples in baseline and post-baseline.
3. The computed pattern variables demonstrate statistically significant baseline to post-baseline changes.

4.2 Materials and methods

4.2.1 Dataset

Our dataset derived from a randomized, controlled trial with 22 SAR patients (Baars et al., 2011) that studied the efficacy and safety of Citrus/Cydonia comp.® 1% solution of injection or Gencydo® nasal spray for the treatment of SAR. The two medicinal products are identical in their composition; the objective of the trial was identification of the more favorable route of adminis-

Table 4.1. Baseline characteristics for the two treatment groups

Variable		*Citrus/Cydonia comp. (n = 11)*	*Gencydo (n = 11)*	*p-value*
Sex: Number (percentage)	Male Female	6 (55%) 5 (45%)	2 (18%) 9 (82%)	ns
Age (years) (sd)		37.0 (13.1)	37.6 (10.5)	ns
Height (cm) (sd)		176.3 (9.6)	170.6 (7.4)	ns
Weight (kg) (sd)		69.5 (10.6)	66.4 (8.9)	ns
Smokers: number (percentage)		2 (18%)	1 (9%)	ns
Alcohol consumption: number (percentage)	None Occasionally Regularly	0 (0%) 10 (91%) 1 (9%)	3 (27.3%) 7 (63.6%) 1 (9%)	ns
Ethnic origin: number (percentage)	Caucasian Asian	11 (100%) 0 (0%)	9 (82%) 2 (18%)	ns
Blood pressure at screening (mmHg) (sd)		119 (24)/ 73 (14)	104 (16)/ 72 (9)	ns/ns
Heart rate at screening (beats per minute)		72 (10)	70 (6)	ns
RAST grass pollen		3.8 (1.3)	3.9 (1.1)	ns
RAST birch pollen		1.9 (1.8)	2.3 (2.1)	ns
Usual SAR symptom severity during the pollen season (total score anamnestically) (sd)	Sneezing Itching nose Watery nasal discharge Total score	1.9 (0.5) 1.8 (0.8) 1.9 (0.9) 5.6 (1.9)	2.3 (0.5) 2.1 (0.5) 2.1 (0.5) 6.5 (1.2)	ns
SAR symptom severity scores in the morning during the wash-out period (total score) (sd)		6.6 (4.5)	8.0 (4.6)	ns
SAR symptom severity scores in the evening during the wash-out period (total score) (sd)		6.1 (3.7)	9.7 (5.2)	ns

ns = not significant

tration (subcutaneous injections versus nasal spray). The trial was a national (The Netherlands), stratified (age: 18-40 years or 41-60 years; and radioallergosorbent testing (RAST) scores (Quillen and Feller, 2006) for birch pollen: > 2 or < 3; with a balanced randomization for age and RAST scores), comparative, single-blind laboratory clinical trial with two parallel groups with 11 patients in each group. All eligible patients were recruited from a single center, the Louis Bolk Institute in Driebergen, NL. The first patient was enrolled on May 19, 2009 and the last patient completed the study on August 11, 2009.

4.2.2 Pollen counts

Pollen counts were acquired on a daily basis for grass pollen and birch pollen from the Leiden University Medical Centre (http://www.lumc.nl/con/1070/85683/105795/105824/) during both the wash-out period and the treatment period.

4.2.3 Patients

Eligible participants were all adults, aged 18 to 60 years, suffering from SAR for at least two years, with a RAST for grass pollen ≥ 2, and suffering from sneezing, itching nose, and watery nasal discharge, with a severity score of at least two of the three symptoms ≥ 2 (ranging from 0 = not present to 3 = severe) and requiring the use of antihistamines and/or corticosteroids for treatment of SAR symptoms for at least the preceding two years. Exclusion criteria were chronic inflammatory autoimmune diseases, allergy (hypersensitive) to one of the constituents of Citrus/Cydonia comp.® or Gencydo® nasal spray, pharmacological treatment of allergic rhinitis or use of other preparations containing Citrus and/or Cydonia extracts within the two weeks prior to study enrollment, use of cromoglycates in the last month before study onset, pregnancy or lactation, and severe internal or systemic disease. We also excluded patients with concomitant pharmacological treatment for SAR such as antihistamines, corticosteroids, or other preparations and patients who participated in another clinical trial simultaneously or within the four weeks prior to enrollment in this study.

Baseline homogeneity of the treatment groups was accomplished with regard to the following SAR related aspects: RAST scores (grass pollen and birch pollen), worst SAR symptom severity during the previous pollen season (anamnestically), SAR symptom severity scores in the morning and the evening during the wash-out period and onset of interventions (data not shown). Homogeneity of the treatment groups was accomplished with regard to the following SAR non-related aspects: sex, age, height, weight, smoking status, ethnic origin, remaining medical history, prior medication, vital signs and physical examination (Table 4.1).

4.2.4 Treatment

After a one-week (for patients that had not been treated for SAR in the week before enrollment) or a two-week (for patients that had been treated for SAR in the week before enrollment) wash-out period, patients were assigned to four strata and then randomized to a six-week treatment period. A randomization list was generated with the Random Allocation Software Program version 1.0 (Saghaei, Isfahan University of Medical Sciences, Iran) using a random block size of two in order to guarantee a balanced allocation.

Patients received the treatment in accordance with the Summary of Product Characteristics; either Citrus/Cydonia comp. 1% subcutaneous injections (1 mL ampoules, available under the trade name Gencydo® 1%, manufacturer Weleda AG, Schwäbisch Gmünd, Germany) twice per week, or the Gencydo nasal spray (available under the name "Gencydo neusspray" in the Netherlands, manufactured by Weleda AG, Schwäbisch Gmünd, Germany) four times per day (1-2 sprays in each nostril). This strategy resulted in the nasal spray group receiving four times the active dose compared with the injection group. Citrus/Cydonia comp. 1% solution for injection and Gencydo nasal spray are medicinal products that contain identical amounts of lemon juice (*Citrus limon*) and aqueous extract from the fruit of a quince (*Cydonia oblonga*). One milliliter of these preparations contains 8-12 mg *C. limon* juice corresponding to 0.65 mg fruit acid, calculated as citric acid, and 30 mg *C. oblonga* aqueous extract (drug-extraction-rate 1:2.1).

For more than eighty years, Citrus/Cydonia comp. has been prescribed for SAR patients. Clinical experiences (De Bruin and Baars, 2001), three *in vitro* studies (Baars and Savelkoul, 2008; Gründemann et al., 2010), two cohort studies (Baars and De Bruin, 2005; Rother and Oexle, 2008), and a randomized controlled trial (Baars et al., 2011) support the efficacy and safety of Citrus/Cydonia comp. and Gencydo, respectively, in SAR treatment.

4.2.5 Efficacy variables

Primary efficacy variables: Cytokine analyses

Primary endpoints were SAR-related changes in immunological parameters between the start of the treatment (baseline) and after six weeks of treatment (post-baseline). From each patient 8 mL of peripheral blood were collected; PBMCs were isolated. PBMCs were cultured in Yssel's medium at 37ºC in a humidified atmosphere with 5% CO2 at a density of 1x106 viable cells/mL. Cells were plated out in 48 well plates at a concentration of 1x106 cells/mL and cultured at 37ºC. After five hours, in which the cells adapted to the culture conditions, various stimuli or a matching volume of medium were added. Cultures were stimulated polyclonally with 150 ng/mL anti-CD3 plus 100 ng/mL anti-CD28 monoclonal antibodies (BD Pharmingen, San Diego, CA, USA) or cultured in medium only (Jeurink et al., 2008). In addition, we performed allergen-specific stimulation of 106 cells/mL in 1 mL cultures with applied pollen extract (Phl p 1 from Timothy grass [Phleum

pratense]; Biomay Vienna, Austria; 10 μg/mL in medium). The proliferation capacity, cell survival, toxicity, and total production capacity of several cytokines in the culture supernatants of the PBMCs were analyzed at day 1 (IL-1β, IL-10, IL-12, IL-17, IFN-γ, and TNF-α: demonstrating the reaction of the innate immune system) and at day 7 (IL-5, IL-10, IL-12, IL-13, IL-17, and IFN-γ: demonstrating the reactions of specialized T cell subsets) (Akdis et al., 2004; Chaplin, 2010) essentially according to well-established procedures (Jeurink et al., 2008). Examples of the reasons to choose these specific cytokines to monitor immunological SAR treatment effect (Ozdemir et al., 2009; Deraz, 2010) (for more detailed information, see: Baars et al., 2011) are:

1. (Grass pollen stimulated minus medium stimulated) IL-10 and TNF-α at day 1: representing the inflammatory state of the SAR-related immune system
2. (Grass pollen stimulated minus medium stimulated) IL-10 at day 7: representing (regulatory) T cells (Tregs)
3. (Grass pollen stimulated minus medium stimulated) IFN-γ at days 7: representing Th1 activity
4. (Grass pollen stimulated minus medium stimulated) IL-1β at day 1; IL-5 and IL-13 at day 7: representing Th2 activity

Cytokine production was detected with the Cytometric Bead Array (CBA, BD Biosciences, San Diego, CA, USA). All buffers used in this protocol were obtained from the BD CBA Soluble Protein Master Buffer Kit (BD Pharmingen, San Diego, CA, USA) and the procedure was performed according to the manufacturer's protocol. The detection limits were 1.1 pg/mL for IL-1β, 2.2 pg/mL for IL-12p70, 0.3 pg/mL for IFN-γ, 0.7 pg/mL for TNF-α, 1.1 pg/mL for IL-5, 0.13 pg/mL for IL-10, 0.6 pg/mL for IL-13, and 0.3 pg/mL for IL-17. Levels below the detection level could not be measured reliably, but still might have exerted a biological effect. Nevertheless for the statistical analyses of the data a real value needed to be entered. For that reason a value of "0" was selected for all production levels below the detection level. The samples were measured on the FACSCanto II, using Flow Cytometry Analysis (FCAP) software (BD Biosciences, San Diego, CA, USA).

Secondary efficacy variables: Symptom severity

Secondary efficacy variables were the change in nasal and non-nasal allergic rhinitis symptom severity before treatment start and after each week of treatment. The severity of nasal symptoms (nasal obstruction, itching nose, sneezing, and watery nasal discharge) and non-nasal symptoms (itchy/burning eyes, watery eyes, redness of eyes, and itching ears/throat) were recorded twice per day (in the mornings and evenings) by the patient. The disease-specific severity questionnaire was provided to the patient as an online questionnaire in Dutch: 0 = no symptom, 1 = mild, 2 = moderate, and 3 = severe. Completion of the online questionnaires by the participants was checked daily. Total symptom scores (TSS) were analyzed during wash-out, and in treatment weeks 1-5. Due to a very low pollen count during week 6, the data from week 6 were excluded from the analyses (for more detailed information see: Baars et al., 2011). Missing values were replaced in two ways, mean week scores and Last Observation Carried Forward (Waladkhani, 2008), and subsequently compared.

4.2.6 Statistical analysis

Software

Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, USA) and with Matlab version 7.11.0 (R2010b, The Mathworks, Natick, MA, USA).

Statistical analyses of the primary efficacy variables: Cytokine analyses

To test the effectiveness of Citrus/Cydonia comp. 1% solution for injection as compared to Gencydo nasal spray with respect to the primary target variables, descriptive statistics, Student's t-tests, and non-parametric Wilcoxon Signed Ranks tests were performed to compare means and mean base-10 log transformed scores of the immunological parameters in both groups.

In order to demonstrate the high variability of the cytokine production levels in homogenous groups means, ranges and skewness ratios of all cytokines were analyzed. A skewness ratio (statistic/ Standard Error) < 2 or >2 was regarded as a non-normal distribution.

Statistical analyses of the secondary efficacy variables: Symptom severity

Mean scores and standard deviations were calculated for each week. Then, Student's *t*-tests, non-parametric Wilcoxon Signed Ranks tests, and multivariate analysis techniques were used to compare the symptom severity mean week scores in the subcutaneous and the nasal spray group for each of the five weeks.

Computation of pattern variables by means of permuted stepwise regression (PStR)

To identify potential pattern variable biomarkers of interest, immunological samples from all 22 SAR patients were analyzed at baseline and after 6 weeks of treatment. In addition, baseline to post-baseline changes were separately analyzed for the two treatment groups (Gencydo nasal spray and Citrus/Cydonia comp. 1% solution for injection) .

The permuted stepwise regression procedure as described in Andersson et al. (2011) was applied on immunological data. Stepwise regressions with five different methods (forward stepwise selection (StepwiseForwardP) and stepwise selection (StepwiseP) using fine tuning with lower p-values to select variables for inclusion in the model (Draper and Smith, 1998), least absolute shrinkage and selection operator (Lasso) (Efron et al., 2004; Tibshirani, 1996), a rescaled version of Lasso (ConeLasso) and orthogonal matching pursuit (OMP) (Mallat and Zhang, 1993)) were computed to find subsets of cytokines that permitted the classification of cytokine samples in baseline and post-baseline. Sets of potential regression models were generated using a minimum of one and a maximum of nine cytokines. Final selection of reliable subsets of explanatory cytokines was based on random permutation tests with permuted subjects and repeated measures permuted within subjects. Random permutation tests were performed on the complete stepwise regression procedure including the selection of subsets of cytokines based on the highest R^2. For each maximum number of cytokines, we tested for 9999 permutations whether the permuted R^2 was equal to or above

90% of the calculated true R^2. In other words, we assessed if there was a significant gap (10%) between the calculated true R^2 and the distribution of all permuted R^2s. Random permutation tests resulting in a p-value below 0.05 were considered significant. The cross-validated correct rate of classification, CV correct, was computed with 5-fold cross-validation averaged over 20 replication

4.3 Theory

4.3.1 Computation of systems biology-orientated biomarkers

Systems biology aims at a more holistic understanding of biology (Chong and Ray, 2002), a systems-level understanding. This objective requires an examination of the structure and dynamics of function in cells and organisms, rather than the characterization of isolated parts of a cell or organism (Kitano, 2002).

In order to develop systems biology-orientated biomarkers capable of monitoring health and disease-specific changes at the systems level, one can compute pattern variables that are either (more) data-driven or (more) hypothesis-driven. Computation of data-driven patterns variables investigates the relationships among all measured immunological components of the studied disease. Computation of hypothesis-driven patterns variables is based on investigating the relationships among a subset of previously, theoretically identified and relevant immunological components of the studied disease.

4.4 Results

4.4.1 Raw immunological data set of a homogeneous group of SAR patients demonstrating a broad range of cytokine production and a non-normal distribution

The hypothesis tested here was that even in a homogeneous group of SAR patients (see 2.3) the individual production of cytokines is highly variable among humans, partly due to an extensive degree of polymorphism in the cytokine genes as a potential, but likely, explanation. This high variability will result in a broad range of cytokine production measurements and a non-normally distributed dataset. Tables 4.2 and 4.3 show the baseline and post-baseline cytokine pro-

Table 4.2. Baseline and post-baseline measurements of cytokine production levels of separate cytokines demonstrating broad ranges and non-normal distributions of data Gencydo group)

	Baseline			*Post-baseline*		
Variable	*Mean (SD)*	*Range*	*Skewness (statistic/ SE)*	*Mean (SD)*	*Range*	*Skewness (statistic/ SE)*
Day 1: innate immunity						
IL-1β#	42 (106.5)	1-362	0.65	70.7 (181.0)	2-615	4.48*
IL-10	79.4 (51.4)	11-187	1.17	62.8 (41.5)	12-136	1.98
IL-12	0.2 (0.5)	0-2	n.a.	0	0	n.a.
IL-17	0.4 (1.0)	0-3	5.02*	0.2 (0.8)	0-3	n.a.
IFN-γ	1.5 (2.4)	0-7	4.58*	2.8 (4.9)	0-15	4.97*
TNF-α	43.4 (46.9)	8.1-170.6	2.02*	48.1 (49.8)	3.8-156.3	3.96*
Day 7: specific T cell subsets						
IL-10	314.8 (250.7)	61.0-890.2	3.87*	172.3 (133.3)	54.4-500.9	3.29*
IL-12	0.6 (0.9)	0-2.5	0.12	1.3 (1.7)	0-5.3	1.40
IFN-γ	2,010.4 (2,361.8)	112.3-6,578.3	1.73	1,089.1 (1,231.8)	52.8-3,345.6	3.69*
IL-5	301.7 (257.3)	12.7-881.2	2.65*	205.4 (126.8)	43.0-450.3	1.79
IL-13	271.6 (281.9)	32.3-912.2	4.78*	176.3 (180.8)	30.7-656.7	3.40*
IL-17	168.4 (150.0)	4.4-483.7	2.08*	200.7 (265.6)	3.5-785.5	1.41

all cytokine measurements are in pg/mL
* Skewness ratio (statistic/SE) < -2 or > 2
SD = standard deviation
SE = Standard error of skewness
n.a. = Not applicable

Table 4.3. Baseline and post-baseline measurements of cytokine production levels of separate cytokines demonstrating broad ranges and non-normal distributions of data (Citrus/Cydonia comp. group)

	Baseline			*Post-baseline*		
Variable	*Mean (SD)*	*Range*	*Skewness (statistic/ SE)*	*Mean (SD)*	*Range*	*Skewness (statistic/ SE)*
Day 1: innate immunity						
IL-1β#	6.5 (3.0)	2-12	4.96*	146.9 (319.9)	2-1079	4.96*
IL-10	113.1 (80.9)	16-252	1.50	122.2 (90.3)	34-330	0.70
IL-12	0	0	n.a.	0.2 (0.4)	0-1	0
IL-17	0.3 (1.0)	0-3	3.93*	0	0	n.a.
IFN-γ	1.0 (2.7)	0-9	2.25*	6.4 (19.8)	0-66	3.06*
TNF-α	51.9 (38.9)	15.7-136.2	3.48*	28.0 (33.4)	4.2-122.2	2.23*
Day 7: specific T cell subsets						
IL-10	252.3 (291.1)	34.5-1,066.8	1.85	297.1 (275.6)	82.6-1,024.5	2.44*
IL-12	1.2 (1.2)	0-2.7	1.89	1.1 (1.2)	0-3.7	1.81
IFN-γ	2,428.4 (2,458.4)	124.2-7,371.9	1,93	1,722.3 (1,206.9)	635.7-5,076.9	1.72
IL-5	455.4 (518.4)	39.4-1,747.4	1.80	329.3 (219.0)	75.0-797.9	0.87
IL-13	706.9 (1,598.8)	16.2-5,458.9	2.26*	412.6 (573.6)	34.7-1,934.0	3.17*
IL-17	61.6 (65.7)	2.7-206.5	1.21	152.6 (144.7)	12.4-433.3	2.15*

all cytokine measurements are in pg/mL
* Skewness ratio (statistic/SE) < -2 or > 2
SD = standard deviation
SE = Standard error of skewness

Table 4.4. The computation of an optimal pattern variable model by means of a permuted stepwise regression (PStR) on all cytokines for the total group

N	R^2	*P-value*	*Method*	*CV correct*	*N of var.*	*Variables in the model*			*Regression weights of variables*			*Intercept*
44	0.47	0.0002	CL	0.84	1	IL10d7			-0.85			2.21
44	0.58	0.0002	StFP	0.88	2	IL10d7	IL10d1		-1.07	0.53		1.67
44	0.56	0.0003	L	0.87	2	IL10d7	TNFd1		-0.89	-0.37		2.83
44	0.66	0.0002	OMP	0.91	3	IL10d7	TNFd1	IL10d1	-1.10	-0.37	0.52	2.29

CL = ConeLasso
L = Lasso
StFP = Stepwise Forward Procedure
OMP = Orthogonal Matching Pursuit

Table 4.5. The computation of an optimal pattern variable model by means of a permuted stepwise regression (PStR) on all cytokines for the Citrus/Cydonia group

N	R^2	*P-value*	*Method*	*CV correct*	*N of var.*	*Variables in the model*				*Regression weights of variables*				*Intercept*
22	0.58	0.0064	L	0.86	1	IL10d7				-0.86				2.23
22	0.66	0.0083	L	0.85	2	IL10d7	IL10d1			-1.05	0.50			1.76
22	0.74	0.0063	L	0.87	2	IL10d7	IL10d1	IL17d7		-1.22	0.60	0.23		1.49
22	0.78	0.0085	L	0.9	3	IL10d7	IL10d1	IL17d7	IFN-G d7	-1.22	0.76	0.26	-0.21	1.75

L = Lasso

Table 4.6. The computation of an optimal pattern variable model by means of a permuted stepwise regression (PStR) on all cytokines for the Gencydo group

N	R^2	*P-value*	*Method*	*CV correct*	*N of var.*	*Variables in the model*			*Regression weights of variables*			*Intercept*
22	0.37	0.0133	StFP	0.79	1	IL10d7			-0.85			2.19
22	0.59	0.0012	StFP	0.86	2	IL10d7	TNFd1		-0.85	-0.60		3.06
22	0.79	0.0018	StFP	0.95	2	IL10d7	TNFd1	IL10d1	-1.21	-0.64	0.80	2.26

CL = ConeLasso
L = Lasso
StFP = Stepwise Forward Procedure
OMP = Orthogonal Matching Pursuit

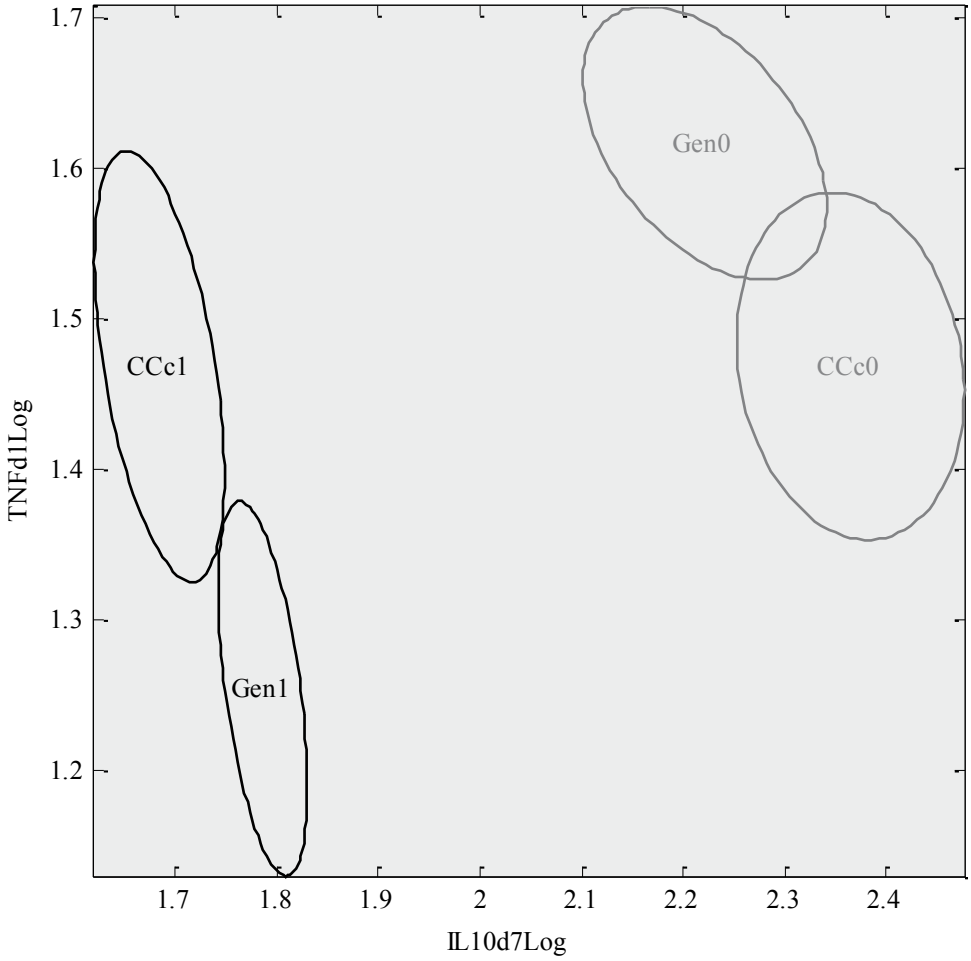

Figure 4.1. The additional effect of TNF-α for the Gencydo group but not for the Citrus/Cydonia comp. group with regard to the classification of cytokine samples in baseline and post-baseline as compared to the effect of IL-10 for both groups; CCc = Citrus/Cydonia comp.; Gen = Gencydo; 0 = baseline; 1 = post-baseline

duction levels of the patients included in the SAR study. The tested hypothesis was confirmed, since more than 55.8% (24/43) of the cytokine data were not normally distributed, and the broad range of all cytokine measurements in both treatment groups demonstrated the large variations from subject to subject. The non-normal distribution of the dataset implied the necessity of base-10 log transformation of all data.

4.4.2 The computation of pattern variables using PStR that would demonstrate larger CV correct values than the separate cytokines and statistically significant baseline to post-baseline changes

It was hypothesized that we were able to compute (data-driven) pattern variables that would demonstrate larger CV correct values than the separate cytokines with regard to the classification of cytokine samples in baseline and post-baseline. In addition it was hypothesized that these pattern variables would demonstrate statistically significant baseline to post-baseline changes. After base log 10 transformation of all cytokine data, we performed a permuted stepwise regression (PStR) on all cytokines for the total group, the Gencydo group and the Citrus/Cydonia group with all five different regression approaches. Only the statistically significant results are presented here.

For the total group a pattern variable was computed with three variables: IL-10 (day 7), TNF-α (day 1) and IL-10 (day 1) (CV correct: 0.91; $p < 0.001$; $R2 = 0.66$) (Table 4.4, 4th row: N of variables = 3), demonstrating a small improvement compared to the model with IL-10 (day 7) only (CV correct: 0.84; $p < 0.001$; $R2 = 0.47$) (Table 4.4, 1st row: N of variables = 1).

For the subcutaneous injection group a pattern variable was computed with four variables: IL-10 (day 7), IL-10 (day 1), IL-17 (day 7) and IFN-γ (day 7) (CV correct: 0.90; $p < 0.01$; $R2 = 0.78$) (Table 4.5, 4th row: N of variables = 4), demonstrating a very small improvement compared to the model with IL-10 (day 7) only (CV correct: 0.86; $p < 0.01$; $R2 = 0.58$) (Table 4.5, 1st row: N of variables = 1). For the nasal spray group a pattern variable was computed with three variables: IL-10 (day 7), TNF-α (day 1) and IL-10 (day 1) (CV correct: 0.95; $p < 0.01$; $R2 = 0.79$)) (Table 4.6, 3rd row: N of variables = 3), demonstrating a moderate improvement compared to the model with IL-10 (day 7) only (CV correct: 0.79; $p < 0.05$; $R2 = 0.37$) (Table 4.6, 1st row: N of variables = 1). The random permutation tests for the three pattern variables resulted in p-values of 0.0002 (total group), 0.0085 (Citrus/ Cydonia comp.) and 0.0018 (Gencydo), demonstrating valid, robust prediction models with significant differences between baseline and post-baseline.

Figure 4.1 demonstrates the importance of TNF-α (day 1) in addition to IL-10 (day 7) for the classification of cytokine samples in baseline and post-baseline, for the Gencydo group but not for the Citrus/Cydonia comp. group. For both groups there is a significant difference between baseline (0) and post-baseline (1) for IL-10 (day 7) (horizontal level of Figure 4.1). For TNF-alpha (vertical level of Figure 4.1) this was only the case for the Gencydo group.

The tested second and third hypotheses were confirmed, since we were able to compute three robust statistically significant pattern variables that demonstrated larger CV correct values than

the separate cytokines with regard to the classification of cytokine samples in baseline and post-baseline. The computed pattern variables demonstrated also statistically significant baseline to post-baseline changes.

4.4.3 Summary of the results of the secondary efficacy variables symptom severity and safety

Both routes of administration demonstrate a statistically significant reduction in SAR symptom severity, with larger effects of the subcutaneous route of administration. In the Citrus/Cydonia group a statistically significant SAR symptom reduction was measured already after one week and two weeks of treatment, in the morning and the evening, respectively. In the Gencydo group a statistically significant SAR symptom reduction was measured already after two weeks of treatment in the evening. TSS reduction of the subcutaneous route of administration was larger in the morning, but not in the evening. Cohen's delta effect sizes were larger for the subcutaneous route of administration (= large) than the nasal spray route of administration (= medium), both in the morning and the evening. During the treatment period, a total of 9 adverse events (AEs) were observed with none of the AEs classified as serious. Also the *in vitro* analyses demonstrated acceptable cell survival, with no signs of toxicity. The overall conclusion of the safety analysis in this study is that both routes of administration of Gencydo and Citrus/Cydonia comp. are safe for use by SAR patients (for more detailed information about the secondary efficacy variables, see: Baars et al., 2011).

4.5 Discussion

In this study at first we confirmed the problem of high inter-individual variability of cytokine production among humans for the use of separate cytokines as biomarkers. Even in this study with a controlled, homogeneous group of SAR patients, the individual production of cytokines was highly variable. This wide individual biological variability in cytokine production levels may arise from factors such as circadian rhythm, sex, age, and smoking status (De Roos & MacArdle, 2008; Duramad et al., 2004). Alternatively, the observed inter-individual variability might be partly due to an extensive degree of polymorphism in the cytokine (receptor) genes as a potential, but likely, explanation (Kubistova et al., 2006). Therefore three robust, systems biology-oriented, pattern variable biomarkers for the monitoring of SAR were computed, that demonstrated small to moderate improvement in the classification of cytokine samples in baseline and post-baseline compared to the monitoring of the separate cytokine (IL-10 (day 7)) (CV correct improvement: 0.07 (total group), 0.04 (Citrus/Cydonia group), 0.16 (Gencydo group)). This study therefore provides a new and solid strategy in the field of immunology for the development of objectively quantifiable, high-

quality biomarkers that are relevant to the analysis of potential activity of new therapeutics for the treatment of allergic diseases. The procedure that is successfully used in other new scientific areas like for example proteomics (Kim et al., 2010), is expected to be widely applicable in immune-mediated diseases.

Currently, in immunological research practice, multiple cytokines in particular are often measured in relation to immune responses under diseased conditions or in response to treatments. However, the interpretation of these observed data is most often subjective and based on the relative importance of some, but not all, different cytokines. This obscures the validity of the observed immune responses and compromises the comparison of different studies in literature. The presented approach in this study provides an improvement for this situation by offering an objectively handling of the *complete* cytokine response and making the data available for objective and standardized analysis in patients.

The presented approach is also an improvement for statistical procedures that analyze several predictive variables at the same time and by doing that, provide more information than by analyzing separate variables. Like in proteomics, genomics, metabolomics, etcetera, extracting useful multivariate relations from measured data is not so straightforward and an ongoing learning process. We think that this relative new methodology, combining stepwise regressions with permutation tests, can offer valuable insights in immunological multivariate relations. With respect to the stepwise regressions relationships among multiple variables predicting a response can be modeled additive in a linear or multiplicative way. The resulting regression weights define the optimal balance between the predictive variables for obtaining the most accurate prediction. Imagine an example with two predictors where the difference between the variables or the product of the variables (the interaction) is related to the response. The specific additive or multiplicative relation can be found by applying respectively linear or loglinear regression with the two predictors. One can intuitively understand that the relations found in regression models can compensate for the high variability in the predictors by realizing that the predicted values are proportional to the weighted mean of the predictors with weights fixed to the regression weights. As long as there is no overfitting the weighted mean will have less variability than the separate predictors. In the methods presented here the permutation tests monitor the robustness of the stepwise regressions and prevent overfitting.

The results provide also relevant information for the comparison of the immunological effects of the nasal (Gencydo) and the subcutaneous (Citrus/Cydonia comp.) route of administration of these effective treatments for hay fever. For both groups the most important effects come from IL-10 (day 7), which reflect modulation of differentiated Treg activity. However, for the nasal spray route of administration in addition TNF-α (day 1) and IL-10 (day 1) contribute to the treatment effect (CV correct improvement of 0.16). The production level of monocyte-derived TNF-α and IL-10 reflects the local chronic SAR-related inflammatory activity between baseline and post-baseline In addition, the observed kinetics at day 7 after allergen-specific stimulation, reflects the activation state of the immune system due to the activity of monocytes, which are the largest producers of IL-10 in the PBMC, and induced already by day 1 after allergen exposure. Subsequently, also the gradual and delayed induction of regulatory T cell subset (Treg) by day 7 will be inhibited as these

cells use the IL-10 as a selective autocrine growth factor (Akdis et al., 2004; Akdis and Akdis, 2009; Kant, 2010). The observed kinetics can be interpreted as a decrease in the activation state of the immune system due to a decrease in the activity of monocytes and a reduction of the chronic inflammation (TNF-α (day 1)). This additional effect can be attributed to the acute local effect of the nasal spray route (for more detailed information, see: Baars et al., 2011).

There are two major theoretical arguments for an increasing focus on a pattern-oriented or a systems biology approach for the development of valid immune system biomarkers. First, focusing on the pattern of relationships overcomes the large inter-individual variations in cytokine production. Since these relationships, other than the calculated mean scores of separate immunological components, are independent of the absolute magnitude of the component's production, individual changes can be compared more precisely. Second, studying the systems-level changes in the relationships among separate immunological components accounts for the observation that changes in the production of one immunological component can lead to compensatory changes in others (Atkinson et al., 2001).

The systems biology-oriented approach for developing biomarkers may also innovate outcome studies in SAR treatment (Kitano, 2002). Until now, in designing SAR outcome studies, clinical symptoms have been regarded as primary efficacy parameters, and immunological parameters are relegated to a secondary status (EMEA, 2002); indeed, measurements of separate immunological parameters are often unreliable. The systems biology-oriented pattern variables proposed in this study offer a first step toward reliable biomarkers of immune activity that could be used in SAR outcome research. Similar approaches could result in the development of reliable immune system biomarkers for use in different studies and different diseases. Additionally, our results support a holistic, systems-biology view of the immune system in terms of practical application.

The major limitations of this study are the small group sizes (11 patients in each group) and the absence of data from healthy persons and from categories of SAR patients with varying symptom severities. The small group sizes may have resulted in imprecise computation of the pattern variables, since larger samples provide more precise calculations. The absence of data from healthy persons and categories of SAR patients prevented to study in a more controlled setting the differences between health and (levels of) disease. We expect that computation with a larger and better dataset will provide larger differences between results based on computing pattern variables and results based on analyzing only separate variables. The presented study must therefore be regarded as a first study exploring the possible contribution of permuted stepwise regression approaches to the development of pattern variables that reflect immunological activity on the system level, with promising results.

Future (replication) studies on the applicability of this PStR procedure to SAR are indicated with larger populations, with categories of SAR patients with varying symptom severities, and with a control group of healthy persons. These study design changes will allow the computation of pattern variables that are able to distinguish among disease severities and between disease and health more precisely. This PStR procedure may also be applicable to other immune-mediated diseases; datasets describing rich cytokine sets will determine the critical parameters that can be

combined in the various pattern variables.

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PART 3

Empirical studies on the efficacy and safety of the curative health promotion treatment of seasonal allergic rhinitis with Citrus/Cydonia comp.

Chapter 5

Citrus/Cydonia Comp. for Seasonal Allergic Rhinitis

Use in General Practice

Practice-based evidence from a survey among anthroposophic doctors*

E.W. Baars & A. de Bruin

*** This is a summary and arrangement from the publication by A. de Bruin & E. Baars (2001), "Citrus/Cydonia comp. use in general practice. A survey among anthroposophic physicians. Driebergen: Louis Bolk Instituut" for use in this thesis.**

Abstract

Background: In order to become currently accepted in conventional medicine, CAM streams have to develop practice-based evidence and subsequently evidence-based practices.

Objective: To obtain an overview of the experiences of Dutch Citrus/Cydonia comp. prescribing anthroposophic doctors with the treatment of seasonal allergic rhinitis (SAR). This provides the first practice-based evidence on anthroposophic SAR treatment.

Methods: A survey was sent to 80 doctors of the Dutch Association of Anthroposophic Physicians (NVAA). After two weeks a reminder was sent to the non-responders.

Results: The subcutaneous route of administration of Citrus/Cydonia comp. is the most favoured, with the nasal spray route of administration coming a close second. There is much variation between doctors in the way they prescribe Citrus/Cydonia comp. regarding the period of the year, the concentration, and frequency of the prescribed drug. Doctors combined the use of Citrus/Cydonia comp. with other medicinal or non-medicinal therapies for a large group of their patients. More than 50 percent of the doctors expect an effect within two weeks, and 94 percent of the doctors expect to see an effect within three months after treatment onset. One-third of the doctors indicated that they had sometimes observed side effects on administration of Citrus/Cydonia comp. (most often pain (due to the injection or injection fluid) or local skin reactions).

Conclusion/ Discussion: There is first practice-based evidence based on the expert knowledge of prescribing doctors that the anthroposophic SAR treatment with Citrus/Cydonia comp. (with or without additional therapies) is safe and effective. Further intervention studies are indicated.

5.1 Introduction

Citrus/Cydonia compositum (comp.) is an anthroposophic medicine, which contains extracts of lemon (Citrus limon (L.) Burm. f.) and quince (Cydonia oblonga Mill.) (De Bruin and Baars, 2001). It has been prescribed in clinical practice in Europe for more than 80 years to patients with hay fever or seasonal allergic rhinitis (SAR). In this period, anthroposophic doctors have empirically developed expert knowledge regarding the (side) effects, routes of administrations, and the necessity of co-medication/ co-therapies of Citrus/Cydonia comp. in SAR treatment.

However, in this evidence-based medicine period, expert knowledge is insufficient fundament for medical professionalism. In order to become scientifically accepted in medicine, expert knowledge must be explicated, embedded in valid theories, systematized in protocols, guidelines or healthcare programmes, and subsequently tested in increasingly more controlled ways (e.g. starting with routine outcome monitoring on effects, safety and patient satisfaction up to placebo-controlled randomized clinical trials).

This study took the first step in the development of practice-based evidence by explicating the expertise of anthroposophic doctors experienced in prescribing Citrus/Cydonia comp. in SAR treatment.

5.2 Methods

A new questionnaire was designed by the researchers of the Louis Bolk Instituut in Driebergen, the Netherlands. The questionnaire consisted of both open and closed questions, covering three domains: (1) general information on practice and expertise of the doctors; (2) experiences with diagnosis, prescription behaviour and side effects of Citrus/Cydonia comp. in SAR treatment, and (3) experiences with the effectiveness of Citrus/Cydonia comp. in SAR treatment.

After a thorough evaluation on face-validity by a small group of anthroposophic doctors, the questionnaire was approved and distributed by mail amongst anthroposophic general practitioners as well as to consultative working doctors who are all connected to the Nederlandse Vereniging van Antroposofische Artsen (NVAA) (Dutch Association of Anthroposophic Physicians). In total, 80 questionnaires were sent out. After two weeks a reminder was sent to the non-responders.

5.3 Results

5.3.1 Response rate

A total of 39 of 80 sent questionnaires were completed and returned to the research institute, providing a response rate of 49 percent.

5.3.2 Practice and patient characteristics

The responding doctors were both general practitioners and consultative working doctors. The average number of patients in their practices was 1,960 (range: 100 - 3,500). Seventy percent of the doctors treated, on average, between 10 and 50 SAR patients annually per season per practice. Based on this doctor population (n=39), this means that the experiences with SAR treatment is based on, in total, between 570 and 1,650 SAR patients treated per season. Both GPs and (of course) consultants treat patients on a consultative basis. However, 78 percent of the GPs state that the proportion of patients treated on a consultative basis is less than 10 percent of the total number of patients treated for SAR.

5.3.3 Forms of administration

Citrus/Cydonia comp. was available in several forms on the Dutch market: as ampoules, nasal spray, salve, and eye drops. In general practice, the main forms prescribed were ampoules, eye drops and nasal spray. The salve was rarely prescribed. Of the three commonly prescribed forms, the doctors demonstrated a preference for ampoules administrated as subcutaneous injections (30 doctors). The nasal spray was their second choice (17 doctors). The main reason for this preference was the anticipated effect of the subcutaneous route of administration. The main reason for prescription of the nasal spray was the local effect, since the SAR symptoms are restricted to nasal symptoms in most patients. Another reason for prescribing the nasal sprays was that this method of administration is free of the (sometimes) painful side effects of the injections.

5.3.4 Use of Citrus/Cydonia comp. in SAR treatment

Eighty percent of the doctors used Citrus/Cydonia comp. as a basic remedy in SAR treatment. There was a great deal of variation in *the time of the year doctors prescribe* Citrus/Cydonia comp. More than 25 percent of the doctors only treated patients prophylactically with Citrus/Cydo-

nia comp., while more than 60 percent treated patients both before and during the pollen season. In addition, approximately 50 percent of the doctors treated patients with Citrus/Cydonia comp. only during the pollen season. The reason was that many patients only visit the doctor when they have symptoms, and this is, of course, during the pollen season.

Not only the timing of the treatment by doctors, but also the *concentrations* of Citrus/Cydonia comp. ampoules used to treat SAR patients varied between doctors. All the concentrations (0.1% - 7%) were administered in practice, but the 0.1%, 1% and 5% concentrations were particularly commonly prescribed (by 19, 30 and 21 doctors, respectively). The two lowest concentrations (0.1% and 1%) together accounted for 65 percent of the prescribed ampoules.

Regarding the *frequency* of the administered injections, the doctor's preference depended partly on the time of treatment: prior to or during the pollen season. Prior to the pollen season, eighty percent of the doctors administered the injections twice per week, and only 13 percent once per week. During the pollen season, the frequency of injections varied between two and three times per week, given to 37 percent and 47 percent of SAR patients, respectively. However, the frequency did not depend only on the period in which the injections were administered; it could also be influenced by the patient's reaction to the injections. No or little reduction of symptoms could lead to an increase of the frequency of administration.

5.3.5 Simultaneous use of other therapies

Ninety-eight percent of the doctors sometimes or always prescribed another medication for simultaneous use with Citrus/Cydonia comp. The percentage of doctors who sometimes or always prescribed Citrus/Cydonia comp. simultaneously with another non-medicinal therapy was slightly lower, but still high (74%). The simultaneous use of other forms of therapy was mainly due to the fact that the doctors wished to increase the efficacy of the SAR treatment.

Concerning simultaneous use of other medicinal therapy, the doctors mainly indicate that they prescribe other medicines to reduce the symptoms if Citrus/Cydonia comp. does not produce the desired results. They also introduce other anthroposophic medicines that can affect the systems level of the organization. These 'other' medicines can be conventional drugs (80%), anthroposophic remedies (80%), or other remedies (homeopathic remedies (15%), etc.). The use of these conventional drugs is justified on the grounds of the strong symptom-reducing nature of these remedies.

5.3.6 The effectiveness of Citrus/Cydonia comp. in SAR patients

On average, an improvement is anticipated in 50 percent of patients after one year of monotherapy with Citrus/Cydonia comp. The majority of the doctors believe that this percentage of patients would rise if: (1) the treatment was continued for several years in succession, and (2) other therapies were used simultaneously.

In the absence of any effect *during* treatment with Citrus/Cydonia comp., the doctor can (1) vary the frequency and/or concentration of Citrus/Cydonia comp., or (2) switch to a different therapy such as eurythmy therapy, diet, or art therapy. Sixty-six percent of the doctors indicated that increased frequency was always or sometimes worthwhile if the results were only moderate. Twenty-five percent of the doctors indicated that in the case of inadequate results, they always increased the concentration, and 56 percent of the doctors stated they sometimes did so.

Of the doctors who switch to another therapy when therapy with Citrus/Cydonia comp. is ineffective, fifty-one percent resort to prescribing conventional remedies, thirty-six percent opt for other anthroposophic 'SAR medication,' and forty-nine percent will prescribe other anthroposophic non-SAR medication as an alternative therapy.

Concerning the effect of treatment with Citrus/Cydonia comp. during the season, ninety-four percent of doctors indicated that the effect would appear within three months, and of these, fifty percent believed that the effect would occur within two weeks.

5.3.7 Side effects

One-third of the doctors indicated that they had sometimes observed side effects on administration of Citrus/Cydonia comp. Side effects were most often pain due to the injection or injection fluid (especially the higher concentrations that contain more Citrus) or local skin reactions (irritation, itching, redness).

5.4 Discussion

The aim of the survey was to obtain an overview of the experiences of Dutch, Citrus/Cydonia comp. prescribing doctors with SAR treatment. The results demonstrate that the subcutaneous route of administration of Citrus/Cydonia comp. is the most favoured, with the nasal spray route of administration coming a close second. There is much variation between doctors in the way they prescribe Citrus/Cydonia comp. regarding the period of the year, the concentration, and frequency of the prescribed drug. Doctors combined the use of Citrus/Cydonia comp. with other medicinal or non-medicinal therapies for a large group of their patients. More than 50 percent of the doctors expected an effect within two weeks, and 94 percent of the doctors expected to see an effect within three months after treatment onset. One-third of the doctors indicated that they had sometimes observed side effects on administration of Citrus/Cydonia comp. (most often pain and local skin reactions).

Several CAM streams have a long history in clinical practice, and have developed a lot of expert (tacit) knowledge, but they lack sufficient scientific evidence of quality, safety, efficacy, effectiveness, and cost-effectiveness. In order to provide sufficient evidence, this tacit knowledge

can be converted into explicit knowledge and then systematized into healthcare programs, guidelines and/or protocols, and subsequently tested on the quality, safety, efficacy, effectiveness, and cost-effectiveness in order to become 'evidence-based' (Baars and Van der Bie, 2008). The results of the survey provide important practice-based evidence that can be used to take the next steps in the development of evidence-based practice of anthroposophic SAR treatment integrated with conventional SAR treatment.

The major limitation of this study is that it provides results based on expert knowledge only. Expert knowledge in the hierarchy of quality of evidence in outcome studies is regarded as very low-quality evidence. Nevertheless, this study provides positive practice-based evidence that provides a justification for designing and executing controlled trials to obtain high-quality evidence of the safety, efficacy, effectiveness, and cost-effectiveness of the anthroposophic SAR treatment.

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Chapter 6

Citrus/Cydonia Comp. Can Restore the Immunological Balance in Seasonal Allergic Rhinitis-Related Immunological Parameters In Vitro

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**Erratum: After publication we changed the following error in the text in paragraph 3.1 in accordance with our experimental findings:*
"Citrus/Cydonia comp. had no effect on cell survival and did appear to be toxic for PBMC cell subpopulations." is changed into "Citrus/Cydonia comp. had no effect on cell survival and did not appear to be toxic for PBMC cell subpopulations."

Abstract

In two *in vitro* studies, we examined the immunological (pathways of the) effects of Citrus/Cydonia comp. from, respectively, a healthy and an allergic donor; peripheral blood mononuclear cells (PBMCs) were isolated out of peripheral blood and analyzed *in vitro* after polyclonal stimulation of T-cells. The differentiation capacity and the influence with regard to Th1 (IFN-γ) and Th2 (IL-5) cells were examined. Citrus/Cydonia comp. has a selective effect on the differentiation of T-cells by producing relatively more IL-10 than IL-12. By that, it also seems to have an effect on the induction of regulatory (IL-10 producing) T-cell subsets. It is *in vitro* capable of neutralizing (to some extent) the changes, characteristic to allergic rhinitis, with regard to the maturation, differentiation, and activity of the immune system. Thus, Citrus/Cydonia comp. can potentially restore the disturbed immune state of rhinitis patients, which essentially could be sufficient to make allergic symptoms disappear permanently.

6.1 Introduction

Allergic rhinitis is a condition characterized by sneezing, watery nasal discharge, and nasal obstruction and itching. It is an increasingly prevalent condition, particularly in the Western world where it affects around 20% of the adult population. Allergic rhinitis is divided into seasonal allergic rhinitis (hay fever) which is triggered by pollens and moulds, and perennial allergic rhinitis in which house dust mites and pet dander are the predominant triggers. The spectrum of severity is wide and includes a significant number of sufferers with severe symptoms that are resistant to treatment with usual pharmacotherapy (antihistamines and topical nasal corticosteroids) [1]. The mean prevalence of allergic rhinitis in several Western countries is 12% [2].

Seasonal allergic rhinitis or hay fever is a type I immediate hypersensitivity reaction mediated by specific IgE antibody to a seasonal allergen, leading to mucosal inflammation characterized by sneezing, itching, rhinorrhoea, and nasal blockage. Pollens (6–40 m in diameter) from wind-pollinated grasses, trees, weeds, and spores from fungi are the most common aeroallergens. Grass pollen is the most common cause of seasonal allergic rhinitis. The highest levels of pollen in the atmosphereare found in May to July and pollen concentrations of 50 grains/mm3 are associated with symptoms in all susceptible people. The treatment of choice of seasonal allergic rhinitis is the symptomatic treatment with local or oral antihistamines and/or local corticosteroids. Immunotherapy, including sublingual immunomodulation therapy, is indicated in a limited subpopulation of patients [3].

Citrus/Cydonia comp. is an anthroposophic medicine, which contains extracts of lemon (Citrus lemon) and quince (Cydonia oblongata) [4]. For over eighty years now, the medicine "Citrus/Cydonia comp." is being prescribed as a subcutaneous injection or as a nasal spray for patients who suffer from seasonal allergic rhinitis. A survey on clinical experiences, carried out among a group of 39 active Dutch general practitioners [4], indicates that the subcutaneous treatment with Citrus/Cydonia comp. ampoules is profoundly effective. Firstly, a permanent effect from the treatment with Citrus/Cydonia comp. tends to be experienced, which indicates that the patients in question are claiming to lastingly suffer less from hay fever or even that they are free from complaints. Secondly, the effect is occurring within a period of two weeks, up to three months, after the actual treatment. Thirdly, the effect is optimal after a treatment of several years. Moreover, the survey pointed out that preventive administration before the start of the pollen season with Citrus/Cydonia comp. may be even more efficacious to the patients in question. Recently, positive effects by Citrus/Cydonia comp. were obtained among a group of 13 patients with the following characteristics: (a) allergic to grass pollen, (b) suffering from hay fever, on average, for nine years, and (c) the necessity for the use of antihistamines with regard to the nature of the complaints [5]. In addition, a prospective, observational study on the effect of Citrus/Cydonia comp. nasal spray on hay fever symptoms reported positive results without side effects in 140 patients [6]. We now performed *in vitro* studies to study the possible immunological (pathways of the) effects of Citrus/Cydonia comp.

6.2 Materials and Methods

6.2.1 Blood Donors

From a healthy and an allergic individual, 8 mL of blood was collected in sodium heparinate-coated vacutainers (BD Biosciences, San Diego, Calif, USA). The allergic individual was sensitized for birch pollen (RAST 6+) and grass pollen (RAST 4+), and he was also food allergic with a positive skin prick test on apple and cherry. The blood was subsequently diluted 1:1 with IMDM containing GlutaMAX (IMDM; Gibco-BRL, Paisley, Scotland) before the density gradient centrifugation on Ficoll-Paque PLUS (Amersham Biosciences, Uppsala, Sweden). The PBMC layer was washed twice with IMDM and the cell viability and cell concentration were determined by Trypan blue exclusion. An informed consent was obtained before the sample collection and the performed experiments were approved by the local ethical committee.

6.2.2 Culture Conditions

PBMCs were cultured in Yssel's medium at in a humidified atmosphere with 5% at a density of viable cells/mL. Yssel's medium consisted of IMDM supplemented with 1% Penicillin-Streptomycin (Gibco BRL), extra additions according to Yssel et al. [1984], and 1% human AB serum (Gibco BRL). Cells were plated out in 48 well plates at a concentration of cells/mL and cultured at . After five hours of adaptation to the culture conditions, the various stimuli or a matching volume of medium were added. Cultures were stimulated with 150 ng/mL anti-CD3 plus 100 ng/mL anti-CD28monoclonal antibodies (BD Pharmingen, San Diego, Calif, USA) or cultured in medium only [7].

6.2.3 Citrus/Cydonia Comp. Stimulation

Both conditions (negative and positive controls) took place in the presence of Citrus/Cydonia comp. 100 L/1 mL culture in two dilutions (undiluted and 1:3 dilution in culture medium). The extract (Gencydo) was obtained from (Weleda, Zoetermeer, The Netherlands).

6.2.4 Cell Viability

Half a million PBMCs were washed and subsequently incubated with 2 L Annexin V-APC (BD Biosciences) in 200 L Annexin V buffer according to the manufacturer's protocol. After an incubation period of 15 minutes on ice, the cells were spun down (400 g for 10 minutes) and re-

suspended in 200 L Annexin V buffer and 2 L PI (1 mg/mL; Sigma, St. Louis, Mo, USA). The cells were then analyzed on a flow cytometer (FACS array, BD Biosciences).

6.2.5 Immunological Phenotype

The immunological phenotype of PBMC subsets was determined by staining the surface antigens with the following two monoclonal antibody () mixtures: (1) -hCD3 (PE-Cy7), -hCD4 (PE), -hCD8 (APC), and -hCD25 (APC-Cy7); (2) -hCD3 (PE-Cy7), -hCD14 (APC), -hCD16 (PE), -hCD19 (APC-Cy7), and -hCD56 (PE). All antibodies were purchased at BD Biosciences.

Per well, cells were spun down in a 96 wells U-bottom plate. The cells were incubated with staining buffer (1% FCS and 0.1 M in PBS) containing the surface markers or the matching isotype controls for 30 minutes on ice in the dark. The cells were washed once with PBS and resuspended in PBS for flow cytometry. The four-color flow cytometric acquisition was performed on an FACS array, using the BD FACS-array software. An electronic gate was set to exclude debris and at least 10 000 events/samples were acquired. The percentages of positive cells were corrected for the isotype control.

6.2.6 Proliferation Capacity

The proliferation capacity of the PBMC was studied by intracellular expression of the nuclear Ki-67 antigen (Ki-67; BD Pharmingen). The Ki-67 antigen is absent in the nuclei of resting cells, but present in all other phases of the cell division cycle as well as in the mitosis phase [8]. In each well, PBMCs were incubated with 100 L Cytofix/Cytoperm (BD Pharmingen) for 15–20 minutes on ice to fix and permeabilize the cells. Cells were washed twice with perm/wash buffer (BD Pharmingen) and incubated with anti-Ki-67 PE antibody, or the matched isotype control, diluted in perm/wash buffer for 30 minutes on ice in the dark. Hereafter, the cells were washed with perm/wash buffer, resuspended in PBS, and measured on the flow cytometer. Values are expressed as cells positive for the Ki-67 mAb corrected for the isotype control.

6.2.7 Cytokines

PBMC culture supernatants were analyzed for their IL-1β, IL-12, IFN-γ, TNF-α, IL-4, IL-5, IL-10, and IL-13 contents. The cytokine production was measured with Cytometric Bead Assay Flex Sets (BD Pharmingen). All buffers used in this protocol were obtained from the BD CBA Soluble Protein Master Buffer Kit (BD Pharmingen). Supernatants were collected, stored at –20, and tested within 2 weeks. The procedure was performed according to the manufacturer's protocol. The samples were measured on the FACS array, using the FCAP software. The sensitivity limits for quantitative determi-

nations, according to the manufacturer, were 1.1 pg/mL for IL-1, 0.3 pg/mL for IL-4 and IFN-γ, 0.5 pg/mL for IL-5, 2.3 pg/mL for IL-10, 2.2 pg/mL for IL-12, 0.6 pg/mL for IL-13, and 0.7 pg/mL for TNF-α.

6.3 Results

6.3.1 Effect of Citrus/Cydonia Comp. on Healthy Donor Pbmc

PBMCS were isolated and analyzed for their subset composition. The percentages of the subsets PBMC were 63% CD3+ T-cells (with 50.4% CD4+ Th-cells and 12.6% CD8+ Tc-cells), 8% CD19+ B cells, 5% CD14+ monocytes, and 12% CD16/CD56+ NK cells. After one day (Table 6.1) and four days (Table 6.2) of culture, the results showed that Citrus/Cydonia comp. not only induced T-cell proliferation directly, but also activated monocytes resulting in a selective cytokine production (TNF-α, IL-1β, IL-10, and IL-12). However, Citrus/Cydonia comp. induced more IL-10 than IL-12 production most likely derived from monocytes, thereby stimulating the outgrowth of immunoregulatory (IL-10) monocytes more than the immunoreactive (IL-12) subsets of monocytes. These monocyte-related effects of Citrus/Cydonia comp. were detectable within one day and were found in cultures with a normal therapeutic dose. Subsequently, T-cell activation and proliferation were suprastimulated by Citrus/Cydonia comp. over the polyclonal stimulation alone. Citrus/Cydonia comp. had no effect on cell survival and did not[1] appear to be toxic for PBMC cell subpopulations.

Table 6.1. Mean scores after one day (healthy donor).

	Medium	*Medium + Citrus/Cydonia comp. 1*	*Medium + Citrus/Cydonia comp. 1:3*
Proliferation (%)	3 (1)*	13 (3)	1 (1)
Cell death (%)	88 (12)	82 (11)	87 (5)
Cytokines (pg/mL)			
TNF-α	13 (4)	3315 (129)	13 (1)
IL-1β	25 (6)	3535 (147)	15 (2)
IL-10	22 (9)	918 (52)	12 (1)
IL-12	12 (3)	46 (12)	12 (2)
IFN-γ	15 (2)	55 (8)	25 (3)
IL-4	10 (2)	12 (1)	10 (1)
IL-5	12 (2)	28 (3)	10 (1)

1 After publication we added the word “not” in the text in accordance with our experimental findings.

Percentages of proliferating cells and cells in apoptosis of PBMC cultures of a healthy donor stimulated for one day with medium or Citrus/Cydonia undiluted and 1:3 diluted. In the supernatants of these cultures, cytokines were measured by flow cytometric analysis in the Bead Assay Flex Sets system. Cytokine levels in pg/mL. *All results are described with standard deviations (SDs).

Table 6.2. Mean scores after four days (healthy donor).

	Medium	*Anti-CD3/28*	*Medium + Citrus/ Cydonia comp. 1*	*Medium + Citrus/ Cydonia comp. 1:3*	*Stimulation + Citrus/Cydonia comp. 1*	*Stimulation + Citrus/ Cydonia comp. 1:3*
Proliferation (%)	3 (1)*	42 (8)	3 (1)	1 (1)	48 (11)	40 (2)
Cell death (%)	88 (12)	61 (11)	87 (8)	92 (11)	65 (12)	60 (6)
Cytokines (pg/mL)						
TNF-α	13 (2)	8483 (987)	15 (2)	13 (2)	9647 (733)	8117 (566)
IL-1β	15 (3)	4134	35 (11)	15 (1)	4858 (247)	4037 (138)
IL-10	12 (2)	9553	18 (8)	12 (2)	12276 (566)	5662 (931)
IL-12	12 (1)	84	26 (9)	12 (2)	238 (87)	185 (77)
IFN-γ	15 (2)	34355	15 (1)	15 (2)	48800 (12778)	37750 (12501)
IL-4	12 (2)	134	12 (1)	12 (2)	55 (12)	106 (28)
IL-5	12 (2)	375	12 (2)	12 (1)	118 (45)	266 (56)

Percentages of proliferating cells and cells in apoptosis of PBMC cultures of a healthy donor stimulated for four days with medium alone, polyclonal stimulation with anti-CD3 plus anti-CD28 antibodies, or polyclonal stimulation in the presence of Citrus/Cydonia preparation undiluted and 1:3 diluted. In the supernatants of these cultures, cytokines were measured by flow cytometric analysis in the Bead Assay Flex Sets system. Cytokine levels in pg/mL. *All results are described with standard deviations (SDs).

6.3.2 Effect of Citrus/Cydonia Comp. on an Allergic Donor Pbmc

After four days (Table 6.3), the results demonstrated that Citrus/Cydonia comp. was able to restore the reduced IL-10 production in PBMC cultures of the allergic individuals. The stronger immunoregulatory balance (IL-10) and a curbed augmented Th2 response (a decrease of IL-4 and IL-5 production) was accompanied by an increased production of IL-12 and a reduced production of IFN-γ. Even after 4 days, monocyte stimulation by Citrus/Cydonia comp. was detected by the production of TNF-α and IL-10.

Table 6.3. Mean scores after four days (allergic donor).

	Medium	*Stimulation with anti-CD3/28*	*Stimulation + Citrus/Cydonia comp.*
Proliferation (%)	1 (1)*	48 (12)	57 (12)
Cell death (%)	78 (8)	59 (16)	55 (8)
Cytokines			
TNF-α	10 (1)	5782 (154)	6893 (738)
IL-1β	10 (1)	2275 (339)	3772 (665)
IL-10	10 (1)	2331 (452)	7634 (1299)
IL-12	10 (1)	134 (26)	335 (89)
IFN-γ	10 (1)	9778 (452)	11668 (1638)
IL-4	10 (1)	456 (87)	138 (18)
IL-5	10 (1)	667 (154)	227 (85)

Percentages of proliferating cells and cells in apoptosis of PBMC cultures of an allergic donor stimulated for four days with medium alone, polyclonal stimulation with anti-CD3 plus anti-CD28 antibodies, or in the presence of Citrus/Cydonia undiluted preparation. In the supernatants of these cultures, cytokines were measured by flow cytometric analysis in the Bead Assay Flex Sets system. Cytokine levels in pg/mL.*All results are described with standard deviations (SDs).

6.4 Discussion

Here, we show that Citrus/Cydonia comp. has a selective effect on the differentiation of T-cells with regard to the production of cytokines; the production of IL-10 is relatively larger than that of IL-12. By that, Citrus/Cydonia comp. also seems to have an effect on the induction of regulatory (IL-10 producing) T-cell subsets. Hence, as a consequence, Citrus/Cydonia comp. might be producing an allergy reducing effect, at which it does not concern Th1 induction and the reduction of the allergen-specific Th2 response, bearing the risk of induction of a chronic inflammation and very likely even an increased risk for autoimmunity.

Recent developments mainly concern the field of allergen-specific immunotherapeutic protocols. This immunotherapy is widely believed to occur through restoration of the disturbed Th1-Th2 balance [9–12], either linked to the induction of allergen-specific (blocking) IgG4 antibodies, or to the induction of regulatory T-cell subsets. The exact role which the regulatory T-cell subsets play with regard to these mechanisms is not yet indistinct. With regard to the development of these immunotherapeutic protocols, special allergen preparations, obtained from purified natural or recombinant produced allergens, are necessary and need to be developed.

Citrus/Cydonia comp. is likely to induce more regulatory T-cells, whether CD4+CD25+Fosp3+ natural or antigen-induced IL-10 and/or TGF-b producing Tr-cells, that are, therefore, very im-

munosuppressive, and which are capable of reducing allergen specifically activated Th2 cells. Our results imply that Citrus/Cydonia comp. does not induce a complete state of immunosuppression, resulting in a diminished resistance against infections and a reduced protection against tumors. This is consistent with the long-term clinical experiences and the results of the empirical studies on the use of Citrus/Cydonia comp.

Based on these *in vitro* investigations, we hypothesized that Citrus/Cydonia comp. is capable of neutralizing (to some extent) the changes, characteristic to allergic rhinitis, with regard to the construction, the maturation, the differentiation, and the activity of the immune system. By that, it is possible to explain the therapeutic positive effects on allergic rhinitis patients treated with Citrus/Cydonia comp. found in previous studies and clinical practice.

The conclusions based on this study are of great importance, since the standard treatment of allergic rhinitis is based on the long-term use of antihistamines, potentially in combination with a local application of corticosteroids, in case of persisting and/or serious symptoms. Those treatments tend to reduce the symptoms, but they do not possess any immunotherapeutic potency themselves. This implies that it is compulsory for individual patients to keep on using such medicines for many years. Based on our pilot data, indicating that *in vitro* Citrus/Cydonia comp. is capable of modulating the Th1-Th2 balance, we actually expect Citrus/Cydonia comp. to have an immunotherapeutic potency. This adds to the clinical therapeutic effect from Citrus/Cydonia comp., both as an injection and as a topical application. This implies that a long-term treatment with Citrus/Cydonia comp. injections during several years, before the start of the pollen season, can potentially restore the disturbed immune state of rhinitis patients, which essentially could be sufficient to make the allergic complaints disappear.

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Chapter 7

A comparative *in vitro* study of the effects of separate and combined products of *Citrus e fructibus* and *Cydonia e fructibus* on immunological parameters of seasonal allergic rhinitis

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Abstract

This study examined the effects of the combined product, *Citrus e fructibus/Cydonia e fructibus* (Citrus/Cydonia) (Citrus and Cydonia: each 0.01g/mL), and separate products of Citrus (0.01 g/mL) and Cydonia (0.01 g/mL) on the immunological pathways involved in seasonal allergic rhinitis (SAR). Peripheral blood mononuclear cells (PBMCs) from five healthy and five grass pollen-allergic donors were isolated and analyzed *in vitro* after polyclonal and allergen-specific stimulation of T-cells in the presence of the three extracts. The analyses demonstrated acceptable cell survival with no signs of toxicity. Citrus mainly had a selective effect on reducing allergen-specific chronic inflammatory (TNF-α; Citrus compared to Cydonia and Citrus/Cydonia: -87.4 ($p < 0.001$) and -68.0 ($p < 0.05$), respectively) and Th2 pathway activity (IL-5; Citrus compared to Cydonia: -217.8 ($p < 0.01$); while, both Cydonia and Citrus/Cydonia mainly affected the induction of the allergen-specific Th1 pathway ((IFN-γ; Cydonia and Citrus/Cydonia compared to Citrus: 3.8 ($p < 0.01$) and 3.0 ($p < 0.01$), respectively). Citrus and Cydonia demonstrated different working mechanisms in the treatment of SAR and the combination product did not demonstrate larger effects than the separate preparations. Further effectiveness and efficacy studies comparing the effects of the products on SAR *in vivo*, are indicated.

Keywords: seasonal allergic rhinitis, Citrus/Cydonia, in vitro study, immunological pathways

7.1 Introduction

Citrus/Cydonia is an anthroposophic medicine, which contains extracts of lemon (Citrus limon (L.) Burm. f.) and quince (Cydonia oblonga Mill.) [1]. For over eighty years, the combination of preparations from Citrus and Cydonia has been prescribed as a subcutaneous injection or nasal spray for patients who suffer from seasonal allergic rhinitis (SAR). Both *in vitro* [2,3] and clinical studies [4-6] have demonstrated that Citrus/Cydonia might be effective in treating SAR. Previous immunological analyses demonstrated it has an effect on the induction of regulatory (IL-10 producing) T-cell subsets and on the suppression of the Th2 pathway cytokines, IL-4 and IL-5 [2], *in vitro*. Another *in vitro* study demonstrated that Citrus/Cydonia significantly reduced the histamine production and the inflammatory mediator release from mast cells in a dose-dependent manner [3]. Although positive immunomodulating activity has been reported for the combination product, Citrus/Cydonia, the extent that each of the two active substances, Citrus and Cydonia, contribute to the observed effects is not known.

In this *in vitro* study, we compared the effects of the combination product, Citrus/Cydonia, and the separate products, Citrus and Cydonia, on SAR-related immunological components. The primary hypothesis of the present study, based on traditional use in clinical practice, was that the combination preparation Citrus/Cydonia would demonstrate larger SAR-related treatment effects *in vitro* than the single preparations of Citrus and Cydonia. The secondary hypothesis was that treatment with Citrus, Cydonia and Citrus/Cydonia would demonstrate larger treatment effects (as expected in SAR patients) *in vitro,* in the healthy group compared to the SAR group. This hypothesis was based on the assumption that the three products support the SAR-related self-healing capacity of the organism and that this capacity is already stronger in healthy persons than in SAR patients before treatment.

7.2 Materials and Methods

This study was conducted according to the study protocol previously accepted by the medical ethical committee. No violations or deviations to the study protocol were noticed.

7.2.1 Investigational Products

The three preparations were obtained from WALA Heilmittel GmbH (Bad Boll/Eckwälden, Germany). Citrus/Cydonia (Citrus e fructibus / Cydonia e fructibus, batch no 008 704C) is a solution for subcutaneous injection. One ampoule of 1 mL contains: 0.1 g Citrus medica ssp. limonum e fructibus ferm 33c Dil. D1 HAB, methode 33c and 0.1 g Cydonia oblonga e fructibus ferm 33b Dil. D1 HAB, methode 33b. Excipients were sodium chloride, sodium hydrogen carbonate and

water for injection. Citrus (Citrus e fructibus, batch no 008 702C) is a solution for subcutaneous injection. One ampoule of 1 mL contains: 1 g of Citrus medica ssp. limonum e fructibus ferm 33c Dil. D2 HAB, methode 33c in a sodium chloride, sodium hydrogen carbonate solution for injection. Cydonia (Cydonia e fructibus, batch no 008 703C) is a solution for subcutaneous injection. One ampoule of 1 mL contains: 1 g of Cydonia oblonga e fructibus ferm 33b Dil. D2 HAB, methode 33b in a sodium chloride, sodium hydrogen carbonate solution for injection.

7.2.2 Blood donors and preparation of blood samples

Blood was collected from two groups of participants: SAR patients and healthy persons. The experiments performed were approved by the local medical ethical committee and informed consent was obtained before sample collection.

Eligible participants from the SAR group were all adults of both sexes; aged 18 to 40; who gave written informed consent; suffered from SAR for at least two years; had a RAST score ≥ 2 for both grass pollen and birch pollen; suffered from the following nasal symptoms: sneezing, itchy nose, nasal obstruction, and watery nasal discharge; with a severity score ≥ 2 for at least three of the four symptoms (ranging from 0 = not present to 3= severe) and the necessity to use antihistamines and/or corticosteroids for the treatment of symptoms for at least the last two years. Exclusion criteria were: chronic inflammatory autoimmune diseases such as Type I - diabetes mellitus, rheumatoid arthritis, multiple sclerosis, psoriasis or Crohn's disease; allergic (hypersensitive) to one of the constituents of Citrus e fructibus / Cydonia e fructibus; asthma; use of other preparations containing Citrus and/or Cydonia extracts within the last two weeks prior to enrolment into the study; use of cromoglycates in the last month before study onset; concomitant pharmacological treatment indicated for seasonal allergic rhinitis such as antihistamines, corticosteroids or other preparations in the last two weeks before study onset; anti-allergy immunotherapy in the previous two years; participation in a further clinical trial at the same time or within the previous 4 weeks prior to enrolment into this study; pregnancy or lactation; severe internal or systemic disease (e.g. cardiac, hepatic, renal diseases); a known history of drug, alcohol and/or medication dependence or addiction.

Eligible participants from the healthy group were men and women; aged 18 to 40 who gave written informed consent; had a RAST score for SAR related pollen = 0; did not have the following nasal symptoms during the pollen season: sneezing, itching nose, nasal obstruction, and watery nasal discharge; and no history of SAR symptoms for at least two years. Exclusion criteria: chronic inflammatory autoimmune disease such as Type I - diabetes mellitus, rheumatoid arthritis, multiple sclerosis, psoriasis or Crohn's disease; allergic (hypersensitive) to one of the constituents of Citrus e fructibus / Cydonia e fructibus; asthma; participation in a further clinical trial at the same time or within the previous 4 weeks prior to enrolment into this study; pregnancy or lactation; severe internal or systemic disease (e.g. cardiac, hepatic, renal diseases); drug, alcohol and/or medication dependence or addiction.

All eligible participants were recruited from a single center, the Louis Bolk Institute (Drieber-

gen, NL). The first participant was included on June 24, 2010 and the last participant completed the study on August 3, 2010. Blood samples were taken on July 22, 2010 (2 participants), July 30, 2010 (5 participants), and August 3, 2010 (3 participants). From each person in the SAR group and healthy group, 3 x 8 mL of blood was collected in sodium heparinate-coated vacutainers (BD Biosciences, San Diego, Calif, USA). The blood was subsequently diluted 1:1 with Iscove's Modified Dulbecco's Media (IMDM) containing GlutaMAX (IMDM; Gibco-BRL, Paisley, Scotland) before density gradient centrifugation using Ficoll-Paque PLUS (Amersham Biosciences, Uppsala, Sweden). The PBMC layer was washed twice with IMDM and the cell viability and cell concentration were determined by Trypan blue exclusion.

7.2.3 Culture conditions and stimulations

PBMCs were cultured in Yssel's medium at 37°C in a humidified atmosphere with 5% CO2 at a density of $1x10^6$ viable cells/mL. Immunological phenotyping of freshly isolated PBMC was performed on a FACS Canto II (BD Pharmingen, San Diego, USA), using monoclonal antibodies and the procedure from BD Pharmingen (San Diego, USA). Cells were plated out in 48 well plates at a concentration of $1x10^6$ cells/mL and cultured at 37°C. After five hours, in which the cells adapted to the culture conditions, various stimuli or a matching volume of medium were added. Cultures were stimulated polyclonally with 150 ng/mL anti-CD3 plus 100 ng/mL anti-CD28 monoclonal antibodies (BD Pharmingen, San Diego, Calif, USA) or cultured in medium only [7]. In addition, we performed allergen-specific stimulation of 10^6 cells/mL in 1 mL cultures with applied pollen extract (Phl p 1 from Timothy grass, Phleum pretense; Biomay Vienna, Austria; 10 µg/mL in medium). Supernatants of the stimulated cultures were harvested after 1, 4 and 7 days of culture and stored at -80°C for later cytokine analysis.

7.2.4 Stimulation with Citrus /Cydonia, the single products of Citrus and Cydonia, relative to medium control

Three experimental conditions and one control condition were evaluated. Experimental conditions: (a) Citrus, 0.01 g/mL; (b) Cydonia, 0.01 g/mL; and (c) Citrus/Cydonia, 0.02 g/mL (Citrus and Cydonia: each 0.01g/mL). The (negative) control condition and dilution medium was Yssel's medium. Samples of culture supernatants were taken after 24 hrs to elucidate monocyte reactivity; after four days to evaluate the effects of medium and polyclonal stimulation; and after seven days to evaluate the effects of the three extracts, Citrus, Cydonia, and Citrus/Cydonia, relative to a medium control for grass pollen-specific stimulation. Cytokine concentrations were measured in culture supernatants using labeled antibody preparations and flow cytometry measurements.

7.2.5 Cell viability

Early apoptosis and late apoptosis/necrosis was assessed on freshly prepared and 7 day cultured cells using double staining with APC-Annexin V and propidium iodide (PI) [8]. On day 0 half a million cells from the isolated PBMCs of the individuals were washed and subsequently incubated with 2 μL Annexin V-APC (BD Biosciences, San Diego, Calif, USA) in 200 μL Annexin V buffer according to the manufacturer's protocol. After a 15 min incubation period, the cells were spun down (400 g for 10 min) and resuspended in 200 μL Annexin V buffer and 2 μL PI (1 mg/mL; Sigma, St. Louis, Mo, USA). The cells were then analyzed on a flow cytometer (FACS array, BD Biosciences, San Diego, Calif, USA) as detailed in sections 2.6 and 2.7.

7.2.6 Immunological phenotype

On day 0 the immunological phenotype of PBMC subsets was determined by staining the surface antigens with the following two monoclonal antibody (α) mixtures: (1) α-hCD3 (PE-Cy7), α-hCD4 (PE), α-hCD8 (APC), and α-hCD25 (APC-Cy7); (2) α-hCD3 (PE-Cy7), α-hCD14 (APC), α-hCD16 (PE), α-hCD19 (APC-Cy7), and α-hCD56 (PE). All antibodies were purchased at BD Biosciences (San Diego, Calif, USA). For each well, $5x10^5$ cells were spun down in a 96 well U-bottom plate. The cells were incubated with staining buffer (1% FCS and 0.1M NaN_3 in PBS) containing the surface markers or the matching isotype controls for 30 min on ice in the dark. The cells were washed once with PBS and resuspended in PBS for flow cytometry. The four-color flow cytometric acquisition was performed on a FACS array, using the BD FACS-array software. An electronic gate was set to exclude debris and at least 10,000 events/samples were acquired. The percentage of positive cells was corrected for the isotype control.

7.2.7 Proliferation capacity

On days 4 and 7 the proliferation capacity of the PBMCs was studied by intracellular expression of the nuclear Ki-67 antigen (Ki-67; BD Pharmingen, San Diego, Calif, USA). The Ki-67 antigen is absent in the nuclei of resting cells, but present in all other phases of the cell division cycle as well as in the mitosis phase [8,9]. In each well, $5x10^5$ PBMCs were incubated with 100 μL Cytofix/Cytoperm (BD Pharmingen, San Diego, Calif, USA) for 15–20 min on ice to fix and permeabilize the cells. Cells were washed twice with perm/wash buffer (BD Pharmingen, San Diego, Calif, USA) and incubated with anti-Ki-67 PE antibody or the matched isotype control, diluted in perm/wash buffer, for 30 min on ice in the dark. Hereafter, the cells were washed with perm/wash buffer, resuspended in PBS, and measured on the flow cytometer. Values are expressed as cells positive for the Ki-67 mAb, corrected for the isotype control.

7.2.8 Cytokines

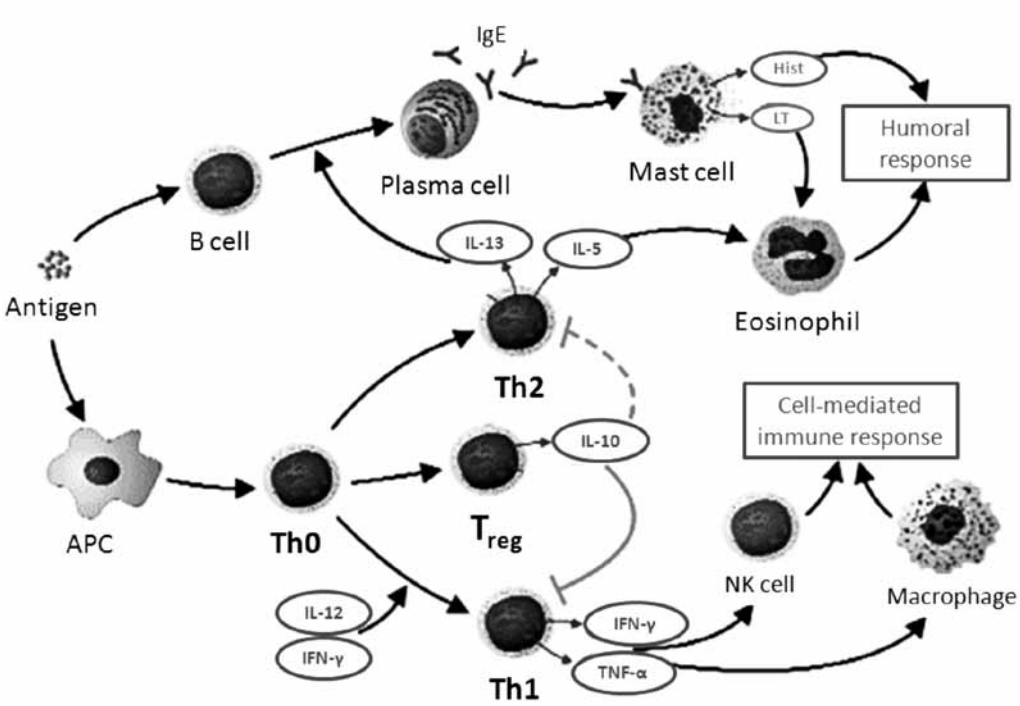
On days 1, 4 and 7 PBMC culture supernatants were analyzed for their IL-1β, IL-12, IFN-γ, TNF-α, IL-5, IL-10, and IL-13 contents. The cytokine production was measured with Cytometric Bead Assay Flex Sets (BD Pharmingen, San Diego, Calif, USA). All buffers used in this protocol were obtained from the BD CBA Soluble Protein Master Buffer Kit (BD Pharmingen, San Diego, Calif, USA). Supernatants were collected, stored at –20°C, and tested within 2 weeks. The procedure was performed according to the manufacturer's protocol. The samples were measured on the FACS array, using the FCAP software. The sensitivity limits for quantitative determinations, according to the manufacturer, were 1.1 pg/mL for IL-1β, 0.3 pg/mL for IFN-γ, 0.5 pg/mL for IL-5, 2.3 pg/mL for IL-10, 2.2 pg/mL for IL-12, 0.6 pg/mL for IL-13, and 0.7 pg/mL for TNF-α.

7.2.9 Definition of positive effects

A non-allergic, healthy state is hypothesized to represent a balanced state within the immune system with a relatively high level of IL-10 (demonstrating sufficient immunoregulation) and a balance between the Th1 pathway (e.g. IFN-γ) and the Th2 pathway (e.g. IL-5 and IL-13). SAR is associated with relatively low levels of IL-10, a chronic inflammatory activity (e.g. TNF-α), an overproduction of Th2 pathway cytokines and an imbalance between the Th1 and Th2 pathways [9, 10]. The following changes in cytokine production levels were therefore regarded as positive immunological SAR treatment effects [9-14] (Figure 7.1):

1. The induction of (regulatory) T-cells (Treg): increase of (grass pollen stimulated minus medium stimulated) IL-10 on day 7, often accompanied by monocyte-derived IL-10 on day 1.
2. The induction of Th1 activity: increase of (grass pollen stimulated minus medium stimulated) IFN-γ on day 7, often accompanied by monocyte-derived IFN-γ on day 1.
3. The reduction of Th2 activity: reduction of (grass pollen stimulated minus medium stimulated) IL-5 and IL-13 on day 7, often accompanied by monocyte-derived IL-1β on day 1, which is essential for the outgrowth of Th2 cells.
4. The reduction of chronic inflammatory activity: reduction of (grass pollen stimulated minus medium stimulated) monocyte-derived TNF-α on day 1.
5. The restoration of the Th1/Th2 balance: an increase in (grass pollen stimulated minus medium stimulated) IFN-γ/IL-5 and IFN-γ/IL-13 ratios on day 7.
6. The restoration of the Treg/Th2 balance: an increase in the (grass pollen stimulated minus medium stimulated) IL-10/TNF-α ratio on day 1; the increase of (grass pollen stimulated minus medium stimulated) IL-10/IL-5 and IL-10/IL-13 ratios on day 7.

Figure 7.1. Seasonal allergic rhinitis related immunological subsystems, pathways and cytokines



7.2.10 Statistics

Pearson Chi-square tests were performed on all relevant donor characteristics. For the PBMC subsets, we calculated all immunological outcome parameters (day 1: IL-1β, IL-10, IL-12, TNF-α, and IFN-γ; day 7: IL-5, IL-13, IL-10, and IFN-γ), the relevant ratios (day 1: IL10/TNF-α and IFN-γ/TNF-α; day 7: IL10/IL-5, IL-10/IL-13, IFN-γ/IL-5, and IFN-γ/IL-13), the mean scores, and 95% confidence intervals of cytokine production levels after grass pollen stimulation by subtracting the medium stimulation values. Subsequently, ANOVA (with Tamhane correction in case of unequal distribution) and unpaired t-tests were used to determine statistically significant differences between (1) the means of the three SAR groups that were stimulated in the presence of the three experimental extracts, (2) the means of the three healthy groups that were stimulated in the presence of the three experimental extracts, and (3) the means of the SAR groups and the healthy groups that were stimulated in the presence of the three experimental extracts. GLM Repeated Measures tests were performed to determine statistically significant differences between the means of the three total groups (total group = SAR group and healthy group) that were stimulated in the presence of the three experimental extracts. All statistical analyses were performed with SPSS 18.0 (SPSS Inc., Chicago, USA).

7.2.11 Blinding

All researchers were blinded to the identity of the products, which were numbered A, B, and C by the manufacturer. Unblinding took place after all statistics had been performed.

7.3 Results

7.3.1 Blood donors

The SAR group and the healthy group demonstrated no statistically significant differences with regard to: sex (both groups: 3 women and 2 men); ethnicity (both groups: all Caucasian); age (means and standard deviations): 35.2 (10.5) and 43.4 (10.9) years, respectively; height (means and standard deviations): 1.79 (0.1) and 1.77 (0.1) meters, respectively; weight (means and standard deviations): 75 (12.6) and 74 (9.2) kg, respectively; systolic blood pressure (means and standard deviations): 113 (13.2) and 117 (12.0) Hg, respectively; diastolic blood pressure (means and standard deviations): 71 (7.5) and 78 (5.7) Hg, respectively; and heart rate (means and standard deviations): 65 (3.8) and 65 (8.7), respectively. RAST scores (means, standard deviations and ranges) for grass pollen and birch pollen respectively were 3.6 (1.5, range: 2-6) and 3.6 (0.9, range: 3-5) for the SAR group, and 0 and 0 for the healthy group (Table 7.1).

The mean percentages of the PBMC subsets for the SAR group were: 58.8% CD3+ T cells (with 40.4% CD4+ Th cells and 18.4% CD8+ Tc cells), 6.2% CD19+ B cells, 16% CD14+ monocytes, and 9.6% CD16/CD56+ NK cells. The mean percentages of the PBMC subsets for the healthy group were: 60.0% CD3+ T cells (with 39.4% CD4+ Th cells and 20.6% CD8+ Tc cells), 7.0% CD19+ B cells, 15.8% CD14+ monocytes, and 8.8% CD16/CD56+ NK cells. There were no statistically significant differences in the mean scores of the subsets between the SAR group and the healthy group.

7.3.2 Effects on viability

Citrus (Fig. 7.2A), Cydonia (Fig. 7.2B), and Citrus/Cydonia (Fig. 7.2C) had no effect on *in vitro* cultured blood mononuclear cell survival and did not appear to be toxic to the PBMC subpopulations by Annexin V-PI staining.

Table 7.1. Baseline characteristics seasonal allergic rhinitis (SAR) group and Healthy group

Variable		SAR group (n = 5)	Healthy group (n = 5)	p-value
Sex: Number (percentage)	Male Female	3 (60%) 2 (40%)	3 (60%) 2 (40%)	n.s.
Ethnicity: number (percentage)	Caucasian Asian	5 (100%) 0 (0%)	5 (100%) 0 (0%)	n.s.
Age (year) (sd)		35.2 (10.5)	43.4 (10.9)	n.s.
Height (cm) (sd)		179 (10)	177 (10)	n.s.
Weight (kg) (sd)		75 (12.6)	74 (9.2)	n.s.
Blood pressure at screening (systolic/ diastolic) (mmHg) (sd)		113 (13.2)/ 71 (7.5)	117 (12)/ 78 (5.7)	n.s.
Heart rate at screening (beats per minute)		65 (3.8)	65 (8.7)	n.s.
RAST grass pollen		3.6 (1.5)	0	
RAST birch pollen		3.6 (0.9)	0	

n.s. = not significant

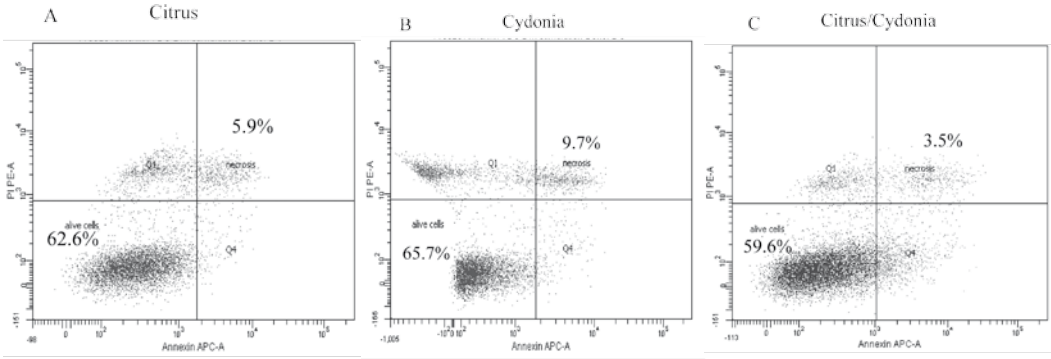


Figure 7.2. Representative example of a flow cytometric analysis profile of live, necrotic and apoptotic cells after *in vitro* culture of human PBMC stimulated in the presence of either Citrus (A) or Cydonia (B) or their combination (C). Citrus (100 µL/mL), Cydonia (100 µL/mL) or the combination Citrus/Cydonia (100 µL/mL) were added to 0.5 x 106 PBMC in 1 ml cultures which were stimulated with grass pollen extract (10 mg/mL) for 7 days. Staining was performed with Annexin V-APC and PI and measured on a BD flow cytometer.

Table 7.2. Differences in mean cytokine scores after one day PBMC cultures in the SAR group, healthy group, and total group (grass pollen stimulation minus medium stimulation)

		Citrus	*Cydonia*	*Citrus/Cydonia*	*Citrus vs. Cydonia p-value*	*Citrus vs. Citrus/ Cydonia p- value*	*Cydonia vs. Citrus/ Cydonia p- value*
IL-1β*	SAR	8.2 (7.0)	20.6 (5.0)	14.6 (8.9)	$p < 0.05$	ns	ns
	Healthy	2.4 (2.3)	17.6 (6.7)	18.8 (8.0)	$p < 0.01$	$p < 0.01$	ns
	Total group	5.3 (5.8)	19.1 (5.8)	16.7 (8.3)	$p < 0.001$	$p < 0.01$	
IL-10	SAR	64.0 (14.4)	59.2 (10.1)	54.0 (8.2)	ns	ns	ns
	Healthy	53.0 (15.2)	65.2 (14.4)	69.6 (3.9)	ns	ns	ns
	Total group	58.5 (15.1)	62.2 (12.1)	61.8 (10.2)	ns	ns	ns
TNF-α	SAR	94.8 (9.0)	182.2 (15.2)	162.8 (37.4)	$p < 0.001$	$p < 0.05$	ns
	Healthy	105.6 (25.2)	160.2 (13.2)	143.2 (23.9)	$p < 0.01$	$p < 0.05$	ns
	Total group	100.2 (18.7)	171.2 (17.7)	153.0 (31.3)	$p < 0.001$	$p < 0.001$	
IFN-γ	SAR	3.0 (1.9)	6.8 (1.1)	6.0 (1.2)	$p < 0.01$	$p < 0.01$	ns
	Healthy	3.0 (1.6)	6.0 (1.4)	5.4 (1.1)	$p < 0.01$	$p < 0.05$	ns
	Total group	3.0 (1.6)	6.4 (1.3)	5.7 (1.2)	$p < 0.001$	$p < 0.001$	
IL-10/ TNF-α	SAR	0.7 (0.1)	0.3 (0.1)	0.4 (0.1)	$p < 0.001$	$p < 0.01$	ns
	Healthy	0.5 (0.2)	0.4 (0.1)	0.5 (0.1)	ns	ns	ns
	Total group	0.6 (0.2)	0.4 (0.1)	0.4 (0.1)	$p < 0.01$	$p < 0.01$	

* The mean score (SD) in pg/mL is presented for all cytokine measurements; ns, not significant.

7.3.3 Effects on PBMCs from allergic donors

The mean cytokine scores after one day culture and seven days culture are presented in Table 7.2 and Table 7.3, respectively, and Figure 7.3 and Figure 7.4, respectively. Citrus demonstrated a larger reduction of chronic inflammatory activity than Cydonia and Citrus/Cydonia (TNF-α (day 1): -87.4 (95% CI: -120.3 to -54.5), $p < 0.001$) and -68.0 (95% CI: -100.9 to -35.1), $p < 0.05$, respectively) and had a larger effect on the restoration of the allergen-specific Treg/Th2 balance (IL-10/TNF-α (day 1): 0.34 (95% CI: 0.20 to 0.49), $p < 0.001$ and 0.32 (0.17 to 0.47), $p < 0.01$, respectively). Citrus was also more powerful than Cydonia in the reduction of Th2 pathway activity in both the innate reaction (IL-1β (day 1): -12.4 (95% CI: -22.3 to -2.5), $p < 0.05$) and the outgrowth of the allergen-specific specialized T cell subsets (IL-5 (day 7): -217.8 (95% CI: -361.9 to -73.7), $p < 0.01$). Citrus also demonstrated larger effects than Citrus/Cydonia on the reduction of the Th2

Table 7.3. Differences in mean scores after seven days PBMC cultures in the SAR group, healthy group, and total group (grass pollen stimulation minus medium stimulation)

		Citrus	*Cydonia*	*Citrus/Cydonia*	*p-value Citrus vs. Cydonia*	*p-value Citrus vs. Citrus/Cydonia*	*p-value Cydonia vs. Citrus/Cydonia*
IL-10	SAR	88.4 (28.5)	101.4 (19.5)	115.2 (36.2)	ns	ns	ns
	Healthy	76.4 (38.2)	107.4 (16.5)	102.4 (26.1)	ns	ns	ns
	Total group	82.4 (32.4)	104.4 (17.3)	108.8 (30.5)	ns	ns	$p < 0.05$
IL-5	SAR	509,8 (126.6)	727.6 (95.5)	619.0 (87.7)	$p < 0.01$	ns	ns
	Healthy	358.0 (81.3)	575.8 (98.6)	711.4 (144.7)	$p < 0.01$	$p < 0.001$	ns
	Total group	433.9 (128.3)	651.7 (121.6)	665.2 (122.9)	$p < 0.01$	$p < 0.001$	ns
IL-13	SAR	1470 (390.3)	1823.0 (185.7)	1917.2 (277.7)	ns	$p < 0.05$	ns
	Healthy	1589.2 (300.2)	1864.2 (102.0)	1765.4 (186.6)	ns	ns	ns
	Total group	1529.6 (334.2)	1843.6 (142.9)	1841.3 (237.0)	ns	ns	ns
IFN-γ	SAR	666.8 (126.2)	724.0 (105.2)	621.4 (90.8)	ns	ns	ns
	Healthy	558.0 (130.1)	692.0 (58.4)	571.4 (57.1)	ns	ns	$p < 0.05$
	Total group	612.4 (133.7)	708.0 (82.0)	596.4 (76.2)	$p < 0.05$	ns	$p < 0.05$
IFN-γ/ IL-5	SAR	1.4 (0.5)	1.0 (0.2)	1.0 (0.2)	ns	ns	ns
	Healthy	1.6 (0.4)	1.2 (0.2)	0.8 (0.2)	ns	$p < 0.01$	$p < 0.05$
	Total group	1.5 (0.4)	1.1 (0.2)	0.9 (0.2)	$p < 0.01$	$p < 0.001$	ns
IFN-γ/ IL-13	SAR	0.5 (0.1)	0.4 (0.1)	0.3 (0.1)	ns	ns	ns
	Healthy	0.4 (0.1)	0.4 (0.1)	0.3 (< 0.1)	ns	ns	ns
	Total group	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)	ns	ns	ns
IL-10/ IL-5	SAR	0.2 (0.1)	0.1 (< 0.1)	0.2 (0.1)	ns	ns	ns
	Healthy	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	ns	ns	ns
	Total group	0.2 (0.1)	0.2 (< 0.1)	0.2 (0.1)	ns	ns	ns
IL-10/ IL-13	SAR	0.1 (< 0.1)	0.1 (< 0.1)	0.1 (< 0.1)	ns	ns	ns
	Healthy	< 0.1 (< 0.1)	0.1 (< 0.1)	0.1 (< 0.1)	ns	ns	ns
	Total group	0.1 (< 0.1)	0.1 (< 0.1)	0.1 (< 0.1)	ns	ns	ns

*** The mean score (SD) in pg/mL is presented for all cytokine measurements; ns, not significant.**

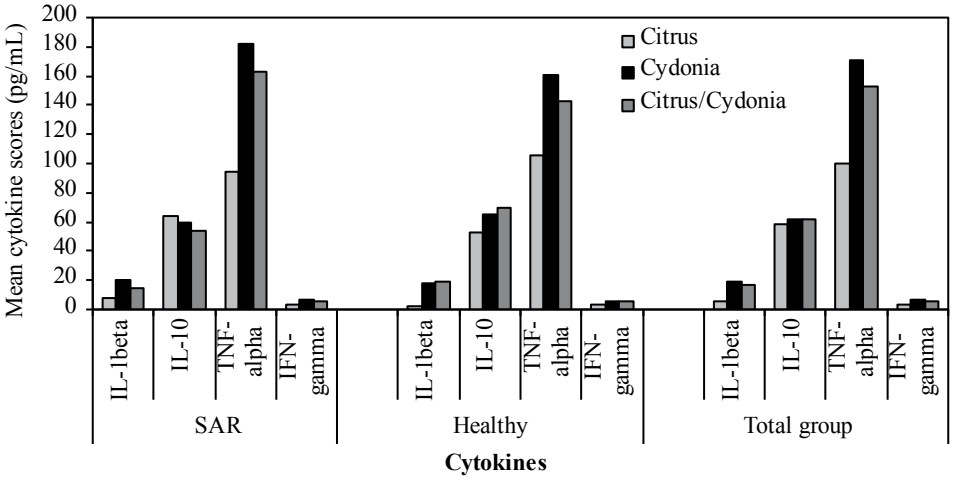


Figure 7.3. Summary of differences in mean scores after one day PBMC cultures in the SAR group, healthy group and total group

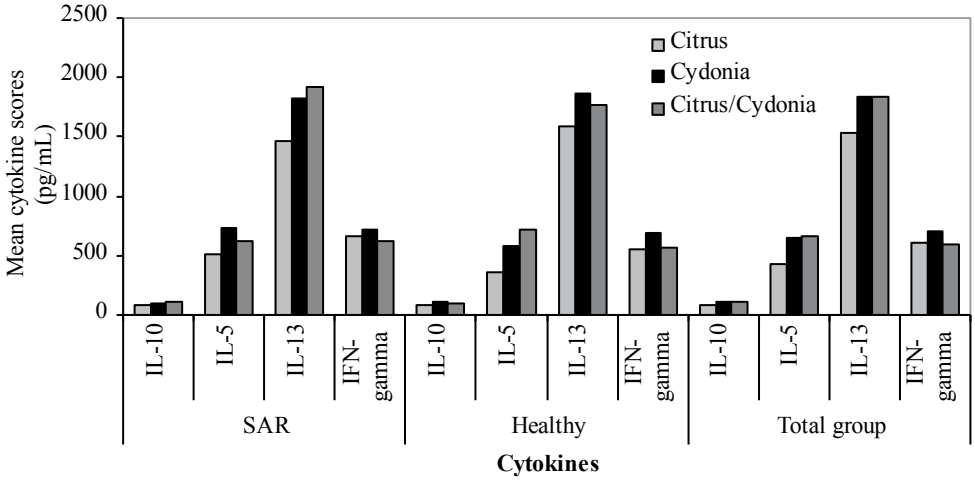


Figure 7.4. Summary of differences in mean scores after seven days PBMC cultures in the SAR group, healthy group and total group

pathway activity of the allergen-specific specialized T cell subsets (IL-13 (day 7): -447.2 (95% CI: -855.9 to 38.5), $p < 0.05$). Both Cydonia and Citrus/Cydonia demonstrated larger effects than Citrus on the innate induction of Th1 pathway activity (IFN-γ (day 1): 3.8 (95% CI: 1.8 to 5.8), $p < 0.01$ and 3.0 (95% CI: 1.0 to 5.0), $p < 0.01$, respectively.

7.3.4 Effects on the PBMCs from healthy donors

Similar to the results in the allergic donor group (Table 7.2), Citrus demonstrated a larger effect in the healthy donor group than Cydonia and Citrus/Cydonia on the reduction of chronic inflammatory activity (TNF-α (day 1): -54.6 (95% CI: -84.2 to -25.0), $p < 0.01$ and -37.6 (95% CI: -67.2 to -8.0), $p < 0.05$, respectively) but not on the restoration of the Treg/Th2 balance (IL-10/TNF-α - day 1). As in the allergic donor group, Citrus was also more powerful than Cydonia in the healthy donor group in reducing Th2 pathway activity in both the innate reaction (IL-1β (day 1): -15.2 (95% CI: -23.7 to -6.7), $p < 0.01$) and the outgrowth of the allergen-specific specialized T cell subsets (IL-5 (day 7): -217.8 (95% CI: -371.4 to -64.2), $p < 0.01$). Unlike the results in the allergic donor group, Citrus also demonstrated larger effects than Citrus/Cydonia on the reduction of Th2 pathway activity in both the innate reaction (IL-1β (day 1): -16.4 (95% CI: -24.9 to -7.9), $p <0.01$) and the outgrowth of the specialized T cell subsets (IL-5 (day 7): -353.4 (95% CI: -507.0 to -199.8), $p < 0.001$). Finally, Citrus was more powerful than Citrus/Cydonia in restoring the Th1/Th2 balance (IFN-γ/ IL-5 (day 7): 0.75 (95% CI: 0.38 to 1.12), $p < 0.01$).

Both Cydonia and Citrus/Cydonia also demonstrated larger effects than Citrus on the innate induction of the Th1 pathway (IFN-γ (day 1): 3.0 (95% CI: 1.1 to 4.9), $p <0.01$ and 2.4 (95% CI: 0.5 to 4.3), $p < 0.05$, respectively). In addition, Cydonia was more powerful than Citrus/Cydonia on the allergen-specific T cell subset related induction of the Th1 pathway (IFN-γ (day 7): 120.6 (95% CI: 10.8 to 230.4), $p < 0.05$) and the restoration of the Th1/Th2 balance (IFN-γ/IL-5 (day 7): 0.38 (95% CI: -0.02 to 0.78), $p < 0.05$) (Table 7.3).

7.3.5 Effects on the total group

As in the allergic donor group and the healthy donor group, Citrus demonstrated a larger reduction of chronic inflammatory activity than Cydonia and Citrus/Cydonia (TNF-α (day 1): -71.0 (95% CI: -92.5 to -49.5), $p < 0.001$ and -52.8 (95% CI: -74.3 to -31.3), $p < 0.001$, respectively). As in the SAR group, Citrus demonstrated larger effects than Cydonia and Citrus/Cydonia in the restoration of the Treg/Th2 balance (IL-10/TNF-α (day 1): 0.23 (95% CI: 0.11 to 0.35), $p < 0.01$ and 0.17 (95% CI: 0.05 to 0.29), $p < 0.01$, respectively). As in the allergic donor and healthy donor groups, Citrus was also more powerful than Cydonia in the reduction of Th2 pathway activity in

Table 7.4. Summary of effects on days 1 and 7 in the SAR group, healthy group, and total group

Cytokines (immunological SAR related subset) on day 1 and day 7	*SAR group*			*Healthy group*			*Total group*		
Day 1	CI vs CY	CI vs CI/CY	CY vs CI/CY	CI vs CY	CI vs CI/CY	CY vs CI/CY	CI vs CY	CI vs CI/CY	CY vs CI/CY
IL-1β (Th2)	CI*			CI**	CI**		CI***	CI**	
TNF-α (Chr. Inf.)	CI***	CI*		CI**	CI*		CI***	CI***	
IFN-γ (Th1)	CY**	CI/CY**		CY**	CI/CY*		CY***	CI/CY***	
IL-10 /TNF-α (Treg/ Chr. Inf)	CI***	CI**					CI**	CI**	
Day 7									
IL-10 (Treg)								CI/CY*	
IL-5 (Th2)	CI**			CI**	CI***		CI**	CI***	
IL-13 (Th2)		CI*							
IFN-γ (Th1)						CY*	CY*		CY*
IFN-γ /IL-5 (Th1/Th2)					CI**	CY*	CI**	CI***	

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$
CI = Citrus; CY = Cydonia; CI/CY = Citrus/Cydonia
Treg = regulatory T cells; Th2 = SAR related Th2 pathway; Th1 = SAR related Th1 pathway; Chron. Inf. = SAR related chronic inflammatory activity
Example: CI** (CI vs CY): Citrus (in relation to Cydonia) demonstrates a SAR related treatment effect with a statistically significant difference < 0.01

both the innate reaction (IL-1β (day 1): -13.8 (95% CI: -20.0 to -7.6), $p < 0.001$) and the outgrowth of the allergen-specific T cell subsets (IL-5 (day 7): -217.8 (95% CI: -331.8 to -103.8), $p < 0.01$). As in the healthy donor group, Citrus was also more powerful than Citrus/Cydonia in the reduction of Th2 pathway activity in both the innate reaction (IL-1β (day 1): -11.4 (95% CI: -17.6 to -5.2), $p < 0.01$) and the outgrowth of the allergen-specific T cell subsets (IL-5 (day 7): -231.3 (95% CI: -345.3 to -117.3), $p < 0.001$).

Both Cydonia and Citrus/Cydonia demonstrated larger effects than Citrus on the innate induction of the Th1 pathway activity (IFN-γ (day 1): 3.4 (95% CI: 2.1 to 4.7), $p <0.001$ and 2.7 (95% CI: 1.4 to 4.0), $p < 0.001$, respectively), which was also found in the allergic donor and healthy donor groups. In addition, Cydonia was more powerful than both Citrus (unlike the results of the other groups) and Citrus/Cydonia (like the results in the healthy donor group) on the allergen-specific T cell subset related induction of Th1 pathway activity (IFN-γ (day 7): 95.6 (95% CI: 3.2 to 188.0), $p < 0.05$) and 111.6 (95% CI: 19.2 to 204.0), $p < 0.05$, respectively). In addition, as in the healthy donor group, both Citrus and Cydonia were more powerful than Citrus/Cydonia in the restoration of Th1/Th2 balance (IFN-γ/IL-5 (day 7): 0.37 (95% CI: 0.06 to 0.68), $p < 0.01$ and 0.56 (95% CI: 0.26 to 0.87), $p < 0.001$, respectively. Finally, unlike the results in the allergic donor group and the healthy donor group, Citrus/Cydonia demonstrated a larger effect on the allergen-specific T cell subset related induction of Treg activity (IL-10 (day 7): 26.4 (95% CI: 1.1 to 51.7), $p < 0.05$) (Table 7.4).

7.3.6 Comparison of the effects on PBMCs from allergic and healthy donors

Statistically significant differences between the allergic donor group and the healthy donor group were demonstrated on day 1 with regard to: TNF-α in the Cydonia group (182.2 vs. 160.2, respectively, $p < 0.05$) and IL-10 (day 1) in the Citrus/Cydonia group (54.0 vs. 69.6, respectively, $p < 0.05$); and on day 7 with regard to IL-5 in the Citrus group (509.9 vs. 358.0, respectively, $p < 0.05$).

7.3.7 Comparison of the results in the different groups

In all three groups (allergic donor, healthy donor, and total group) Citrus consistently demonstrated a selective effect on the reduction of chronic inflammatory activity compared to the other two extracts and on the reduction of allergen-specific Th2 pathway activity (Table 7.4: IL-1β, TNF-α, and IL-5). Cydonia and Citrus/Cydonia consistently demonstrated a selective effect on the induction of the innate Th1 pathway activity compared to Citrus (Table 7.4: IFN-γ - day 1) in all three groups.

In the allergic donor group and the total group, Citrus demonstrated a larger restoration of Treg/Th2 balance compared to the other two extracts (Table 7.4: IL-10/ TNF-α). In the healthy donor group and the total group, Citrus induced a reduction in the activity of an allergen-specific,

specialized T cell subset related Th2 pathway compared to Citrus/Cydonia (Table 7.4: IL-5). Citrus also demonstrated a restoration of the Th1/Th2 balance compared to Citrus/Cydonia (Table 7.4: IFN-γ/ IL-5) and Cydonia demonstrated a larger effect on the induction of the allergen-specific, specialized T cell subset related Th1 pathway compared to Citrus/Cydonia (Table 7.4: IFN-γ-day 7). All analyses with regard to cell viability demonstrated acceptable cell survival, with no signs of toxicity, providing evidence for the safety of the three extracts (data not shown).

7.4 Discussion

In this *in vitro* study we examined the immunological effects of the combined product, Citrus e fructibus/Cydonia e fructibus (Citrus/Cydonia), and separate products Citrus and Cydonia on PBMCs from a group of five healthy and five grass pollen allergic donors to study possible differences in the working mechanisms and magnitude of the effects. Previous *in vitro* studies and clinical studies already repeatedly demonstrated positive treatment effects of Citrus/Cydonia on immunological and clinical SAR related elements [2-6]. The primary hypothesis of the present study was that the combination product Citrus/Cydonia would demonstrate larger SAR-related immunological treatment effects *in vitro* than each of the single products. The secondary hypothesis was that the healthy group would demonstrate larger SAR-related immunological treatment effects *in vitro* than the SAR group.

In grass pollen allergies, a state of chronic inflammation in the upper airways is reminiscent of allergen-induced activity of the innate immune system, which can be analyzed by the presence of TNF-α and IL-1β on day 1 in allergen-induced *in vitro* PBMC cultures. The allergen-induced outgrowth of various T-cell subsets *in vitro* is widely considered to be reminiscent of the presence of selected cytokines after seven days of culture, including IFN-γ (a signature Th1 cytokine), IL-5 (a signature Th2 cytokine), and IL-10 (a signature Treg cytokine) [9, 10, 13, 14]. Based on our results, we conclude that Citrus and Cydonia appear to have different working mechanisms. Citrus has mainly a selective effect on the reduction of chronic inflammatory activity and the reduction of allergen-specific Th2 pathway activity; while, Cydonia has mainly a selective effect on the induction of the innate related, allergen-specific Th1 pathway activity. In theory the combination product Citrus/Cydonia would, therefore, provide an effective therapy that targets SAR from different and possibly additive or synergistic working mechanisms. However, the empirical results demonstrated that Citrus/Cydonia does not provide larger effects than the separate components, both with regard to the reduction of the allergen-specific Th2 pathway activity and the induction of the innate related, allergen-specific Th1 pathway activity *in vitro*. Thus, the first hypothesis needs to be rejected. Based on these results, further effectiveness and (placebo)controlled efficacy studies are indicated to compare the effects of the three products on SAR in vivo.

The differences between the SAR group and the healthy group (TNF-α in the Cydonia group, IL-10 (day 1) in the Citrus/Cydonia group, and IL-5 in the Citrus group) and the comparison of the

results of both groups (Table 7.3) demonstrates overall larger and more effects in the healthy group. These results implicate effects on both the innate arm of the immune response (monocyte-derived TNF and IL-10 production) and the potential Th2 arm of the adaptive immune response (reflected by the IL-5 effect). The second hypothesis of this study could therefore be confirmed, providing empirical underpinning of the assumption that the products support the SAR-related self-healing capacity of the organism and that this capacity is already stronger in healthy persons than in SAR patients before treatment.

The positive effects of Citrus and Cydonia are in line with fundamental studies on the immunomodulating compounds of these fruits. The extracts of Citrus [15] and Cydonia [16, 17] contain several immunologically active compounds, including organic acids, polyphenols, flavonoids, and pectins. For example, flavonoids are mainly present in Citrus fruits as their glycosyl derivatives [18]. Flavonoids appear to be able to regulate acute and chronic inflammatory responses and to suppress production of tumor necrosis factor-α (TNF-α), by macrophages, microglial cells, and mast cells stimulated with lipopolysaccharide (LPS) and others via toll-like receptors (TLRs), and TNF-α-mediated acute and chronic inflammatory responses [19-21]. And for example, the pectins in Cydonia extract generally contain a galacturonic acid. This immunomodulatory compound may strengthen innate-immune responses that may be beneficial in specific conditions, such as attenuation of allergic disease [22,23].

The major limitation of this study is that it is an *in vitro* study that provides, within the classic evidence hierarchy framework, only limited evidence on the effectiveness of the examined extracts in vivo. However, the results are in line with previous *in vitro* studies [2,3] and an *in vitro* study within an in vivo randomized controlled trial [5], demonstrating the positive SAR related immunological effects of Citrus/Cydonia.

7.5 Conclusion

It appears that Citrus and Cydonia have different working mechanisms in the treatment of SAR *in vitro*. Citrus mainly inhibits the chronic inflammatory activity and the SAR related Th2 pathway activity, whereas Cydonia mainly promotes the SAR related Th1 pathway activity. Theoretically, the combination of both extracts would provide an optimal treatment that would target SAR from different directions. However, in this *in vitro* study, the combination product Citrus/Cydonia did not demonstrate larger effects than Citrus and Cydonia separately.

The primary hypothesis of the present study, that the combination preparation Citrus/Cydonia as a whole would demonstrate larger SAR-related treatment effects *in vitro* than each of the single preparations, was rejected. The secondary hypothesis, that the healthy group would demonstrate larger SAR-related treatment effects *in vitro* than the SAR group, was confirmed.

Future studies could focus on the comparison of the effects of the separate extracts and the combination product *in vivo.*

7.6 Acknowledgements

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Chapter 8

The Effect of Gencydo® Injections on Hayfever Symptoms: A Therapeutic Causality Report

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Abstract

Objectives: The aim of this study was to study the effect of Gencydo® (Weleda AG, Arlesheim, Switzerland) injections on hayfever symptoms.

Design: This is a therapeutic causality report based on the practices of 13 Dutch general practitioners.

Subjects: Thirteen (13) patients with a mean history of hayfever with grass pollen allergy of 9 years duration, who in previous years used conventional hayfever medication because of the severity of symptoms during the pollen season.

Interventions: Gencydo injections were given in 12 patients before the onset of and during the grass pollen season, and in one patient during the grass pollen season only.

Outcome measures: Nasal and non-nasal hayfever symptom severity, use of rescue medication (antihistamines or corticosteroids), and subjective experiences of patients were used as outcome measures.

Results: Nine (9) of 13 patients showed no increase of symptom severity during the so-called pollen season. Both the mean maximal total nasal symptom score per patient and the mean total nasal symptom score for the entire study group during the days with a grass pollen count >100 were mild. Percentages of individual "strong" or "severe" nasal symptom scores were low. There was no statistically significant difference in mean total nasal symptom score between the period when the pollen count was <100 and the period when it was >100. Conventional rescue medication for hayfever was used only eight times, all by one person. Nine (9) patients (69%) reported an improvement of symptoms (eight patients cited strong improvement and one patient noted slight improvement).

Conclusions: There are clear indications that Gencydo treatment was effective in a large subgroup of the research population.

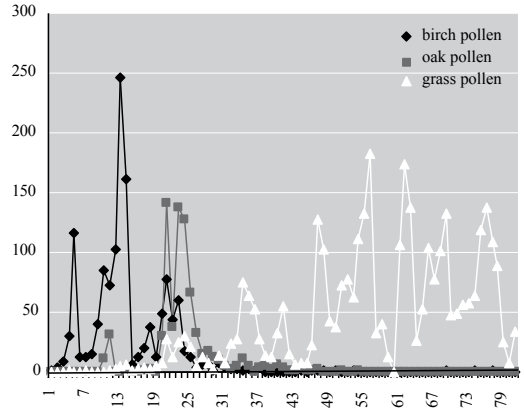
8.1 Introduction

Gencydo® is an anthroposophical medicine that has been specifically prescribed for the treatment of hayfever for more than 80 years. A survey of experienced doctors who often prescribe Gencydo, concerning their experience in practice, showed that Gencydo is expected to produce an effect within a few days to a maximum of 3 weeks. The doctors estimated the maximal efficacy of Gencydo as a monotherapy for hayfever at no greater than 50%.[1] Because the effect of Gencydo on hayfever has not adequately been studied, an outcome study was performed. The central research question was whether Gencydo injections are effective in patients with hayfever and known grass pollen allergy during the grass pollen season.

8.2 Materials and methods

Fig. 8.1. Overview of study patients from first to last weeks of study, in relation to pollen count (birch, grass, and oak), type of seasonal allergy, and subjective assessment of the effect of the treatment.

Black lines indicate birch pollen; grey lines, oak pollen; white lines, grass pollen.



	wk1	wk2	Wk3	wk4	wk5	wk6	wk7	Wk8	wk9	wk10	wk11	wk12	wk13	wk14	wk15	wk16	wk17	wk18	wk19	wk20	wk21	Type of seasonal allergy	Evans 1	Evans 2
DATE	19/2 – 25/2	26/2 – 4/3	5/3 – 11/3	12/3 – 18/3	19/3 – 25/3	26/3 – 1/4	2/4 – 8/4	9/4 –15/4	16/4 - 22/4	23/4 – 29/4	30/4 – 6/5	7/5 – 13/5	14/5 – 20/5	21/5 – 27/5	28/5 – 3/6	4/6 – 10/6	11/6 – 17/6	18/6 – 24/6	25/6 –1/7	2/7 –8/7	9/7-10/7			
1			x																	x		?, grass	1	1
2													x				x					trees, grass	6	6
3				x																x		trees, grass	1	1
4							x													x		trees, grass	1	1
5					x															x		grass	1	1
6						x													x			grass	3	3
7				x															x			grass	1	1
8					x																x	grass	1	1
9										x											x	grass	6	6
10									x												x	grass	2	2
11											x										x	trees, grass	1	1
12														x						x		birch, grass	6	6
13																x					x	grass	1	1

8.2.1 Design

To determine the effect of Gencydo injections on hayfever symptoms an outcome study was designed based on therapeutic causality report (TCR) methodology.[2,3]* Central in this study design was the methodologic element that the relationship between the time spans of an illness (or symptom) before and after intervention can be an important guide to detect the causal relationship between intervention

and subsequent effect. The methodologic principle here is that if a symptom has existed for a long time and disappears shortly after the application of a therapy, this indicates the efficacy of the treatment. In this situation there is a pictorial correspondence between (the onset of) treatment and (the onset of) improvement. Besides that, more certainty can be achieved when each of the times of symptom start, treatment start, and symptom disappearance is not synchronous among the random selected cases in the study.[3]

8.2.2 Study population and eligibility criteria

Patients were randomly recruited for the study through various participating doctors. Thirteen (13) patients were recruited, 12 before the start of the grass pollen season and the remaining patient during the grass pollen season (Fig. 8.1).

Inclusion was based on the following criteria: age > 18 years; history of grass pollen hayfever > 2 years; severity of symptoms: the presence of the need to use conventional medication (antihistamines and/ or corticosteroids) for the treatment of hayfever symptoms during the pollen season in previous years; and willingness to comply with the study (i.e., to have injections as prescribed and to complete questionnaires). The study population consisted of two men and 11 women with a mean age of 38 years range: 13–69 years). The mean history of grass pollen allergy was 9 years (range: 2–30 years).

8.2.3 Intervention

During the study period (March to July 2001), patients were treated with Gencydo injections with a dose and frequency as prescribed by their doctor over an average period of 8 weeks. Adaptation of the prescribed dose and frequency was permitted. The use of other prophylactic medication was not allowed. Rescue medication was twofold: first patients were asked to use Gencydo injection fluid as a nasal spray; if this was not sufficient treatment of the symptoms (according to the subjective view of the patient), conventional symptomatic medication (such as antihistamines and/or corticosteroids) was allowed. Use of all medication was recorded.

8.2.4 Instruments

During the study the patients were requested to complete several questionnaires at conventional intervals over an average period of 12 weeks. All questionnaires and symptom scores were recorded in a patient diary containing all applicable forms. The questionnaires were completed as described below.

Daily symptom score sheet.[4] This questionnaire was completed each morning and evening, noting the following items: nasal (blocked nose, itchy nose, sneezing or a runny nose) and nonnasal symptoms (itchy/ burning eyes, watery eyes, redness around the eyes, itchy ears/ palate) on a 4-point scale (0 = none, 1 = mild, 2 = strong, and 3 = severe). Both total nasal score (TNS) and total nonnasal score (TNNS) could vary between 0 and 12. The overall symptom score was defined as the sum of TNS and TNNS. Using symptom scores remains a reliable method for measuring the level of symptoms.[5]

Final assessment in accordance with Evans.[6] This questionnaire was completed at the end of the intervention period and is a yardstick for the patient's subjective view on the efficacy of the study treatment.

Pollen count. Grass, birch, and oak pollen counts were recorded daily, based on data from the Leiden University Hospital (Fig. 8.1).[7]

8.2.5 Data analyses

Data were entered using SPSS Data Entry, version 2.0 (SPSS Inc., Chicago, IL) and both statistically analyzed with SPSS, version 10.0, and Microsoft Excel (Microsoft Corp., Redmond, WA) and visually analyzed. The analyses performed are described below.

Symptom curve score analyses. In the cases of patients who started treatment before the grass pollen season, effective treatment would result in a symptom curve in which the symptoms remained relatively mild during the "hayfever days" or days with a grass pollen count > 100 ("horizontal symptom curve") in comparison to the previous period of low birch, oak, and grass pollen counts. In the cases of patients who started during the pollen season, effective treatment would also be expressed in a falling symptom curve ("falling symptom curve"). The choice for this form of analysis is based on TCR methodology3 combined with the literature that shows that in general it may be concluded that pollen count level is strongly related to the onset and severity of symptoms experienced by patients during the hayfever season.[8–11] Medication use has been reported to increase until the grass pollination peaks.[12] A grass pollen count >100 is related to severe hayfever symptoms.[7]

Mean maximal total nasal score during high pollen count. Effective treatment would result in a low percentage of patients who individually only have mild symptoms on average.

Mean total nasal score for the entire study population during high pollen count. Effective treatment

would result in only mean mild symptom scores during high pollen count for the entire study population.

Difference in TNS means between high and low pollen count periods. Effective treatment would result in the absence of a difference in TNS means between the period with high pollen count (>100) and the period with low pollen count (<100).

Percentage of strong or severe nasal symptoms in relation to the overall number of nasal symptoms scores. Effective treatment would result in a low percentage of strong or severe symptom scores, both throughout the study period and during the period with a grass pollen count >100.

Rescue medication. Effective treatment would result in a low number of use of antihistamines and corticosteroids.

Subjective judgment of treatment effect. Effective treatment would result in a high percentage of patients indicating that symptoms are improved by Gencydo treatment.

8.3 Results

8.3.1 Symptom score curve

Horizontal symptom curve. Nine (9) patients (69%) showed a horizontal symptom curve. In two patients (15.5%) the symptom score in the high grass pollen count was higher than the score in the low pollen count period. In two patients (15.5%) the symptom curve could not be analyzed because of missing data (Fig. 8.2).

Falling symptom curve. Only one patient started the treatment with Gencydo injections during the high grass pollen count period. The symptom curve of this patient was falling (Fig. 8.3).

8.3.2 Mean maximal total nasal score during high pollen count

During 14 days of the study period the grass pollen count was >100. Two (2) patients did not complete their questionnaires during this period, so they were taken out of this part of the analysis. In the other 11 patients the maximal total nasal symptom scores in the mornings and the evenings on each of the days were described as mild (Fig. 8.4).

8.3.3 Mean total nasal score for the entire study group during high pollen count

Both in the morning and in the evening the mean total nasal symptom score per week remained <4. Thus on average the entire study group experienced only mild symptoms at most experienced (Fig. 8.5).

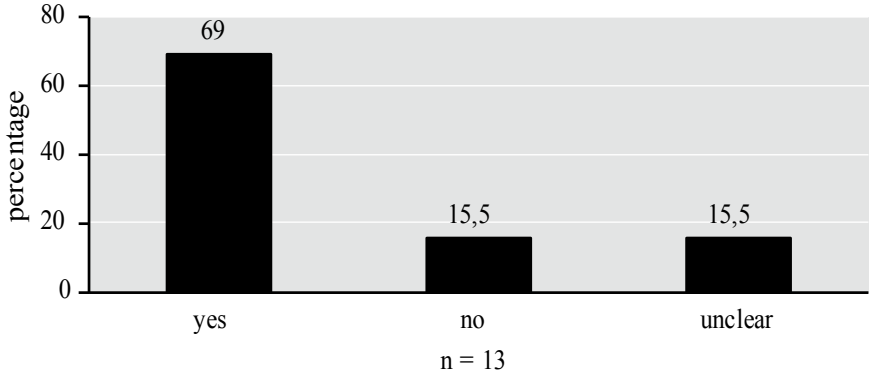


Fig. 8.2. Horizontal symptom curve.

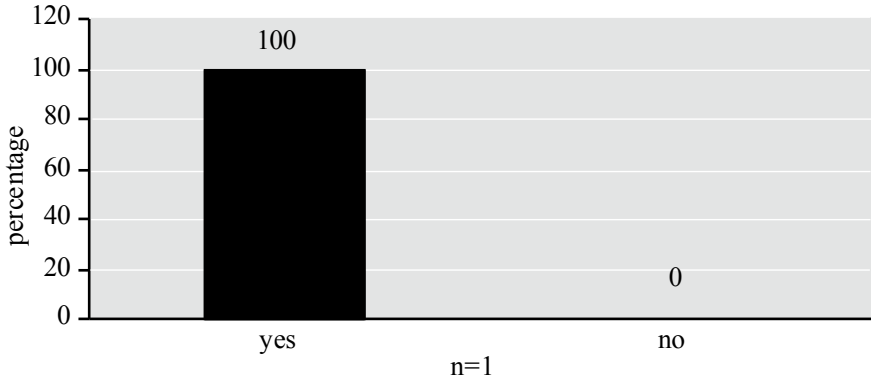


Fig. 8.3. Falling symptom curve.

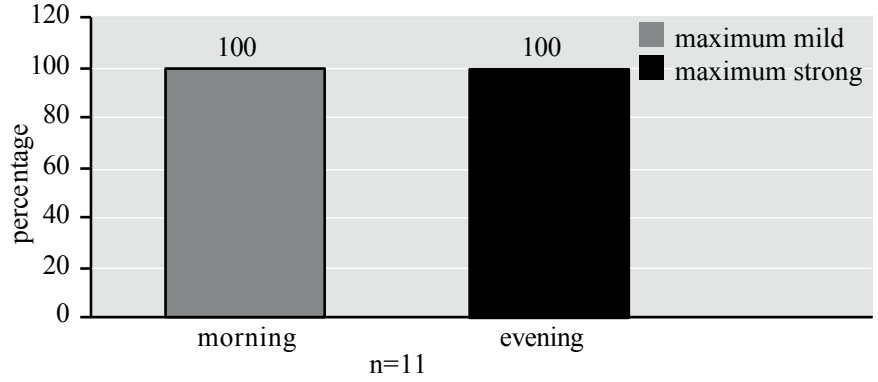


Fig. 8.4. Total nasal score during pollen days with a grass pollen count >100.

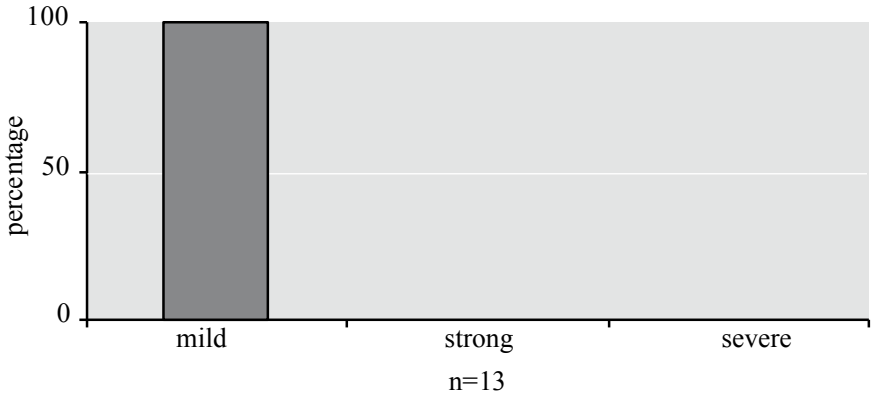


Fig. 8.5. Group mean total nasal symptom score during grass pollen count >100.

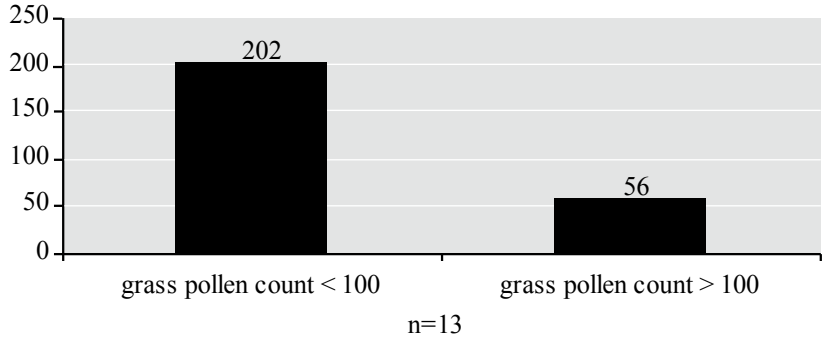


Fig. 8.6. Incidence of the use of Gencydo®-like rescue medication.

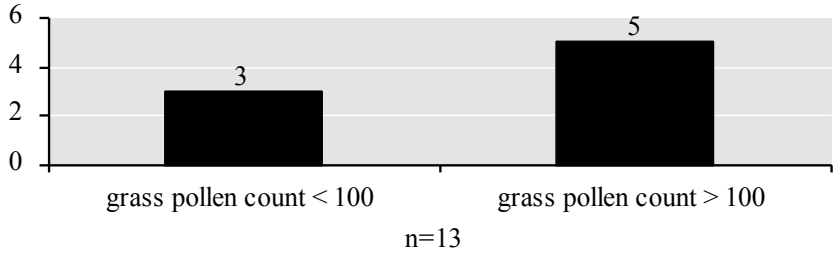


Fig. 8.7. Incidence of the use of conventional rescue medication.

8.3.4 Differences in TNS means between high and low pollen count periods

The total nasal symptom scores (morning) in weeks 13–15, the weeks before the period when the grass pollen count was >100, were compared to the total nasal symptom scores (morning) for the 14 days that the grass pollen count was >100. One patient was excluded from the analysis because of several missing values. The few remaining missing values were replaced with the series means. The Wilcoxon test (nonparametric method) for paired observations in small numbers showed no significant difference between these two periods.

8.3.5 Rescue medication

In periods with a pollen count <100, Gencydo-like rescue medication was used 202 times. Gencydo-like medication was used 56 times during the periods with a pollen count >100 (Fig. 8.6). A total of eight times applied to one patient only (Fig. 8.7).

8.3.6 Percentage of strong or severe nasal symptoms in relation to overall number of nasal symptoms scores

The percentages of individual nasal symptom scores reported as strong or severe over the entire study period were low (Tables 8.1 and 8.2).

8.3.7 Subjective judgment of treatment effect

Nine (9) of the 13 patients (69%) judged that symptoms had been reduced. Eight (8) patients (61.5%) indicated that there had been a strong improvement, and one patient (7.5%) indicated a slight improvement. One patient (7.5%) reported that symptoms had remained the same. Three (3) patients

(23%) said that they did not know whether their symptoms had been reduced (Fig. 8.8). Nine (9) of the 13 patients (69%) also said that it had been the treatment with Gencydo injections that was responsible for the reduction of symptoms. Eight (8) patients (61.5%) found that Gencydo had greatly improved their symptoms, and 1 patient (7.5%) indicated that Gencydo had slightly reduced symptoms. One (1) patient (7.5%) reported that Gencydo had had no effect, and three patients (23%) stated that they did not know whether the product had had any effect (Fig. 8.9).

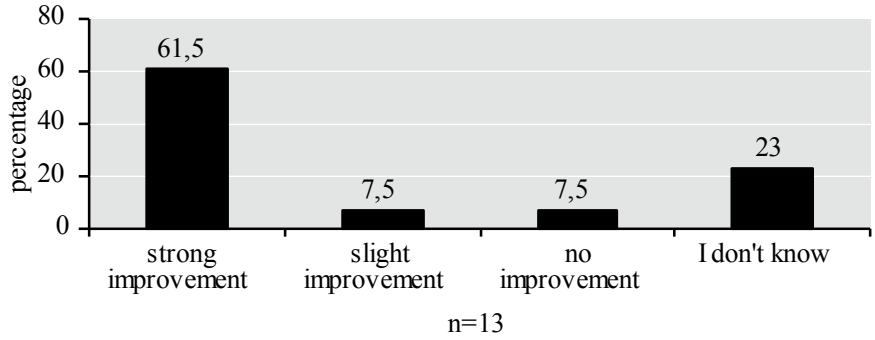


Fig. 8.8. Patients' subjective assessment of degrees of improvement in their symptoms.

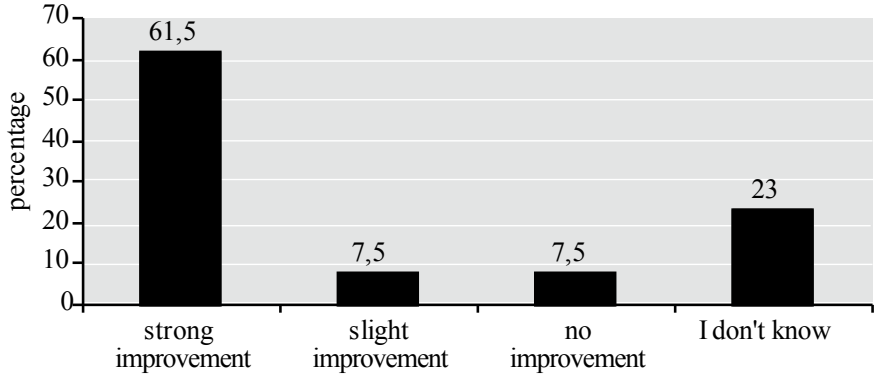


Fig. 8.9. Patients' subjective assessments of effects of Gencydo injections on their symptoms.

Table 8.1. Percentages of individual nasal symptom scores reported as strong

Symptom	*Morning (%), entire period*	*Morning (%), period grass pollen count >100*	*Evening (%), entire period*	*Evening (%), period of grass pollen count >100*
Blocked nose	10.9	12.7	11.9	13.0
Itchy nose	8.9	13.2	9.5	13.6
Sneezing	10.9	11.7	9.6	18.2
Runny nose	11.5	21	9.9	19.1

Table 8.2. Percentages of individual nasal symptom scores reported as severe

Symptom	*Morning (%), entire period*	*Morning (%), period of grass pollen count >100*	*Evening (%), entire period*	*Evening (%), period of grass pollen count >100*
Blocked nose	0.8	0	0.9	0.9
Itchy nose	0.5	0.3	0.7	0
Sneezing	3.1	1.5	2.6	4
Runny nose	3.5	6.1	2.5	3.8

8.4 Discussion

In this study the effect of Gencydo injections on patients with hayfever and a grass pollen allergy were examined. The research population had a mean hayfever history of 9 years, and in former years the necessity to use conventional hayfever medicines to control symptom severity. The expectation was therefore that without conventional hayfever medications the effect of Gencydo injections would be expressed in low total nasal and non-nasal symptom scores during the pollen season and especially during the period with a grass pollen count >100.

The analyses of symptom curves, the use of rescue medication, and patients' own subjective assessments show that there was an improvement in the symptoms in many of the patients both individually and in the group as a whole. Of particular importance here are the various analyses of the severity of the overall nasal symptoms during the period with a grass pollen count >100. These analyses show that (1) on average during this period, the sample group as a whole experienced only mild symptoms; and (2) the highest total nasal score in this period was reported to be mild in 100% of patients. Thus despite the high pollen count none of the individual patients had strong or even severe symptoms. This also points to only a small increase in symptoms despite the increase in pollen count.

Although this study design did not control for the influence of a placebo effect, this appeared not to have influenced the observed effect to a great extent. The main reason for this assumption is that results of Danish empirical research recently cast serious doubt on the existence of a placebo effect.[13]

Because the patients were aware of the purpose of the research it is possible that the patients under-reported their symptoms or reported the severity of these symptoms as being less severe than they actually were. This possibility certainly cannot be excluded, but it does not seem likely that this contributed significantly to the ultimate effect. The study used the same symptom score questionnaire,[4] which has often been used in clinical studies with good results.

On the basis of this study it can be concluded that there are clear indications that Gencydo injections was effective in the treatment of hayfever, at least in some of the population. Future re-

plicated studies, possibly with larger numbers of patients, greater checks for possible information bias, and control groups will clarify whether the effect is replicable and whether there are specific prognostic subpopulations of hayfever patients in whom Gencydo injections have a greater or lesser effect. It would also be worthwhile, given the anthroposophical nature of the medication and the experience of the prescribing doctors, to carry out multiyear research to determine the optimal outcome of Gencydo injections. The reason for this is that Gencydo injections are expected to achieve their optimum outcomes of treatment of hayfever only after several years. A multiyear study could determine the outcomes of this treatment.

Acknowledgements

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Chapter 9

Citrus/Cydonia comp. Subcutaneous Injections Versus Nasal Spray for Seasonal Allergic Rhinitis: a Randomized Controlled Trial on Efficacy and Safety

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Abstract

Background: Clinical experiences, *in vitro* and clinical studies have demonstrated the curative potency and safety of Citrus/Cydonia compositum in seasonal allergic rhinitis treatment.

Objectives: To compare the efficacy and safety of two routes of administration (nasal spray versus subcutaneous injections).

Methodology: Design: a national, randomised, comparative clinical trial with two parallel groups. Participants: 23 patients fulfilled the study requirements. Intervention: after a one- or two-week wash-out period, 23 patients were randomized to a 6 weeks treatment period.

Outcomes: Immunological and symptom severity changes, and safety. Immunologic outcome assessments were blinded to group assignment. 23 patients were randomized and from 22/23 patients (11 in each group) blood samples were analyzed before and after treatment.

Conclusion: Both routes of administration demonstrate immunological and clinical effects, with larger inflammatory and innate immunological effects of the nasal spray route and larger allergen-specific clinical effects of the subcutaneous route, and are safe.

Keywords: seasonal allergic rhinitis, Citrus/Cydonia comp., Gencydo, subcutaneous injection, nasal spray, randomized clinical trial, curative health promotion

9.1 Introduction

Seasonal allergic rhinitis (SAR) or hay fever is a type I immediate hypersensitivity reaction mediated by specific IgE antibody formation to a seasonal allergen, leading to mucosal inflammation characterized by sneezing, itching, rhinorrhoea, and nasal blockage. Pollen from wind pollinated grasses, trees, weeds, and spores from fungi are the most common aeroallergens. The estimated prevalence of SAR in adults in several Western countries is 8 - 15% [1,2]. The treatment of choice is the symptomatic treatment with antihistamines and/or local corticosteroids. Immunotherapy is indicated in a limited subpopulation of patients that are insufficiently treated with antihistamines and/or local corticosteroids [3]. Since SAR is a chronic disease and the treatment of choice for most patients is purely symptomatic, most SAR patients must be treated for decades.

Citrus/Cydonia compositum (comp.) 1% solution for injection and Gencydo nasal spray are medicinal products, which contain exactly the same ratio of constituting substances, lemon juice (Citrus limon, succus) and an aqueous extract from quince (Cydonia oblonga, fructus rec.): 1ml contains 8-12 mg Citrus limon, succus, corresponding to 0.65 mg fruit acid, calculated as citric acid and 30 mg aqueous extract from Cydonia oblonga, fructus rec. (1:2.1). For more than eighty years, Citrus/Cydonia comp. has been prescribed for SAR patients.

The experiences of prescribing general practitioners (GP's) is that SAR patients are claiming to permanently suffer less from hay fever symptoms or even that they are free from complaints after the treatment with Citrus/Cydonia comp. [4]. Positive effects, without side effects, were also observed in two cohort studies: a group of 13 patients suffering from grass pollen SAR treated with subcutaneous injections [5] and in a group of 140 patients, who were treated with nasal spray [6]. Recently, the immunological pathways underlying the positive effects of Citrus/Cydonia comp. in SAR patients were studied *in vitro* [7, 8]. These studied demonstrated a restoration of the disturbed immune state of allergic rhinitis patients by direct modulation of the Th1/Th2 balance. Such a perturbed Th1/Th2 balance is widely considered the hallmark of allergic disease [9]. In addition, it was demonstrated that Citrus/Cydonia comp. significantly reduced the histamine production and the inflammatory mediator release from mast cells in a dose-dependent manner [8].

The objectives of this study were to assess and compare the immunological and clinical effects and to assess the safety of two routes of administration (subcutaneous injection versus nasal spray) of Citrus/Cydonia comp. 1% in order to determine which route of administration demonstrated superior efficacy and safety.

9.2 Methods

This is a national, stratified (age: 18-40 or 41-60; and RAST (radioallergosorbent testing) scores for birch pollen: > 2 or < 3; with a balanced randomization), comparative, single-blind (laboratory) clinical trial with two parallel groups conducted in The Netherlands.

9.2.1 Participants

Eligible participants were all adults aged 18 to 60, suffering from SAR for at least two years, with a RAST for grass pollen ≥ 2, suffering from the following nasal symptoms: sneezing, itching nose and watery nasal discharge, with a severity score of at least two of the three symptoms ≥ 2 (ranging from 0 = not present to 3= severe) and the necessity to use antihistamines and/or corticosteroids for treatment of symptoms for previous (at least two) years. Exclusion criteria were: chronic inflammatory autoimmune diseases; allergic (hypersensitive) to one of the constituents of Citrus/Cydonia comp. or Gencydo nasal spray; pharmacological treatment of allergic rhinitis or use of other preparations containing Citrus and/or Cydonia extracts within the last two weeks prior to enrolment into the study; use of cromoglycates in the last month before study onset; concomitant pharmacological treatment indicated for seasonal allergic rhinitis such as antihistamines, corticosteroids or other preparations; participation in a further clinical trial at the same time or within the previous 4 weeks prior to enrolment into this study; pregnancy or lactation; and severe internal or systemic disease.

9.2.2 Ethics

The medical ethical committee (STEG-METC, Almere, The Netherlands) approved the study. Individual patients gave written informed consent.

9.2.3 Interventions

Patients received the treatment in accordance with the Summary of Product Characteristics; either Citrus/Cydonia comp. 1% subcutaneous injections (1 mL, ampoules available under the trade name Gencydo® 1%, manufacturer Weleda AG, Schwäbisch Gmünd, Germany) twice per week, or the Gencydo nasal spray (1-2 sprays in each nostril) four times per day (available under the name "Gencydo neusspray" in the Netherlands, manufacturer Weleda AG, Schwäbisch Gmünd, Germany). This application strategy resulted in the nasal spray group receiving four times the active dose compared with the injection group. The composition of Citrus/Cydonia comp. 1% solution for injection and Gencydo nasal spray spray is identical. Both medicinal products contain lemon juice (*Citrus limon*) and an aqueous extract from the fruit of a quince (*Cydonia oblonga*): one milliliter of these preparations contains 8-12 mg *C. limon* juice corresponding to 0.65 mg fruit acid, calculated as citric acid, and 30 mg *C. oblonga* aqueous extract (drug-extraction-rate: 1:2.1).

9.2.4 Objectives

The objectives of the present study were to test (1) the immunological (primary objective) and clinical (secondary objective) superior efficacy of the subcutaneous route of administration compared to the nasal spray route of administration; and (2) the safety of both routes of administration (tertiary objective) in a group of adult, grass pollen SAR patients.

The primary hypothesis was that the subcutaneous route of administration demonstrated superior immunological efficacy; the secondary hypothesis was that the subcutaneous route of administration demonstrated superior clinical efficacy; and the third hypothesis was that both routes of administration were safe. Based on the results of the study, one route of administration will be studied in a future placebo-controlled, randomized trial.

9.2.5 Outcomes

Primary endpoints were SAR-related changes in immunological parameters between the start of the treatment (baseline) and after six weeks of treatment (post-baseline). From each patient 8 ml of peripheral blood was collected from which peripheral blood mononuclear cells (PBMCs) were isolated. PBMCs were cultured in Yssel's medium at 37°C in a humidified atmosphere with 5% CO2 at a density of $1x10^6$ viable cells/mL. Cells were plated out in 48 well plates at a concentration of $1x10^6$ cells/mL and cultured at 37°C. After five hours, in which the cells adapted to the culture conditions, various stimuli or a matching volume of medium were added. Cultures were stimulated polyclonally with 150 ng/mL anti-CD3 plus 100 ng/mL anti-CD28 monoclonal antibodies (BD Pharmingen, San Diego, Calif, USA) or cultured in medium only [10]. In addition, we performed allergen-specific stimulation of 10^6 cells/ml in 1 ml cultures with applied pollen extract (Phl p 1 from Timothy grass, Phleum pretense; Biomay Vienna, Austria; 10 µg/ml in medium).

The proliferation capacity, cell survival, toxicity and total production capacity of several cytokines (e.g., IL-10, TNF-α, IFN-γ, IL-4, IL-5 and IL-13) in the culture supernatants of the PBMCs were analyzed at day 1 (demonstrating the reaction of the innate immune system) and day 7 (demonstrating the reactions of specialized T cells subsets) in the laboratory [10,11]. The following changes in cytokine production levels were regarded as a positive immunological SAR treatment effect [12-14]:

1. The reduction of activation state of the SAR related immune subsystem: reduction of (grass pollen stimulated minus medium stimulated) IL-10 and TNF-α at day 1;
2. The induction of (regulatory) T-cells (Tregs): increase of (grass pollen stimulated minus medium stimulated) IL-10 at days 1 and 7;
3. The induction of Th1 activity: increase of (grass pollen stimulated minus medium stimulated) IFN-γ at days 1 and 7;
4. The reduction of Th2 activity: reduction of (grass pollen stimulated minus medium stimulated) IL-1β at day 1; IL-5 and IL-13 at day 7;

5. The reduction of chronic inflammatory activity: reduction of (grass pollen stimulated minus medium stimulated) TNF-α at day 1;
6. The restoration of the Th1/ Th2 balance: the increase of (grass pollen stimulated minus medium stimulated) IFN-γ/ IL-5 and IFN-γ/ IL-13 ratios at day 7; and/or
7. The restoration of the Treg/Th2 balance: the increase of (grass pollen stimulated minus medium stimulated) IL-10/ TNF-α ratio at day 1; the increase of (grass pollen stimulated minus medium stimulated) IL-10/ IL-5 and IL-10/ IL-13 ratios at day 7.

Secondary efficacy variables were the change in nasal and non-nasal allergic rhinitis symptom severity before treatment start and after each week of treatment. The severity of nasal symptoms (nasal obstruction, itching nose, sneezing, and watery nasal discharge) and non-nasal symptoms (itchy/burning eyes, watery eyes, redness of eyes, and itching ears/throat) symptoms were recorded twice per day (in the mornings and evenings) by the patient. The disease-specific symptom severity questionnaire was provided to the patient as an online questionnaire in Dutch: 0 = no symptom, 1 = mild, 2 = moderate, and 3 = severe. Completion of the online questionnaires by the participants was checked daily.

Blood samples for immunological analyses were taken before and after six weeks of treatment. SAR related symptom severity scores were measured twice a day (morning and evening) during both the one-week or two-week washout and the six-week treatment periods.

Pollen counts were acquired on a daily basis for grass pollen and birch pollen from the Leiden University Medical Centre (http://www.lumc.nl/con/1070/85683/105795/105824/, Figure 9.2) during both the washout period and the treatment period. Safety was measured by means of adverse events surveillance and laboratory parameters (abnormal findings in the immunological analyses).

9.2.6 Sample size

Based on an expected mean difference of IL-10 of 1.834 pg/ml, which is in line with the former *in vitro* studies [7], with a two-sided 5% significance level and a power of 95%, a sample size of 14 patients per group, thus in total 28 patients, was necessary.

9.2.7 Randomization-Sequence generation

Pre-stratification on age (18-40 or 41-60) and RAST scores for grass pollen (> 2 or < 3) was used to divide participants into four subgroups. For allocation of the participants, a computer-generated list of random numbers was used. The randomization list was generated with the Random Allocation Software Program version 1.0 (Saghaei, Isfahan University of Medical Sciences, Iran) using a random block size of two in order to guarantee a balanced allocation.

9.2.8 Randomization-Allocation concealment

After a one-week (for patients that had not been treated for SAR in the week before enrollment) or a two-week (for patients that had been treated for SAR in the week before enrollment) washout period, patients were assigned to four strata and then randomized to a six-week treatment period.

9.2.9 Randomization-Implementation

The two investigational medicinal products were assigned to treatment A and treatment B by the sponsor for all patients who were assigned randomly to either one of the two treatment groups. The label assignment was kept at the sponsor's site until all study data had been entered in the study database.

9.2.10 Blinding

The procedure of label assignment and random allocation to treatment A and treatment B was carried out to guarantee a blinded analysis of the primary efficacy variable, the immunological laboratory parameters. Laboratory personnel had no information about any treatment of patients during the period of all laboratory procedures and analyses. Unblinding took place after all statistics had been performed.

9.2.11 Statistical analyses

Only evaluable patients, with immunological parameters measured before randomization and post-baseline (after six weeks of treatment), were included in the primary analysis for each primary efficacy variable (Per Protocol Set, PPS). In order to eliminate the impact of drop outs on efficacy results, in addition a subset of observed cases (OC) was evaluated. This subset included data only from randomized patients who did not discontinue prematurely and were available for evaluation at the designated assessment times. Missing values of the immunological parameters (e.g. values below cytokine detection limits) were not replaced. Missing values of the symptom severity scores, in absence of a major protocol violation, were replaced in two ways: by week means and last observation carried forward (LOCF) [15], and subsequently compared.

Regular descriptive statistics were performed with regard to demographical and categorical data. To test the primary hypothesis, superiority of Citrus/Cydonia comp. 1% solution for injection as compared to Gencydo nasal spray with respect to the primary target variable changes in immunological parameters, descriptive statistics, Student's t-tests and non-parametric Wilcoxon Signed Ranks tests were performed to compare means and mean base-10 log transformed scores of the

immunological parameters in both groups and to calculate 95% confidence intervals.

To test the secondary hypothesis, superiority of Citrus/Cydonia comp. 1% solution for injection as compared to Gencydo nasal spray with respect to the secondary target variable lower seasonal allergic rhinitis symptom severity scores, mean scores and standard deviations per week were calculated. Then, multivariate analysis techniques were used to compare the symptom severity mean week scores in the subcutaneous and the nasal spray group for each of the 6 weeks and to calculate 95% confidence intervals. In addition, Cohen's delta was calculated for both routes of administration to estimate the effect sizes.

The safety analysis was based on the Full Analysis Set (FAS) of all patients who took at least one dose of the randomised study medication. During the course of the study, all adverse events, irrespective of the relationship to the study medication or study procedure, were recorded on the adverse event forms contained in the Case Report Form (CRF). During each monitoring visit, the person responsible for monitoring of the clinical trial and the investigator reviewed all adverse events. With respect to all adverse events, the investigator was responsible for ensuring that correct and complete information was documented on the adverse event forms in the CRF. The assessment of the severity of an adverse event (AE) (mild, moderate, severe) was also performed by the investigator. The causal relationship with the administration of the investigational drug or a study procedure was assessed according to the categories as described by the Uppsala Monitoring Centre and recommended by the WHO (certain, probable, possible, unlikely, conditional and unassessible), both by the investigator and the sponsor.

9.3 Results

9.3.1 Participants flow

From 34 included patients, 11 patients dropped out before randomization either due to too low RAST score for grass pollen (n = 8), withdrawal of informed consent (n = 1), use of medication during wash-out period (n = 1) or too mild symptoms (n = 1). After randomization at baseline, the Full Analysis Set (FAS) contained 23 patients (12 patients in the Citrus/Cydonia group and 11 patients in the Gencydo group). There was one dropout in the Citrus/Cydonia group after three weeks of treatment due to an adverse event. From another patient in the Citrus/Cydonia group the second blood sample got lost and therefore immunological analyses could not be performed. For the immunological analyses there were 22 patients (11 patients in each group) in the Per Protocol Set (PPS) and 21 patients (10 patients in the Citrus/Cydonia group and 11 patients in the Gencydo group) in the Observed Cases (OC) subgroup (Figure 9.1).

Patients were visited during the screening, before the start of the treatment and at the end of the

treatment. A telephone visit was performed after three weeks of treatment.

A total of 20 out of 23 patients (87%) started treatment in weeks 23 and 24: 10 of the 12 patients in the Citrus/Cydonia comp. group (83%) and 10 of the 11 patients in the Gencydo group (91%). Three other patients started in week 26: two patients in the Citrus/Cydonia comp. group and one patient in the Gencydo group (data not shown).

9.3.2 Recruitment

All eligible patients were recruited from a single centre, the Louis Bolk Institute (Driebergen, NL). The first patient was included on May 19, 2009 and the last patient completed the study on August 11, 2009.

9.3.3 Baseline characteristics and baseline homogeneity

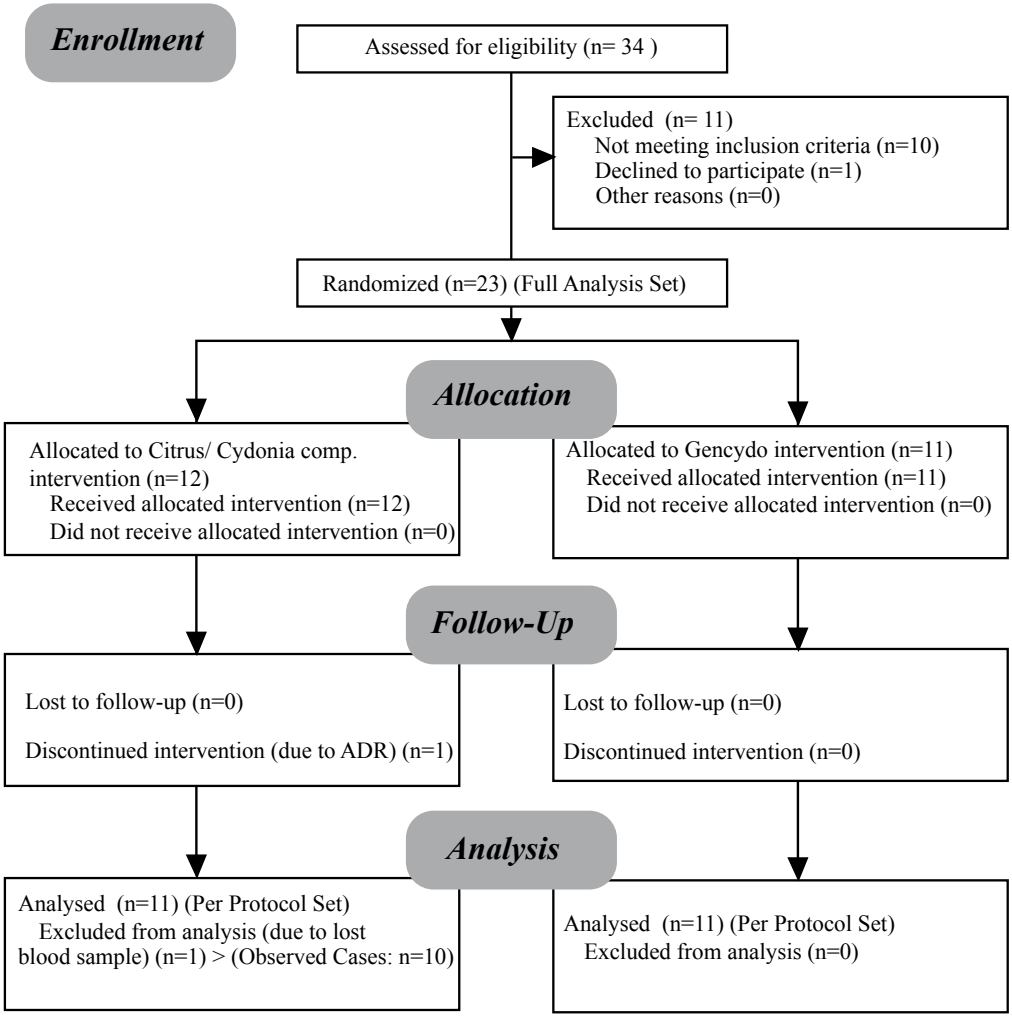
Baseline homogeneity of the treatment groups was accomplished with regard to the following SAR related aspects: RAST scores (grass pollen and birch pollen), worst SAR symptom severity during the previous pollen season (anamnestically), SAR symptom severity scores in the morning and the evening during the wash-out period (Table 9.1) and onset of interventions (data not shown). Homogeneity of the treatment groups was accomplished with regard to the following SAR non-related aspects: age, height, weight, smoking status, ethnic origin, remaining medical history, prior medication, vital signs and physical examination. Homogeneity of the treatment groups was not accomplished with regard to gender as a SAR non-related aspect.

9.3.4 Pollen counts

The grass pollen counts during the wash-out period and the treatment period demonstrated that the grass pollen season in this period, apart from week 22, was not severe and the influence of the birch pollen was almost null (number of grains of pollen in a cubic meter of air/ 24 hours) (Figure 9.2). Grass pollen counts higher than 100 were measured in only four days in week 22, thus before the treatment period. After week 27, the week means were lower than 15. Since pollen counts higher than 80 – 100 are correlated with severe symptoms, and pollen counts higher than 10 - 15 are correlated with mild symptoms, the period after week 27 is not clinically relevant for this study [16].

Since patients had their wash-out period for one week or two weeks and in different weeks the mean pollen count during the entire wash-out period was calculated. We calculated the exact mean pollen count in the wash-out period by adding all real pollen counts from day 1 to day 7 of the first wash-out week per patient and subsequently calculating the means of the pollen count. This resul-

Figure 9.1. Participant flow: primary efficacy analysis data sets



ted in a mean pollen count in the wash-out period of 44.6 (sd = 11.6) for the whole population, 46.8 (sd = 17.5) for the Citrus/Cydonia comp. group and 39.1 (sd = 10.7) for the Gencydo group. These differences between both treatment groups were small, not statistically significant and clinically irrelevant, since the categories of symptom severity that are correlated to pollen count are: mild (10/15 – 45/50), moderate (45/50 – 80/100) and severe (80/100 and higher), which implicated that both mean scores (39.1 and 46.8) were mild/ (borderline) moderate.

9.2.5 Numbers analyzed

23 patients were randomized (Full Analysis Set). From 22 patients (11 in each group) blood samples were analyzed before and after treatment (Per Protocol Set) and from 21 patients (10 patients in the Citrus/Cydonia group and 11 patients in the Gencydo group) in the Observed Cases (OC) subgroup (Figure 9.1).

From 20 of the 23 randomized patients (10 in each group) symptom severity scores were analyzed.

9.2.6 Primary outcome variables: immunological analyses

Analyses at day 1 of allergen-specific stimulation

The analyses demonstrated acceptable cell survival, with no signs of toxicity (<5 % apoptotic cells, data not shown). Base-10 log transformations of the data, deemed mandatory due to an unequal distribution.

The cytokine analyses of the PPS comparing medium stimulation versus allergen stimulation, demonstrated statistically significant increases both at baseline vs post-baseline of IL-10 (Gencydo group: 1.29 (95% CI: 1.15 – 1.44), $p < 0.001$ vs. 0.97 (95% CI: 0.56 – 1.37), $p < 0.001$), (Citrus/Cydonia group: 1.03 (95% CI: 0.70 – 1.37), $p < 0.001$ vs. 1.19 (95% CI: 0.64 – 1.74), $p < 0.01$) and TNF-α (Gencydo group: 1.44 (95% CI: 1.18 – 1.71), $p < 0.001$ vs. 1.02 (95% CI: 0.51 – 1.53), $p < 0.01$) (Citrus/Cydonia group: 1.34 (95% CI: 0.76 – 1.91), $p < 0.001$ vs. 0.96 (95% CI: 0.62 – 1.30, $p < 0.001$) in both treatment groups, but not of IFN-γ cytokine production (data not shown).

Comparison of the results of allergen stimulation minus medium stimulation at baseline and post-baseline, demonstrated a reduction in TNF-α production level in the Gencydo group (-0.50 (95% CI: -0.08 to -0.93), $p < 0.05$), (Table 9.2). Comparing results of allergen stimulation at baseline and post-baseline demonstrated also a reduction of TNF-α in the Gencydo group (PPS: -0.36 (95% CI: -0.02 to -0.71), $p < 0.05$ vs. OC: -0.33, $p < 0.05$). IL-10 and IFN-γ levels demonstrated no statistically significant changes. The analyses of the OC subgroup demonstrated no significant differences compared to the analyses of the PPS.

Analyses at day 7 of allergen-specific stimulation

The analyses of the PPS and OC demonstrated only a baseline to post-baseline decrease of IL-10 cytokine production (Citrus/Cydonia comp. group (PPS vs. OC): -0.68 (95% CI: -0.37 to -1.0), $p < 0.01$ vs. 0.67, $p < 0.05$; Gencydo group: -0.44 (95% CI:-0.19 to -0.68), $p < 0.01$) and no statistically significant changes in all other cytokines (IL-12, IL-5, IL-13 and IFN-γ and relevant ratios of cytokines) in both groups (Table 9.3).

9.2.7 Secondary efficacy results: symptom severity

Total symptom scores (TSS) were analyzed during washout, and week 1 until week 5 of treatment. Due to a very low pollen count during week 6, data of week 6 of treatment were excluded from the analyses. Missing values were replaced in two ways: mean week scores and Last Observation Carried Forward. When compared to the dataset without the missing values, the mean week scores demonstrate only small, non-significant differences (data not shown).

A one-way-analysis of covariance (ANCOVA) with parallel regression lines of the TSS *morning* data demonstrated a statistically significant overall reduction of TSS scores in the period from wash-out to respectively 2, 3, 4 and 5 weeks (Figure 9.3). After three weeks of treatment until five weeks of treatment there was also a statistically significant difference between (the level of the parallel regression lines of) the two treatment groups, demonstrating larger effects of the subcutaneous route of administration. Non-parametric tests demonstrated statistically significant differences between wash-out and all weeks of treatment (week 1 - 5) for the whole group; between wash-out and weeks 2 – 5 of treatment for the Citrus/Cydonia group; and no statistically significant differences between wash-out and all separate weeks of treatment for the Gencydo group. The TSS reduction in the Citrus/Cydonia group between washout and week 5 of treatment was 4.8 (95% C.I.: 1.7 to 7.9) (Table 9.4). The analyses of the PPS demonstrated small but not significant differences (data not shown).

A one-way-analysis of covariance (ANCOVA) with parallel regression lines of the TSS *evening* data demonstrates a statistically significant overall reduction of TSS scores in the period from wash-out to 2, 3, 4 and 5 weeks (Figure 9.4). There was a statistically significant difference between (the level of the parallel regression lines of) the two treatment groups at all times of treatment. Non-parametric tests demonstrated statistically significant differences between wash-out and all weeks of treatment (week 1 - 5) for the whole group, the Citrus/Cydonia group and the Gencydo group. The TSS reduction between washout and week 5 of treatment was 4.5 (95% C.I.: 1.7 to 7.2) in the Citrus/Cydonia group, was 4.1 (95% C.I.: -0.4 to 7.8) in the Gencydo group, and was 4.3 (95% C.I.: 2.2 to 6.4) in the total group (Table 9.4). The analyses of the PPS demonstrated small but not significant differences (data not shown).

Cohen's delta effect sizes (wash-out versus five weeks of treatment) were calculated for both the pooled data of all patients, and the Citrus/Cydonia group and the Gencydo group separately. Cohen's delta effect size for the pooled data of all patients in the morning was medium and large

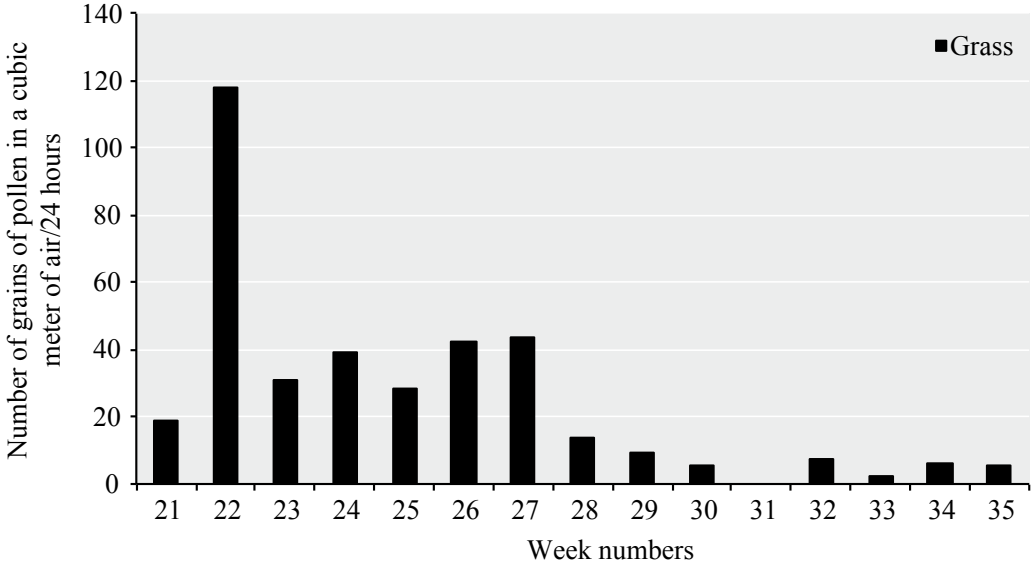


Figure 9.2: Mean grass pollen counts per week. Weeks 21–35 (2009).

in the evening (respectively: 0.65 and 0.99). Cohen's delta effect sizes were large for the subcutaneous route of administration, both in the morning and the evening (respectively: 1.19 and 1.37), and for the nasal spray route of administration medium, both in the morning and the evening (respectively: 0.49 and 0.79) (but the nasal spray morning scores difference was not statistically significant). In order to control for possible bias due to the natural course of the disease (whereas the pollen count was very low after week 27), we post hoc reanalyzed the Cohen's delta effect sizes of all data without the data of the three patients that started treatment in week 26. Cohen's delta effect sizes for the pooled data of all patients now were large both in the morning and the evening (respectively: 0.98 and 0.81). Cohen's delta effect sizes again were large for the subcutaneous route of administration, both in the morning and the evening (respectively: 1.32 and 1.20), and for the nasal spray route of administration were now large in the morning and medium in the evening (respectively: 0.87 and 0.45) (but the nasal spray morning scores difference was still not statistically significant).

9.2.8 Adverse events

The safety analysis set consisted of the 23 randomised patients of the Citrus/Cydonia and the Gencydo group. During the treatment period, a total of 9 adverse events (AEs) were observed in 6/23 patients (26.1%). The number of patients suffering from AEs in the Citrus/Cydonia group (five AEs in 2/12 patients; 16.7%), was lower compared to the Gencydo group (four AEs in 4/11 patients; 36.4%). The incidence of adverse events was comparable between the two treatment groups. None of the AEs were classified as serious. A causal relationship could not be excluded in all of these adverse events. In the Citrus/Cydonia group, three AEs were assessed as 'probably related' and two as 'possibly related'. In the Gencydo group, 3 AEs were assessed as 'certain related' and 1 AE as 'probably related'. One patient in the Citrus/Cydonia group terminated the study prematurely due to an AE (itching skin), which was assessed as 'possibly related' to the study medication. Three of the nine AEs were of mild intensity, they all occurred in the Gencydo group. Two AEs in one patient in the Citrus/Cydonia group and one AE in the Gencydo group were classified as moderate. Three AEs in the Citrus/Cydonia group, all in one patient, were classified as severe. There were no serious AEs.

9.3 Discussion

In this study we compared two routes of administration (nasal spray (Gencydo) versus subcutaneous injections (Citrus/Cydonia comp.) on immunological and clinical effects of SAR treatment and safety in a randomized controlled trial. The primary hypothesis was that the subcutaneous route of administration demonstrated superior immunological efficacy; the secondary hypothesis was that the subcutaneous route of administration demonstrated superior clinical efficacy; and the third hypothesis was that both routes of administration were safe. Based on the results of this study, one route of administration would be selected to be further tested in a placebo-controlled, randomized trial.

9.3.1 Interpretation

The immunological data comparing medium stimulation versus allergen stimulation, reflect a rapid stimulation of the monocyte compartment in the stimulated PBMC fraction and thus indicates the activation of a local innate immune response by the treatment in both treatment groups. In addition, the reduction of the production level of TNF-α in the Gencydo group reflects a decrease in the chronic SAR-related inflammatory activity between baseline and post-baseline. Overall, the observed kinetics at day 1, are consistent with a reduction of an allergic inflammatory condition with larger effects in the Gencydo group. This inhibition of inflammation is substantiated

Table 9.1. Baseline Characteristics (Full Analysis Set)

Variable		*Citrus/Cydonia comp. (n = 12)*	*Gencydo (n = 11)*	*p-value*
Sex: Number (percentage)	Male Female	7 (58%) 5 (42%)	2 (18%) 9 (82%)	0.049*
Age (year) (sd)		36.8 (12.4)	36.9 (10.7)	0.97
Height (cm) (sd)		177 (9.5)	170.6 (7.4)	0.09
Weight (kg) (sd)		70.8 (11.2)	66.4 (8.9)	0.3
Smokers: number (percentage)		3 (25%)	1 (9%)	0.31
Alcohol consumption: number (percentage)	None Occasionally Regularly	0 (0%) 11 (91.7%) 1 (8.3%)	3 (27.3%) 7 (63.6%) 1 (9%)	0.15
Ethnic origin: number (percentage)	Caucasian Asian	12 (100%) 0 (0%)	9 (82%) 2 (18%)	0.12
Childbearing potential: number (percentage)	Capable Sterile Postmenopausal	3 (60%) 1 (20%) 1 (20%)	8 (88.9%) 1 (11.1%) 0 (0%)	0.31
Blood pressure at screening (mmHg) (sd)		120 (23)/ 73 (14)	104 (16)/ 72 (9)	0.09/ 0.77
Heart Rate at screening (beats per minute)		72 (9)	70 (6)	0.56
RAST grass pollen		3.7 (1.2)	3.9 (1.2)	0.76
RAST birch pollen		2.2 (1.8)	2.3 (2.1)	0.82
Usual SAR symptom severity during the pollen season (total score anamnestically) (sd)	Sneezing Itching nose Watery nasal discharge Total score	2.1 (0.3) 1.9 (0.7) 1.9 (0.7) 5.9 (1.4)	2.3 (0.5) 2.1 (0.5) 2.1 (0.5) 6.5 (1.2)	 0.5
SAR symptom severity scores in the morning during the wash-out period (total score) (sd)		6.6 (4.5)	8.0 (4.6)	0.55
SAR symptom severity scores in the evening during the wash-out period (total score) (sd)		6.1 (3.7)	9.7 (5.2)	0.19

*** p < 0.05, ** p < 0.01, *** p < 0.001**

Table 9.2. Log10 transformed allergen stimulation minus medium stimulation at day 1: baseline versus post-baseline cytokine production levels

		Log10 (allergen stimulation minus medium stimulation) at baseline (range)	*Log10 (allergen stimulation minus medium stimulation) at post-baseline (range)*	*Mean difference (95% CI)*
Gencydo	IL-10#	1.91 (1.16 – 2.39)	1.85 (1.23 – 2.51)	ns
	IFN-γ	0.07 (0.01 – 0.34)	0.03 (0.1 – 0.28)	ns
	TNF-α	1.60 (1.16 – 2.12)	1.09 (0.25 – 2.08)	-0.50* (-0.08 to -0.93)
Citrus/ Cydonia	IL-10	1.64 (0.66 – 2.25)	1.60 (0.95 – 2.12)	ns
	IFN-γ	0.09 (0.1 – 0.50)	0.15 (0.1 – 0.84)	ns
	TNF-α	1.20 (-1.10 – 2.23)	1.34 (0.29 – 2.18)	ns
Total group	IL-10	1.77 (0.66 – 2.39)	1.73 (0.95 – 2.51)	ns
	IFN-γ	0.08 (0.1 – 0.50)	0.09 (0.1 – 0.84)	ns
	TNF-α	1.40 (-1.10 – 2.23)	1.21(0.25 – 2.18)	ns

*** = p value < 0.05**

Table 9.3. Changes in cytokine production at day 7 allergen specific stimulation: baseline versus post-baseline

	Citrus/Cydonia comp.			*Gencydo*		
Variable	Baseline (PPS/OC)	Post-baseline (PPS/OC)	Change (PPS/OC) (95% CI)	Baseline	Post-baseline	Change (95% CI)
IL-10#	2.36/2.37	1.68/1.70	-0.68* (-0.37 to -1.00)/-0.67*	2.22	1.79	-0.44* (-0.19 to -0.68)
IL-12	0.15/0.16	0.26/0.29	0.11/0.13	0.27	0.25	-0.02
IFN-γ	3.01/3.1	2.7/2.8	-0.31/-0.3	3.14	3.17	0.03
IL-5	2.21/2.27	2.06/2.05	-0.15/-0.22	2.29	2.3	0.01
IL-13	2.28/2.4	2.22/2.22	-0.06/-0.18	2.40	2.43	0.03

all cytokine scores (IL-10, IL-12, IFN-γ, IL-5 and IL-13) are log10 transformed scores

PPS = Per Protocol Set

OC = Observed Cases

* p < 0.05, ** p < 0.01, *** p < 0.001

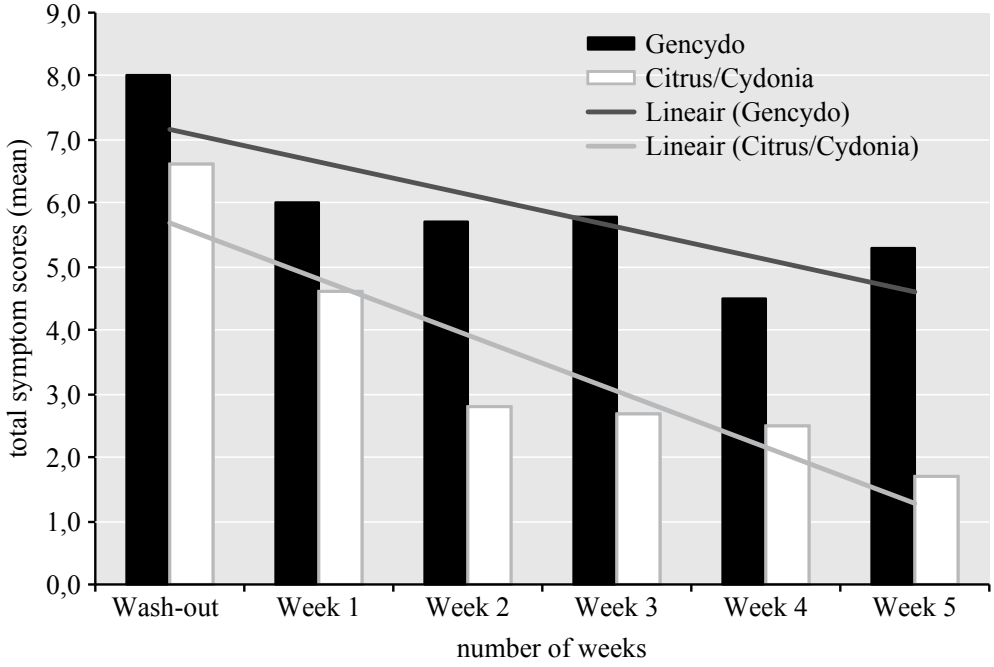


Figure 9.3. Mean total symptom scores from wash-out until five weeks of treatment in the *morning*

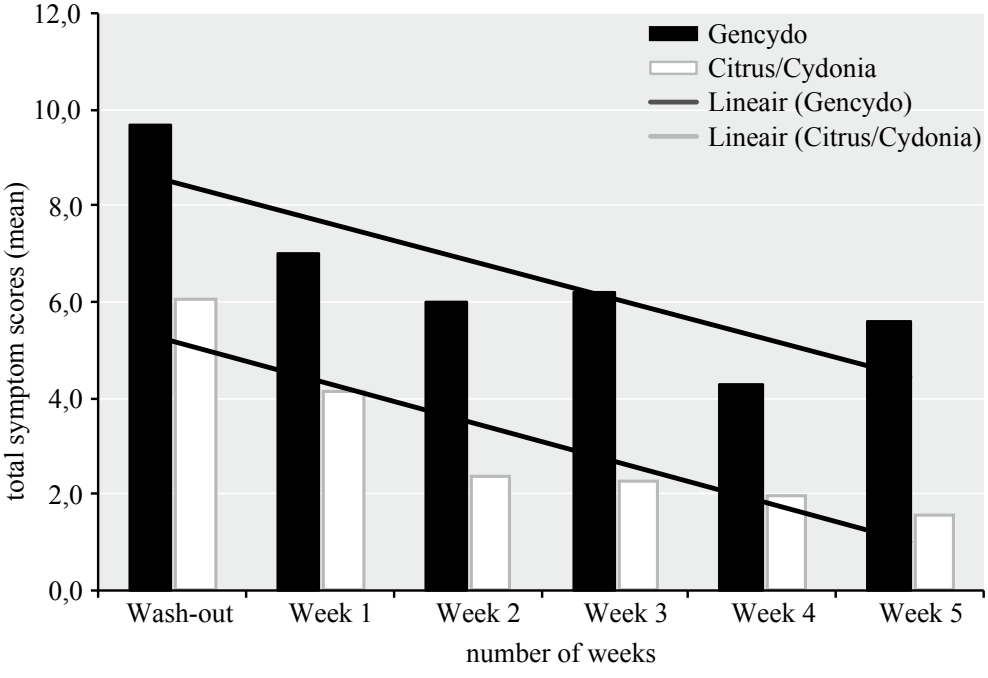


Figure 9.4. Mean total symptom scores from wash-out until five weeks of treatment in the *evening*

Table 9.4. Mean total symptom scores wash-out versus five weeks of treatment: morning and evening scores (n = 20)

	Wash-out week mean (range)	*Treatment week 5 mean (range)*	*Change (95% CI)*
Morning scores			
Citrus/Cydonia comp.	6.6 (2.1 – 15.2)	1.7 (0 – 6.4)	4.8 (1.7 – 7.9)
Gencydo	8.0 (0.6 – 14.3)	5.3 (0.3 – 11.4)	ns
Total	7.3 (0.6 – 15.2)	3.5 (0 – 11.4)	3.8 (1.3 – 6.3)
Evening scores			
Citrus/Cydonia comp.	6.1 (1.7 – 11.8)	1.6 (0 – 5.0)	4.5 (1.7 – 7.2)
Gencydo	9.7 (0.3 – 16.5)	5.6 (0 – 15.0)	4.1 (0.4 – 7.8)
Total	8.0 (0.3 – 16.5)	3.6 (0 – 15.0)	4.3 (2.2 – 6.4)

Total symptom scores can vary from 0 – 24: 0 – 8: mild; 9 – 16: moderate; 17 – 24: severe.

by the concomitant increase in monocyte-derived IL-10 production that profoundly suppresses the TNF-α production. The production level of monocyte-derived TNF-α and IL-10 reflects the local chronic SAR-related inflammatory activity between baseline and post-baseline. In addition, the observed kinetics at day 7 after allergen-specific stimulation, reflects the activation state of the immune system due to the activity of monocytes, which are the largest producers of IL-10 in the PBMC, and induced already by day 1 after allergen exposure. Subsequently, also the gradual and delayed induction of regulatory T-cell subset (Treg) by day 7 will be inhibited as these cells use the IL-10 as a selective autocrine growth factor [17, 18]. The observed kinetics can be interpreted as a decrease in the activation state of the immune system due to a decrease in the activity of monocytes, which are the largest producers of IL-10 in the PBMC, and induced already by day 1 after allergen exposure, and a reduction of the chronic inflammation (TNF-α (day 1)). This additional effect can be attributed to the acute local effect of the nasal spray route. This decreased outgrowth of Tregs must then be the result of the effective treatment installed in these patients. The unaltered production levels of IL-5 and IL-13 (cytokines representing Th2-pathway) are consistent with a slower reacting T-cell compartment in these patients [19]. The frequency of allergen-specific T-cells will still be significant, probably in the order of 1: 300, in these patients as they are in the pollen season and this arm of the immune system will still be triggered in vivo which compromises the Th2 analysis ex vivo [17, 20-22]. The monocyte compartment, being an essential part of the innate immune system, by definition will react faster on induced changes in the patient than the adaptive immune system, which is dependent on the frequency of allergen-specific T-cells and allergen-specific IgE antibody-forming B-cells.

The overall conclusion on the immunological data is that both routes of administration demonstrate positive immunological effects on SAR-related cytokine production levels both in the innate reaction and in the reaction of the allergen-specific T cell subsets, with a larger innate reaction of

the nasal spray route of administration. Both routes of administration appear to stimulate the monocyte compartment into a more immunoregulatory phenotype (day 1: medium stimulation compared to allergen stimulation increase of IL-10 and TNF-α). The day 1 results, reflecting the innate immune reaction, demonstrate more effect of the local Gencydo nasal spray route of administration with a reduction of the chronic inflammatory activity of the allergic Th2 pathway in vivo (baseline to post-baseline reduction of (allergen stimulation) TNF-α). The day 7 results demonstrate comparable effects in reduction of Treg production levels (baseline to post-baseline reduction of IL-10 (allergen stimulation)) of both routes of administration, which can be interpreted as a decrease in activation state of the immune system due to a decrease in the activity of both monocytes and regulatory T-cells.

The overall conclusion of the clinical data is that both routes of administration demonstrate a statistically significant reduction in SAR symptom severity, with larger effects of the subcutaneous route of administration. In the Citrus/Cydonia group a statistically significant SAR symptom reduction was measured already after one week and two weeks of treatment, in the morning and the evening respectively. In the Gencydo group a statistically significant SAR symptom reduction was measured already after two weeks of treatment in the evening. TSS reduction of the subcutaneous route of administration was larger in the morning, but not in the evening. Cohen's delta effect sizes were larger for the subcutaneous route of administration than the nasal spray route of administration, both in the morning and the evening. During the treatment period, a total of 9 adverse events (AEs) were observed with none of the AEs classified as serious. Also the *in vitro* analyses demonstrated acceptable cell survival, with no signs of toxicity. The overall conclusion of the safety analysis in this study is that both routes of administration of Gencydo and Citrus/Cydonia comp. are safe for use by SAR patients.

This study demonstrates that both routes of administration have profound immunological and clinical effects on seasonal allergic rhinitis, with larger clinical effects of the subcutaneous route of administration and larger immunological (innate) effects of the nasal spray route of administration. Therefore the primary hypothesis was rejected and the secondary hypothesis was confirmed. Both AEs analyses and *in vitro* immunological analyses demonstrate that both Citrus/Cydonia comp. and Gencydo are safe treatments, so that also the third hypothesis was confirmed.

The small groups, the relatively low pollen counts during the study period and the absence of placebo groups for both the subcutaneous injection and the nasal spray routes of administration are the most important limitations of this study. The small groups might have led to an underestimation of both clinical and immunological differences between the treatment groups. Larger groups might have provided more precise estimations of means and smaller standard deviations, so that possible other immunological treatment effects might have been detected. The relatively low pollen counts have hampered to evaluate the efficacy of both routes of administration on severe SAR symptoms. However, the differential pollen counts during the short-term intervention during the allergen season did not influence the presence of peripheral blood T-cells and their allergen-specific induced reaction profile. This is consistent with recent evidence showing that immunotherapy of birch pollen hay fever patients left allergen-specific Th2 cells unchanged after one year, but incre-

ased regulatory T-cells that were considered responsible for the observed relief of symptoms. The expected rise in allergen-specific Th1 cells occurs even later after treatment [23]. The absence of placebo groups prevented the estimation of the exact specific effect of both routes of administration by means of controlling for placebo effects.

9.3.2 Generalizability

As the intervention was implemented for both sexes, adults from 18 - 60, both grass pollen and birch pollen SAR the results indicate that a large subgroup of the SAR patient population might benefit from both routes of administration of this treatment.

9.3.3 Overall evidence

The positive results of this study are in line with clinical experiences [4], *in vitro* studies [7,8] and cohort studies [5,6].

Based on the results of this study and previous studies, we can conclude that placebo-controlled clinical trials on short-term and long-term treatment are indicated and adequate to determine the specific effects of both routes of administration. Since the clinical effects (being the primary efficacy variable in SAR trials [24]) were larger in the subcutaneous route of administration and the day 7 immunological results were comparable, we choose to test the subcutaneous route of administration in future placebo-controlled clinical trials.

Since both the antihistamines and/or local corticosteroids treatment are purely symptomatic, and immunotherapy is a treatment with (increasing doses of) pollen allergens, Citrus/Cydonia comp. must be regarded as a new, curative type of treatment that can potentially restore the disturbed immune state of SAR patients permanently. Since there is no stimulation of the immune system with gradually increasing doses of the substances to which a person is allergic, but more a controlled regulation of the activity of the immune system, another working mechanism regarding curative health promotion must be hypothesized.

9.4 Acknowledgements

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Author contributions

Conceived and designed the experiments: EWB MJ AFN HFS. Performed the experiments: EWB MJ IB HFS. Analyzed the data: EWB AFN HFS. Wrote the paper: EWB MJ IB AFN HFS.

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Chapter 10

Adverse drug reactions to anthroposophic and homeopathic solutions for injection: a systematic evaluation of German pharmacovigilance databases

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Submitted: Pharmacoepidemiology and Drug Safety

Abstract

Background: Medicinal solutions for injection are frequently applied in anthroposophic medicine and homeopathy. Despite their extensive use, there is little data published on the safety of these medicinal products.

Objective: The aim of this study was to update the safety status of anthroposophic and homeopathic solutions for injection through a systematic evaluation of the reported Adverse Drug Reactions (ADRs).

Methods: ADRs were extracted from the pharmacovigilance databases of eight German anthroposophic and homeopathic manufacturers covering the period of 2000-2009. These eight manufacturers represented in total more than 94% of the sales of anthroposophic and homeopathic solutions for injection in Germany. Analysed ADRs included reports in humans only, reports from post-marketing surveillance, literature cases and clinical/safety trials and spontaneous reports from healthcare professionals and patients. ADRs derived from medicinal products not prepared according to the HAB (German Homeopathic Pharmacopoeia) were excluded.

Results: Within the time frame of 10 years, in total 303 million solutions for injection (ampoules) were sold. During this same period 486 case reports were identified, corresponding to a total number of 1180 ADRs. Of all case reports, more than 70% (349/486) included ADRs that were listed (e.g. stated in the package leaflet) and 9% (46/486) of the reports were classified as serious. The most frequently reported ADRs were pruritus, followed by angioedema, diarrhoea, erythema and nausea. A total of 27% (322/1180) were localized reactions for example; application or injection site erythema, pain, swelling and inflammation. The overall incidence of ADRs associated with injections was less than 4 per 1 million sold ampoules and classified as very rare.

Conclusions: Our systematic evaluation of ADRs in the German pharmacovigilance databases demonstrated that the rate of ADRs as associated with anthroposophic and homeopathic solutions for injection is very low. Furthermore, most reported ADRs were listed and one quarter consisted of local reactions. These findings suggest an excellent safety profile of solutions for injection as therapeutically applied in anthroposophic medicine and homeopathy, where the overall incidence of ADRs was very rare.

10.1 Introduction

Medicinal solutions for injection, manufactured in accordance with the German Homoeopathic Pharmacopoeia (HAB, 2009), are therapeutically applied in anthroposophic medicine and homeopathy for a wide range of conditions. The therapeutic use of these parenteral administration forms in homeopathy was first described in the 19th century (Kafka, 1867). In 1923/24, Steiner recommended injections as one of the main routes of administration for anthroposophic medication (1925). Nowadays, more than 90 million medicinal ampoules are sold per year worldwide. German anthroposophic and homeopathic manufacturers produce over 90% of these ampoules (ECHAMP, 2003).

Previous studies have shown that anthroposophic and homeopathic practitioners often favour the parenteral dosage form as their first choice of administration in the treatment of acute and chronic diseases (de Bruin, 2001; Baars, 2005). The reason for this preference over other dosage forms is the anticipated better and quicker clinical effect of injections, the possibility to control compliance (injection is general given by physician/therapist or nurse) and the fact that the exact location of administration can be chosen. Other advantages of injections described are that active ingredients do not have to pass the gastrointestinal tract or skin barrier and that the point of injection can be chosen in line with acupuncture trigger points to achieve an optimal systemic or local effect (Stock, 2002).

Despite the extensive use of injections in anthroposophic medicine and homeopathy, there is little data published on the safety of these parenteral dosage forms. Safety data from clinical trials are sparse. A few observational and/or post-marketing surveillance studies with homeopathic medications for injections have reported few side effects (Gottwald, 2000; Zenner, 1990). In the absence of safety data from controlled clinical trials, anthroposophic and homeopathic practitioners throughout Europe were surveyed about their experiences with safety issues of subcutaneous injection of medications. It was demonstrated that more than 98% of the practitioners never, very rarely or rarely observed any adverse reaction caused specifically by the injections. Adverse effects mentioned were local redness, haematoma, local pain, allergic reaction and others (Baars, 2005).

Physicians and other healthcare providers should report possible drug-related adverse events, including adverse drug reaction (ADRs) as associated with anthroposophic and homeopathic medications. It is the obligation of the marketing authorisation holder to analyse drug risks and report those to the regulatory authorities. A first evaluation of ADRs by Stock (2002) in the pharmacovigilance databases of one homeopathic and two anthroposophic German manufacturers reported a very low number of adverse reactions for solutions for injection in the period between 1990 and 1999; approximately one per nine million sold doses. Since 2006, pharmacovigilance procedures and systems have been significantly improved, electronic reporting systems became mandatory for serious case reports and guidelines for pharmacovigilance were published (Eudralex, 2008). The aim of the present study was to update the safety status of parenteral dosage forms of anthroposophic and homeopathic medications.

10.2 Methods

A systematic evaluation was carried out on the ADRs related to solutions for injection and reported between 2000 and 2009 as identified in the pharmacoviligance databases of eight participating German anthroposophic and homeopathic manufacturers.

10.2.1 Data extraction

Retrospective evaluation of adverse drug reactions documented in the pharmacovigilance databases of eight German manufacturers from January 1, 2000 to December 31, 2009 was carried out for the purpose of this study. Of the eight participating manufacturers, six were homeopathic manufacturers and two were anthroposophic manufacturers, all together covering an estimated > 94% (estimated on the basis of sales data) of the total sales of anthroposophic and homeopathic solutions for injection on the German market.

Inclusion criteria for data extraction were: Adverse events for which a causal relationship between the event and a suspected drug could not be excluded and therefore were classified as Adverse Drug Reaction (ADRs) (terminology see ICH-E2A guidelines, 1995); ADRs that were associated with the parenteral dosage forms and with Germany as country of occurrence; ADRs reported between January 1, 2000 and December 31, 2009; ADRs reported by health care professionals and patients (both medically confirmed and medically non confirmed) and ADRs reported from post-marketing surveillance, clinical/safety studies, as well as case reports identified from literature. Excluded from data extraction were ADRs reported for injectables with active ingredients not prepared according to the HAB (2009) and ADRs from solutions for injection for which it was clearly noted that they were applied orally.

The ADRs extracted from the pharmacovigilance database included details on product category (single = one active ingredient, or complex product = more than one active ingredient), calculated dilution of active substance (< 1:10.000 or ≥ 1:10.000), route of parenteral administration (subcutaneous, intramuscular, intravenous, intracutaneous, intraarticular and periarticular), amount administered (1, 2 or 10 ml), date of onset, source of report (spontaneous, literature, clinical studies/safety studies not published, other; such as registries, poison information centre, etc.), qualifications of reporter (health professional, patient; report medically not confirmed, patient; report medically confirmed or other; lawyer etc.), description of ADR including seriousness and listedness, gender and age of the patient. All ADRs were coded in MedDRA including allocations to the respective System Organ Class (SOC).

Patient exposure was estimated from each participating manufacturer by providing yearly total sales data (2000-2009) of the number of units (ampoules), as well as the yearly sales data of the specific product for which the ADR was reported.

10.2.2 Data analysis

Data were extracted from the pharmacovigilance databases in the period from May to September 2010, using a data extraction Excel form and transferred to the SPSS database (IBM, SPSS (PASW) Statistics version 18,0, Somers, NY, USA). Tabulation of the different categories of ADRs was performed by descriptive analysis. Pearsonís Chi-square tests were used to analyse differences between the ADR subgroups and a p-value of <0.05 was regarded as indicating a statistically significant difference. The overall incidence of ADRs of injections was calculated as the sum of ADRs reported in 10 years, per amount of ampoules (estimated patient exposure) sold in the same period. Subsequently ADRs were classified depending on its incidence into very common (≥10%), common (1% < x < 10%), uncommon (0.1% < x <1%), rare (0.01% < x < 0.1%) and very rare < 0.01%.

10.3 Results

Data on ADRs of parenteral dosage forms as reported to eight German anthroposophic and homeopathic manufacturers, collected between January 1, 2000 and December 31, 2009 were extracted from their pharmacovigilance databases. The majority of manufacturers (five out of eight) had systemically evaluated ADRs during this 10-year period. One manufacturer had data available for an 8-year period (2002-2009), one manufacturer for a period of 6 years (2004-2009) and one manufacturer for a period of 3 years (2007-2009). As shown in Figure 10.1, the total number of ampoules sold by the eight participating manufacturers in Germany slightly decreased as from 2005, averaging about 28.8 million ampoules on a yearly basis during the last 5 years. The annual total number of ADRs as reported within the time frame of 2000-2009 is depicted in Figure 10.2. Here it is shown that there are significantly more cases reported in the last three years, 2007-2009 compared to the previous period ($p < 0.001$). Out of 486 case reports, a total number of 1180 ADRs were reported since some patients experienced more than one ADR per case report (Table 10.1). The overall incidence of ADRs with injections in the period 2000-2009 was calculated as < 4 per 1 million sold ampoules and classified as very rare. Although the incidence rate of ADRs calculated for the last 3 years (2007-2009) was doubled in comparison to the ten-years period, e.g. <8 per 1 million sold ampoules, it was still categorized as very rare.

All 486 case reports represented a total of 161 different individual products for injection (some products appeared more than once in the pharmacovigilance database). About 9% (46/486) of all product-related case reports were serious. Furthermore, the majority (72%) (349/486) of all case reports included ADRs that were listed, e.g. the reported ADR is listed in the Summary of Product Characteristics or package leaflet (Table 10.1). A further analysis was carried out to investigate which route of administration was associated most with the reporting of unlisted (unknown) ADRs.

The subcutaneous injections had significantly higher reports of unlisted ADRs compared to the intramuscular injections (n = 97 versus n = 10, respectively; $p<0.01$) and intravenous injections (n= 97 versus n = 20; $p<0.001$). Most case reports were reported spontaneously and by healthcare professionals (Table 10.1). The majority of ADRs (58%; 280/486) occurred in adults in the age of 19 up to 64 years. A total of 27% (132/486) was reported in patients over 65 years. A very low percentage of ADRs were reported in children between 12-18 years of age (1%; 7/486) and 11 years and younger (1%; 5/486). In 13% (62/486) of the cases, the age was unknown.

The most frequently reported ADRs were pruritus, angioedema (swelling of dermis or subcutaneous tissue), diarrhoea, erythema and nausea (Table 10.2). Furthermore, most ADRs reported were classified as skin and subcutaneous tissue disorders and general disorders and administration site conditions. A total of 27% (322/1180) of all reported ADRs was a local reaction, i.e. coded as application or injection site erythema, pain, swelling, inflammation, itching, etc. (results not shown). As shown in Table 10.3, serious case reports on ADRs occurred significantly more frequently as compared to non-serious case reports in (1) male subjects, (2) subjects of 65 years and older, (3) upon injection with complex products, (4) with products of a dilution <1:10.000, and (5) of all parenteral dosage forms, the majority related to intramuscular injection. Pain in extremity (n=15), brain stem infarction (n=4), aphthous stomatitis (n=4), cerebral ischemia (n=4) and tongue disorder (n=4) were labelled most frequently as serious ADRs. The brain stem infarction was reported in one patient only, however, this was presented within the pharmacovigilance database as four separate case reports since the patient was injected simultaneously with a total of 4 different products for injection. With aphthous stomatitis and cerebral ischemia the serious ADR also occurred in one patient only, in which it was not possible to causally relate the ADR to one of the four individual injected remedies. All serious case reports were classified as being serious since the patient in question was either hospitalised or significantly disabled. Although a causal relation to injection of the medications could not be ruled out completely, it was assessed in all cases as more likely that the hospitalisation or disability had occurred in the course of the already ongoing disease (such as multiple sclerosis, diabetes, etc.).

10.4 Discussion

To our knowledge, this is the first study to provide a thorough systematic evaluation of collected and spontaneous reported ADRs for parenteral dosage forms of anthroposophic and homeopathic medications. In the period between 2000-2009, a total of 486 cases were identified in Germany with a total of 1180 reported ADRs. In relation to the overall sales data of ampoules within that same period (303 million ampoules), the incidence rate of ADRs as associated with solutions for injection was found to be very rare, i.e. less than 4 ADRs per 1 million sold ampoules. Compared to other countries worldwide, anthroposophic and homeopathic solutions for injection

Figure 10.1. Total sales of anthroposophic and homeopathic solutions for injection (ampoules) in Germany

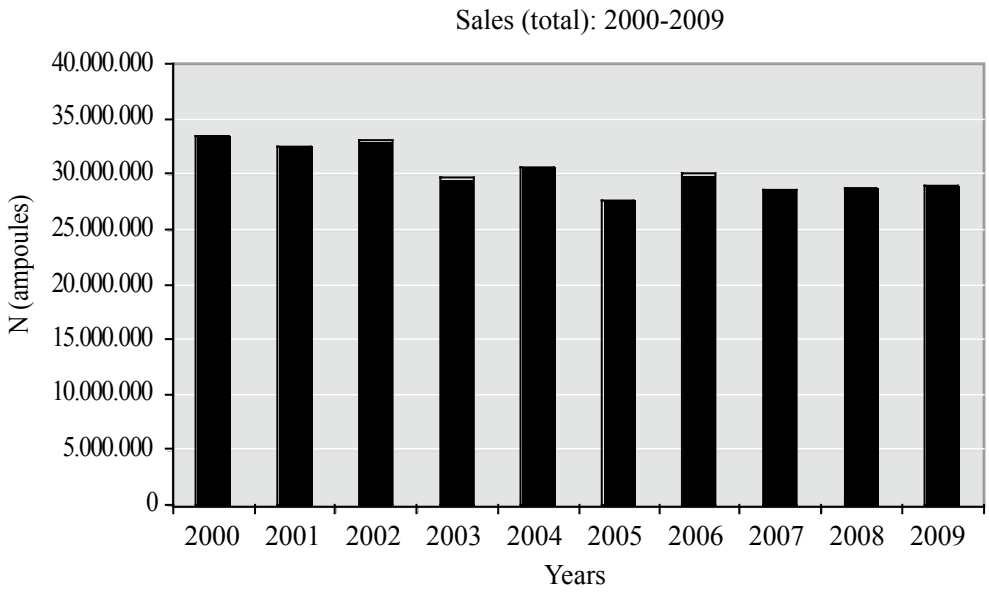

Figure 10.2. Total number of case reports with anthroposophic and homeopathic solutions for injection in Germany

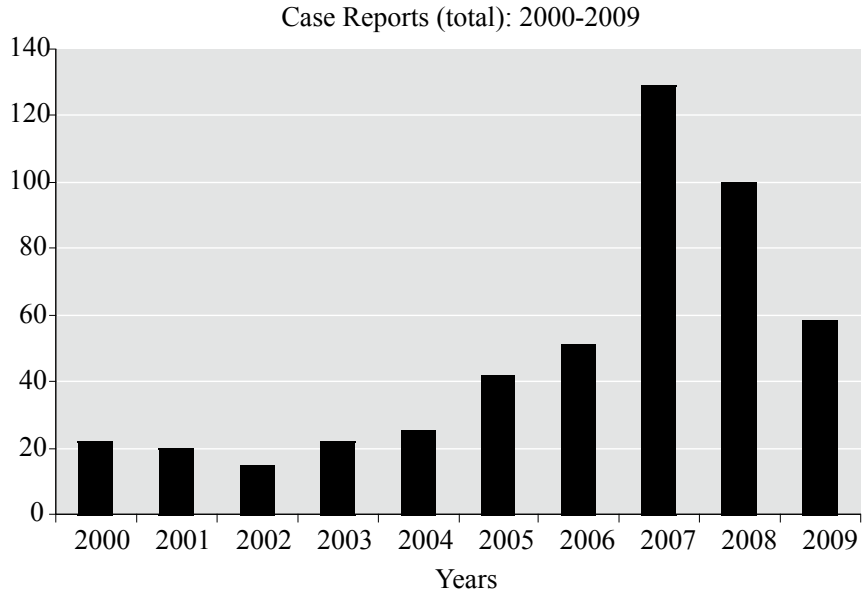

Table 10.1. Overview of number and category of ADRs reported from 2000-2009

Number	*Category*
1180	Total number of reported ADRs
486	Number of case reports*
161	number of individual products
440	non-serious
46	serious
349	listed
135	unlisted
2	not known
	Source
457	spontaneous
1	literature
28	clinical studies
	Reporter
357	healthcare professional
53	consumers
76	not known

* Per case, more than one ADR can be reported

are most frequently prescribed in Germany (ECHAMP, 2003). With the 8 anthroposophic and homeopathic manufactures participating in the study, almost full coverage of the German market was reached. Thus the present findings are expected to be highly representative for the safety status of anthroposophic and homeopathic medications in general.

Most ADRs were reported in adults aged 19 to 64 years, whereas very few ADRs were reported in children. The low number of ADR reports in children can be explained by the current German regulation for authorization of anthroposophic and homeopathic parenteral dosage forms. Those parenteral dosage forms with a therapeutic indication are often not indicated for children under the age of 12 years because the specific indication does not apply to children. Furthermore, physicians usually do not prefer solutions for injection in children.

About 9% of all case reports were classified as serious and seemed to occur significantly more frequently in males, with complex medications, dilutions < 1:10.000 and in adults of 65 years and older. With respect to the latter, a clear correlation between increasing age and higher ADR reporting rates is known (Routlegde, 2003). The finding that 9% of all case reports were classified as being serious seems relatively high, but has to be interpreted with caution. First, all ADRs were categorised as being serious because of hospitalisation or disability of patients. This was most likely due to the ongoing severe disease and/or co-morbidity of the patient rather than to administration

Table 10.2. ADRs* and System Organ Class most frequently reported

ADR	*Frequency*
Pruritus	62
Angioedema	41
Diarrhoea	38
Erythema	35
Nausea	35
System organ class	*Frequency*
Skin and subcutaneous tissue disorders	298
General disorders and administration site conditions	211
Gastrointestinal disorders	149
Nervous system disorders	83
Vascular disorders	75

*Preferred term according to MedDRA

of the anthroposophic or homeopathic solutions for injection itself. Other observational studies on anthroposophic and homeopathic medications did not report of any serious adverse events (Gottwald, 2000; Hamre, 2006; Zenner, 1990). One explanation might be the under-reporting of non-serious and listed ADRs by health care professionals or patients in the present study. Most package leaflets of anthroposophic and homeopathic medications in Germany clearly state that adverse reactions that are not listed in the leaflet should be reported to a physician or a pharmacist. This may lead to under-reporting of listed non-serious adverse reactions. Nevertheless, the incidence rate of serious ADRs with homeopathic injections was extremely rare (less than 4 serious ADRs per 10 million sold ampoules). In comparison, a recent study on the management of knee osteoarthritis with corticosteroid injections reported an incidence of severe infectious complications as high as 1 in 3000 injections (McGarry, 2011).

The ADRs that were identified were mostly related to skin and subcutaneous tissue disorders and or general disorders and administration site conditions. About one quarter of ADRs was related to the injection procedure itself. With respect to the relative safety of the frequently applied parenteral dosage forms, most serious ADRs (in relation to non-serious ADRs) were reported with intramuscular injections. In addition, most unlisted ADRs were reported with the subcutaneous route of administration. No conclusions could be drawn about the overall safety of the different parenteral dosage forms. This was due to the fact that it was not possible to calculate sales data per administration dosage form as most solutions for injections as applied in anthroposophic medicine and homeopathy are registered for more than one administration form.

Our present study suffered from some unavoidable limitations. The first one is that of under-

Table 10.3. Serious versus non-serious case reports

Category	*Serious*	*Non-serious*	*p-value*
Male	33	87	0.001*
Female	12	353	
Not known	1	-	
0-64 years	4	288	0.001*
≥ 65 years and older	26	106	
Not known	16	46	
Single product	0	57	0.05*
Complex product	45	383	
Not known	1	-	
Listed	36	313	0.25
Unlisted	9	126	
Not known	1	1	
<1:10.000	42	320	0.01*
≥1:10.000	3	117	
Not known	1	3	
1 ml	26	306	0.31
2 ml	19	129	
Other/ not known	1	5	
sc injection**	2	142	0.001*
im injection	10	39	
sc injection**	2	142	0.01*
iv injection	20	200	
im injection**	10	39	0.05*
iv injection	20	200	

*Indicating a significant difference ($p < 0.05$) between serious and non-serious case reports, using the Pearsonís Chi-square test. ** Data on the most frequently used parenteral dosage forms are depicted.

reporting of ADRs to the manufacturers. Practitioners and other healthcare providers may not always report on suspected or unsuspected ADRs. However, our findings are in line with data from a prospective observational study by Hamre et al. (2006) in which adverse effects were monitored intensively. In this study an incidence rate of one ADR per 382 patient-months of anthroposophic medications use (all dosage forms) was observed. With a daily dose of 0.7, a calculated ADR incidence rate in the Hamre study would be classified as very rare (<0.01%) as well. Furthermore, comparable results were reported in a European survey among anthroposophic and homeopathic practitioners (Baars, 2005). The majority of practitioners (87%) had never or very rarely (1 or <1:10.000 treated patients) observed an ADR with anthroposophic or homeopathic solutions for injection, whereas 2.6% had rarely observed any ADR and 1.7% occasionally (8.9% gave no re-

sponse). Another limitation is that patient exposure or use was estimated on the basis of the number of ampoules sold. All ampoules sold are not necessarily prescribed and used. Furthermore, case reports for which it was clear that the solutions for injection were administered orally, were excluded from data analysis. However, due to off-label use, some sales data may represent patients that have taken ampoules orally rather than parenteral. Based on practitionerís prescription behaviour (Baars, 2005; Hamre, 2006), the oral use of ampoules is not expected to be high; nevertheless it cannot be excluded. A major limitation of systematic evaluations of pharmacovigilance data in general is that they highly depend on the quality of the reporting and monitoring system. During the last three years in Germany, as well as in other European countries, pharmacovigilance operating procedures have been considerably improved. The introduction of the Volume 9a guidelines for pharmacovigilance which came into force in 2008 has attributed to this improvement. In the present study it became apparent that the collection and spontaneous reporting of ADRs has significantly increased during the last 3 years when compared to the previous years. This increase in reports can partly be explained by the fact that one participating manufacturer submitted data for evaluation only for these last three years. Furthermore, improved pharmacovigilance procedures may also contribute to this increase. Thus repetition of this systematic evaluation of pharmacovigilance data within a time frame of 5 years is warranted.

10.5 Conclusions

This is the first study to provide a systematic evaluation of the safety of anthroposophic and homeopathic solution for injection from pharmacovigilance databases. Our results indicated that solutions for injection as applied in anthroposophic medicine and homeopathy have an excellent safety profile. This included complex products and those that are not highly diluted. These results provide reliable data for risk-benefit ratio calculations of anthroposophic and homeopathic parenteral dosage forms.

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Chapter 11

General discussion

This thesis studies curative health promotion as an additional approach to the fighting disease approach in medicine in general, and specifically, in seasonal allergic rhinitis. The context for the study is the enormous improvement of the health status and life expectancy of individual persons in Western society over the last 150 years, which is, apart from general hygiene measures in society, primarily the result of the success of the fighting disease approach in medicine and society. However, with increasingly more people reaching high ages in the 21st century, rising costs due to the high life expectancy accompanying an increase in chronic diseases and subsequently an increase in medical costs, the issue of the further development of professional health promotion becomes even more relevant. How does one keep the health status of persons high enough in order to ensure a high quality of life for the individual and low costs for society? A second reason for an increased interest in health promotion comes from the changed role of patients in the last decades. More patients are assuming the role of competent patients who regard themselves as responsible for actively managing their own disease and health status and actively using available (health-promoting) resources to influence this health status.

The main strength of medicine has been its fighting disease approach. This involves the fighting of disease-related organisms (e.g. bacteria, viruses), cells and functions in the body (e.g. cancer); the reduction of disease-related symptoms (e.g. hay fever symptoms, pain or headache); and the manipulation and/ or substitution of non-functioning or dysfunctional parts of the body (e.g. hormone therapy or hip replacement surgery) by means of *external* therapeutic resources. In the last decades, however, the interest in an additional, both preventive and curative, health promotion approach has increased in society and healthcare.

Integrating the best of both worlds by combining these two approaches in a logical and professional way is an objective towards which medicine could strive in order to meet the problems of the low health status of the elderly and the increase of medical costs, both of which are due to an ongoing increase in the prevalence of chronic diseases. However, whereas the fighting disease approach already has a long scientific history, science on health promotion as a serious approach in medicine is still relatively young. Preconditions for becoming a professional approach are that there have to be valid concepts of health and health promotion, valid methodologies to monitor (changes in) health states, and empirical studies on the efficacy, effectiveness and cost-effectiveness of concrete preventive and curative health promotion interventions for specific diseases.

This thesis contributes to the further professional development of health promotion by studying the concept of health, the methodology to monitor (changes in) health states, the effects and safety

of Citrus/Cydonia comp. on seasonal allergic rhinitis as an example of curative health promotion, and the cost-effectiveness of integrative medicine in the Netherlands.

11.1 Main findings

The first objective of this thesis is to contribute to the development of a valid definition of health that could serve as a solid, evidence-based fundament for professional preventive and curative health promotion as a supplement to the main (western) biomedical fighting disease approach. Therefore, the authors performed literature studies on health concepts and the holism-reductionism debate (Chapters 2 and 3).

We criticized the WHO definition of health (1948), since it is primarily oriented at end states and does not clarify the mechanisms responsible for reaching and maintaining these states. Therefore, it does not provide sufficient conceptual tools to innovate and professionalize health promotion activities. In order to overcome these shortcomings, the WHO and current health definitions were analyzed and revised into the definition of 'health by self-regulation' (Chapter 2). This definition can best be regarded as a non-atomistic holistic concept of integrity, stating that organisms are self-organizing entities in which ontological (higher organization) levels of wholeness are responsible for maintaining the integrity and organizing of its parts, for the interconnectedness and the balanced harmony of its parts, for its functional and morphological wholeness, and finally, for the balance struck with its environment. This ontological level of wholeness can be distinguished from the levels of the 'parts' of the organism. The definition is preliminarily validated according to the criteria of Oost [1, 2] by means of analyzing (1) its internal consistency (logical inconsistency, use of ambiguous concepts), (2) its accordance with other scientific definitions (a theory "does not fit in" with other theories) (e.g., of epigenetics, systems biology, emergence and self-organisation) and (3) its accordance with empirical facts (a theory "does not fit in" with reality). Key features of the definition are the central position of dynamic and continuous self-regulation as a key organismal function and the holistic, system biological approach. Health promotion supports and enables patients to actively contribute to their own health and disease status towards an optimal functioning of the higher levels of organizing among each other and in the regulation of the lower levels.

The second objective is to develop and test biomarkers for the monitoring of curative health promotion interventions on the systems level. Therefore, pattern variables that should reflect the functioning of the immune system on the system level were computed and tested (Chapter 4). Computation was based on a dataset of cytokines and symptom severity scores from a randomized controlled trial. By means of permuted stepwise regression of the cytokine data, three robust systems biology-orientated biomarkers for the monitoring of SAR were computed, which demonstrated small to moderate improvement compared to monitoring of a single cytokine. Baseline versus post-baseline comparison of mean scores of the pattern variables demonstrated statistically significant baseline versus post-baseline changes.

The third objective of the thesis is to study the effects and safety of Citrus/Cydonia comp. in SAR treatment as an example of a curative health promotion intervention for a specific indication (Chapters 5 to 10). Based on a survey among experienced prescribing general practitioners, two *in-vitro* studies, and two intervention studies (a cohort study and one randomized controlled trial comparing two routes of administration), it can be concluded that evidence exists for both the clinical and immunological effects of Citrus/Cydonia comp. in SAR treatment.

On the immunological level, evidence is provided that Citrus/Cydonia comp. is able to inhibit the chronic inflammatory reaction and overproduction of the Th2 pathway cytokines and promote self-regulation within this SAR-related subsystem of the immune system (Chapter 6). It appears that Citrus and Cydonia have different working mechanisms in SAR treatment *in vitro*: Citrus mainly inhibits the chronic inflammatory activity and the SAR-related Th2 pathway activity, whereas Cydonia mainly promotes the SAR-related Th1 pathway activity (Chapter 7).

Regarding safety of Citrus/Cydonia comp., one can conclude that it is a very safe treatment. In both *in-vitro* studies, the product had no effect on blood mononuclear cell survival and did not appear to be toxic for the PBMC cell subpopulations (Chapters 6 and 7). In the clinical studies, no serious adverse events occurred (Chapters 8 and 9). The systematic evaluation of German pharmacovigilance databases within the time frame of six years (2005-2010), covering a total of approximately 1.1 million sold solutions for injection on the German, Dutch and Belgian market, demonstrated no reported adverse drug reactions for this product (Chapter 10).

11.2 Reflection on the main findings

11.2.1 Reflection on health concepts and their relevance to professional health promotion

As described in Chapter 1, the interest in becoming and remaining healthy if possible, even until an advanced age, is high. However, compared to the fighting disease approach, the scientific fundament of understanding and explaining health, of conceptualizing health and health promotion, and of testing health promotion programs is relatively in its infancy and urgently needs a solid evidence base research. As described, the proposed concept is based on an analysis of empirical studies on several health (promotion) conceptual approaches and is therefore in line with current empirical evidence and literature about health.

The newly developed concept of health (Chapter 2) is at least theoretically important in two ways. First of all, the current knowledge of parts of the health concept is logically integrated, and some of the central questions concerning the theoretical relationships between the positive domains

of health and between health and disease are (partly) answered. Some aspects, for example, the relationship between a sense of coherence and physical health, remain unclear and more research is required to clarify this relationship. Secondly, the concept provides an evidence-based fundament for developing new preventive and curative health promotion interventions and strategies.

The implications for clinical practice are first that sufficient evidence exists to further promote preventive and curative health promotion. However, a lot of scientific and practical work has to be conducted in order to reach a professional integration of both the fighting disease and the health promotion approaches.

Future research should focus on further specifying the relationships between the positive domains, the positive ('health') and the negative ('absence of disease') domain, empirically testing new preventive and curative health promotion interventions and strategies, and the development and empirical testing of the professional, effective, cost-effective and safe integration of both approaches in specific indications.

11.2.2 Reflection on system biology-orientated methodologies

This thesis explores the use of both qualitative and statistical methodologies for pattern exploration and recognition on the system levels of organisms. It describes the Goethean phenomenological method as a qualitative methodology that studies both the underlying lower levels of organizations (the composing parts and their interactions) and the higher level itself, with the aim to arrive at 'correct' knowledge or an understanding of the emergent, higher organization levels of the organism (Chapter 3). In addition, permuted stepwise regression analysis was used to compute pattern variables that measure the relationships between two or more variables, which were able to better distinguish between baseline and post-baseline measurement in a randomized trial than separate variables (Chapter 4).

The search for these types of pattern recognition methodologies fits well with the increasing interest in the systems approach in biology and medicine over the last ten years. Increasingly the 'one gene, one drug, one disease' approach, which has dominated drug development in the last decades, is challenged by decreasing clinical attrition rates and functional genomics studies. This demonstrates the robustness of the phenotype in the single-gene knockout approach paradigm [3]. The two single most important reasons for attrition in clinical development are a lack of efficacy and clinical safety or toxicology, which each account for 30 percent of the failures. Scientists are focusing more on the systems level. For example, Van der Greef et al. [4] characterize systems biology in medicine as the art and practice of mapping patterns of relationships. Ma'ayan et al. [5] describe the search for regulatory patterns during signal propagation in a mammalian cellular network. Van Ommen et al. [6] describe a network biology model of micronutrient-related health. Hopkins [3] presents the network pharmacology as the next paradigm in drug discovery using polypharmacology. Barabasi et al. [7] use the term 'network medicine' as a network-based approach to human disease. Within this system biology approach, new statistical methods are developed

aiming at pattern exploration and pattern recognition [4, 8].

In addition, the value and use of subjective pattern recognition in professional practice (Chapter 3) has received increasing scientific attention. For example, Dalston and Galison [9] describe in their study on the historical development of scientific objectivity that trained judgment, which is able to detect patterns (e.g. in an X-ray), is the latest type of scientific objectivity in history after development of the mechanical and structural objectivity approach. Adequately diagnosing and solving unique complex and context-specific problems can be performed by experts on the basis of so-called tacit knowledge, craftsmanship, the 'clinical look' or 'breeder's eye' [10 – 12].

Experienced workers seem to have learned, most of the time unconsciously, to handle prevailing laws and situations. In doing so, they have developed self-regulation skills based on valid and practical, useful knowledge ('appropriate conclusions and correct predictions'). Self-regulation can be defined as the adaptive use of skill across changing personal and environmental conditions [13]. Expert knowledge is represented at an intermediate level of abstraction and is called the 'moderately abstract conceptual representation': a compromise between different abstractions like comparisons in the disciplines of physics and chemistry and concrete, specific problems [14]. The key element of expert information processing is the intuitive recognition and application of a pattern ('Gestalt') [9, 15]. The professional use of pattern recognition in medicine has been emphasized by, for example, Goudsmit [16], who describes the use of pattern recognition as a phronetic ('practical wisdom') skill in clinical reasoning, and Stolper et al. [17], who describe the gut feelings as a third valid track in general practitioners' diagnostic reasoning (in addition to medical decision-making and medical problem-solving). Smulders [18] emphasized the value of subjectivity and intuition as virtues in treating individual patients in clinical practice next to applying the results of epidemiological studies.

Interestingly, the initial indication of the use of Citrus and Cydonia was performed by Rudolf Steiner and the doctor Ita Wegman in 1925 [19], based on qualitative pattern recognition of the organizing level of these plants. This was performed long before the use of microbiological, epidemiological and immunological studies and the use of statistical pattern recognition methods, also demonstrating the potency of methodologically, subjective qualitative pattern recognition.

Some of the theoretical and practical implications of the development and application of qualitative and statistical methodologies for pattern exploration and recognition on the system levels of organisms are that the development (and further validation) of measuring instruments will be increasingly based on holistic concepts of relationships between parts of the systems relevant for health and disease. In addition, qualitative and statistical pattern recognition methodologies will increasingly be used in the diagnostics and monitoring of effects in clinical practice. These methodologies, as demonstrated in the role of expertise, also have the potential to improve the diagnostic and therapeutic methodologies regarding individualization, as is the aim in current personalized medicine approaches.

The shortcomings of these studies are that the Goethean phenomenological method (Chapter 3) is only used in scientific and clinical practice, but has not been validated as a research methodology according to the current scientific standards. Therefore, further validation of this method for

use in scientific and clinical practice is recommended. The main shortcomings of the development of pattern variables by means of permuted stepwise regression (Chapter 4) are that they are based on small group sizes and that data are absent from healthy persons and from categories of SAR patients with varying symptom severities. In addition, even more sophisticated pattern recognition statistical tools can be developed and applied, which are able to detect patterns of relationships between 'parts of the system' even more precisely.

Future studies on the applicability of permuted stepwise regression to SAR are indicated with larger populations, with categories of SAR patients with varying symptom severities, and with a control group of healthy persons. These study design changes will allow the computation of pattern variables that are able to more precisely distinguish among disease severities and between disease and health more. This procedure and other statistical pattern recognition procedures may also be applicable to other immune-mediated diseases; datasets describing rich cytokine sets will determine the critical parameters that can be combined in the various pattern variables.

11.2.3 Reflection on the effectiveness and safety of Citrus/Cydonia comp.

This thesis presented an overview of the evidence of clinical and immunological effects of Citrus/Cydonia comp. in the treatment of SAR (Chapters 5 to 10). The overall positive clinical results are in line with other *in vitro* studies, which demonstrated that Citrus/Cydonia comp. significantly reduced the histamine production and the inflammatory mediator release from mast cells in a dose-dependent manner [20], and which demonstrated the positive effects of several immunologically active compounds of Citrus and Cydonia like flavonoids and pectins on SAR [21 - 27]. Citrus and Cydonia, at least *in-vitro*, appear to have different working mechanisms that support each other.

Based on the GRADE classification [28, 29], which is developing as an internationally accepted standard for classifying the quality of evidence and the classification of recommendations to patients, doctors and policy makers, one can conclude that the quality of the current evidence of the effect of Citrus/Cydonia comp. on SAR is moderate. The evidence to support this claim comes from two cohort studies (Chapter 8) [30] and a randomized trial (Chapter 9), as well as from *in-vitro* studies [20] that demonstrated a reduction in histamine production and the inflammatory mediator release from mast cells in a dose-dependent manner.

Evidence on the safety of this treatment has been provided by three *in-vitro* studies, two cohort studies, a randomized controlled trial, and a systematic evaluation of German pharmacovigilance databases (Chapters 6 to 10) [20, 30], thus demonstrating that this treatment is very safe.

These results may add to the further conceptualization of SAR treatment, since the working principle of Citrus/Cydonia comp. appears to differ from the various treatments: antihistamines (symptomatic prevention of histamine release), corticosteroids (symptomatic reduction of inflammatory activity) and immunotherapy (curative exposure to increasing doses of allergens) [31]. Based on current clinical knowledge and scientific evidence, it is hypothesized that Citrus/Cydonia

comp. is working on the organizational level of the allergy-related subsystem of the immune system, is promoting the regulation of the Th1/Th2 balance, and might therefore actually be a curative health-promoting remedy (Chapters 6 and 7). Since a difference appears to exist between the subcutaneous and nasal spray routes of administration, the role of the extracellular matrix, which is changed by the subcutaneous route of administration in promoting the self-organization of the immune system, must also be a part of further research and conceptualization (Chapter 9).

The use of Citrus/Cydonia comp. for SAR treatment fits well with the current orientation in medicine and pharmacology, which aims at multi-target treatment (network pharmacology [3, 7] and polypharmacology instead of the 'one-size fits all' approach. As described in Chapter 7, Citrus and Cydonia appear to have different working mechanisms in SAR treatment *in vitro* (Chapter 7): Citrus mainly inhibits the chronic inflammatory activity and the SAR-related Th2 pathway activity, whereas Cydonia mainly promotes the SAR-related Th1 pathway activity. A combination of both working mechanisms is hypothesized to have a cumulative effect on SAR.

Like the combination of immunotherapy (only indicated for a small group of SAR patients), Citrus/Cydonia treatment might be integrated with corticosteroid and antihistaminic treatment in order to develop a SAR treatment strategy that integrates the best of both worlds in fighting disease and health promotion.

The major shortcoming of the studies is the lack of placebo-controlled trials. Therefore, recommendations for future research are the funding, designing and execution of both placebo-controlled RCTs to study the specific effect of Citrus/Cydonia comp. *in vivo,* and pragmatic trials in which the optimal treatment for individual patients can be studied. Then within the context of a research program, the optimal integration of fighting disease and health promotion approaches in SAR treatment must be studied. Finally, cost-effectiveness studies are indicated to compare the cost-effects of treatment of Citrus/Cydonia comp. with current treatments. Based on this additional evidence, a solid recommendation (and subsequent integration into the guidelines) on the use for SAR treatment can be made for patients, clinicians and policy makers [32].

11.3 Cost-effectiveness of health promotion

Since it was hypothesized that health promotion strategies could contribute to cost reduction in healthcare, a summary is presented of a cost-effectiveness study in which the possible contribution of integrative medicine to the reduction of healthcare costs was studied (Appendix).

Since there is a lack of cost-effectiveness data of CAM in the Netherlands, this study compared the performance of general practitioners who have completed certified additional training in CAM after obtaining their conventional medical degree (GP-CAMs) with general practitioners who have not (GPs). More specifically, GP-CAMs with additional training in anthroposophic medicine, homeopathy, or acupuncture (approximately 1 percent of GPs for each of these CAM types) were considered.

A dataset from a Dutch health insurer was used, containing quarterly information on healthcare costs (care by general practitioner (GP), hospital care, pharmaceutical care, and paramedic care), dates of birth and death, gender, and the six-digit postcode of all approximately 150,000 insurees, for the years 2006 to 2009. Data from 1913 conventional GPs were compared with data from 79 GPs with additional CAM training in acupuncture (25), homeopathy (28), and anthroposophic medicine (26).

Patients whose GP has completed additional CAM training have 0–30 percent lower healthcare costs and mortality rates, depending on age groups and the type of CAM. The lower costs result from fewer hospital stays and fewer prescription drugs. Since the differences are obtained while controlling for confounders, including neighborhood-specific fixed effects at a highly detailed level, the lower costs and longer lives are unlikely to be related to differences in socioeconomic status.

Possible explanations include selection (e.g. people with low interest in medical interventions might be more likely to choose CAM) and better practices (e.g. less overtreatment, more focus on preventive and curative health promotion) by GPs with knowledge of complementary medicine. More controlled studies (replication studies, research based on more comprehensive data, cost-effectiveness studies on CAM for specific diagnostic categories) are required.

These findings underscore the fact that the health-promoting methods that are considered CAM today could be effective and might have significant cost-saving potential.

11.4 Reflection on the scientific fundament of anthroposophic medicine

Since Citrus/Cydonia comp. is an example of anthroposophic medicine (AM), and providing evidence of the safety, efficacy, effectiveness and cost-effectiveness is necessary but not sufficient for scientific acceptance, a final reflection on the scientific fundament of AM is also given. Many regard AM as an alternative medicine stream, which has not proven to be effective, is unsafe, and is conceptually not in accordance with science and medical practice [33]. This could be related to the low esteem of AM in the medical profession. Many, if not all, of these characteristics are untrue.

11.4.1 Anthroposophic medicine as a form of complementary and integrative medicine

First of all, AM must be regarded as a complementary, integrative stream of medicine based on and funded in conventional medicine [34]. Doctors, most therapists, and nurses are first

trained in conventional medicine, and then receive AM training. In addition to the conventional use, medical knowledge and observations are also used in qualitative pattern recognition to gain knowledge of higher levels of the human organism and nature in order to diagnose and treat on these higher levels of the organization [34].

11.4.2 Health technology assessment of anthroposophic medicine

Regarding the efficacy, effectiveness and cost-effectiveness of AM, Kienle et al. [35, 36] performed an update of a health technology assessment on AM. This study demonstrated that a total of 265 clinical studies investigated the efficacy and effectiveness of AM: 38 randomized controlled trials, 36 prospective and 49 retrospective non-randomized controlled studies, and 90 prospective and 52 retrospective studies without control groups. These studies investigated a wide spectrum of AM treatments in a multitude of diseases, 38x whole system of AM, 10x non-pharmacological therapies, 133x AM mistletoe products in cancer, and 84x other AM medication treatments. Most studies revealed a positive result for AM. However, the methodological quality differed substantially; some studies showed major limitations, whereas others were reasonably well conducted. Higher-quality trials still showed a positive result, and external validity was usually high. Side effects or other risks are rare and usually mild to moderate. Safety studies generally show high tolerability.

How can these results be judged when compared to conventional medicine? Smulders et al. [37] summarize the epidemiological evidence of common therapeutic actions in conventional medicine: almost half are hardly or never studied, and efficacy has only been demonstrated unequivocally for 13 percent of them. For the rest of common therapeutic actions in conventional medicine, the support from epidemiological studies ranges from ‘probably useful’ (23%) to ‘probably useless or even harmful’ (4%). Until now, a lack of qualitative solid evidence has not been a reason to ban common therapeutic actions from conventional medicine. This should then also not be the case for AM, since this would imply a double standard, especially since the current body of empirical evidence demonstrates positive results on the effects, high safety levels and possible cost-effectiveness. In addition, the AM concept is increasingly in accordance with the systems biology-orientated ‘network medicine’ (see Section 11.4.1). Instead of banning AM from healthcare, current knowledge provides reason for more funding of research and practice in order to further develop and test AM concepts and to acquire high-quality evidence on cost-effectiveness. These considerations are even more applicable when considering complementary medicine in general.

11.4.3 The accordance of the concept of anthroposophic medicine with other theories

Regarding the statement that the concept of AM is theoretically not in accordance with

science and medical practice, the characteristics of AM, as already described in the Introduction (Chapter 1), are summarized:

1. There are non-material organizing principles in nature in addition to the material elements of reality (nature) (ontological aspect)
2. There are higher, more complex levels of organization in organisms, which are responsible for the integrity and organizing of material elements in time, space and function (epistemological aspect)
3. These higher levels of organization in organisms can be examined by means of qualitative-subjective and statistical pattern exploration and pattern recognition methodologies (methodological aspect)
4. Health and disease can be diagnosed by integrating knowledge from the lower and higher levels of the organization of the system (methodological aspect)
5. The anthroposophic treatment of disease must often be systems-oriented (e.g. several substances or therapies) and aimed at:
   a. Influencing different essential aspects of the relevant organizing system level in health and disease at the same time or in phases (poly-target-treatment)
   b. Restoring balance and wholeness within the system by stimulating the higher levels of organization in order to regulate the lower levels (health promotion) (methodological aspect)
6. The anthroposophic treatment of disease requires self-activity of the organism and the patient towards an optimal functioning of the higher levels of organizing among each other and in the regulation of the lower levels. Therefore, it is a health promotion strategy, one that supports and enables patients to actively contribute to their own health and disease status (methodological aspect).

Points 2-6 are conceptually fully in line with the concepts of system biology, epigenetics and emergentism (Chapter 1) and the current scientific and healthcare practice orientation towards 'network medicine', 'polypharmacology' and 'poly-target treatment' [3, 4, 7, 38]. The only aspect in which the anthroposophic formal aspects differ from current scientific thinking is the ontological aspect. Since this is a very delicate discussion (for most scientists, the ontological reductionist materialistic position is *the* basis of science), this topic is separately reflected, thus demonstrating that some of the major scientific questions that remain unanswered are related to the ontological question: 'of what is reality (nature) made of?'

11.4.4 Final reflection on ontology within the holism and reductionism debate

Ontological aspects concern the question of what entities, things or substances are assumed to make up reality (nature), what characteristics are attributed to these things or entities, and what relationships and functions can be assumed to exist between them [38]. On the ontological level,

one can distinguish theoretically between ontological reductionism (atomism) and ontological holism (vitalism, emergentism and organicism). Atomism holds that the entities of the 'lowest' level of organization (atoms, sub-atomic particles, quantum particles, etc.) are somehow 'fundamental', that they have an ontological 'surplus value' over entities of higher levels. They are the 'building blocks' of nature, the 'cement of the universe.' Vitalism claims that the animate nature differs from inanimate nature in that an additional non-material force is operative in living beings. Emergentism is the view that at each higher level of organization, new and irreducible properties appear (emerge) that are not present at lower levels. These are called 'emergent' properties and defined as properties of wholes, which are not possessed by their component parts, neither when taken separately nor when put together in other partial combinations. Organicism is the view that living organisms are complex, hierarchically structured wholes, whose parts are all functionally integrated in and co-ordinated by the whole. Consequently, the parts behave differently than they would when in isolation: they are co-ordinated by the whole. This means that not only do its parts determine the whole, but also that the parts are determined by the whole.

One of the conceptual elements of AM is that there are non-material organizing principles in nature in addition to the material elements of reality (nature). In current science, this position is most often regarded as fully unscientific. Although there is increasingly more scientific consensus that a reductionist explanation of (the development of) complex organisms is too simple, two concepts are regarded as sufficient material explanations for the complexity of organisms: epigenetics and emergentism. Epigenetics is ''the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.' [40] "Emergent phenomena are said to arise out and be sustained by more basic phenomena while at the same time exerting a 'top-down' control, constraint or some other sort of influence upon those" [41].

Although both concepts are currently at the center of scientific debate, this thesis challenges the validity of the central notion that higher organizational, epigenetic levels of organism arise *spontaneously* as a result of the assemblage of the material parts only by describing and discussing some of the unanswered scientific questions that are related to the ontological topic.

Until now, man has not been able to create life experimentally from matter only, and the question remains whether he will ever be able to do so [42]. Man has merely manipulated *existing* life into new forms or phenotypes. Therefore, one also has to consider the non-atomistic holistic hypothesis that life is more than the emergent organization that spontaneously arises from assembling the material parts.

The latest insights into the epigenetic mechanisms state that DNA methylation, histone tail modifications, and noncoding RNAs are responsible for changes in gene expression and the subsequent development of specific cells for specific tissues without changing the underlying DNA sequence [43]. However, this leaves the question unanswered as to how these mechanisms regulated themselves in the spatiotemporal development from the zygote stage to all later stages of the organism. How do the zygote and the further stages know which mechanism to use and which choices to make during mitosis? Where does the orientation towards differentiation in different tissues originate (especially in the zygote stage, which is characterized by a lack of localization

within an organism)?

Is emergentism really able to explain each phase of the development in time and space of the human fertilized egg (one cell) over 25 years, from one zygote into a fully physical developed human being with approximately 10^{27} molecules of human cells, that throughout the development is able to renew each molecule within two weeks, that is able to replace 97 percent of all cells of their body within one year, and that is constantly able to heal smaller and larger physical injuries and wounds of the human body?

The next issue is the field of the relationship between physical matter and the hypothesized production of 'substance' and content of non-material qualities. How is physical matter able to produce ontological non-material qualities like thoughts and feelings, and intuitions? How do neurobiological processes 'produce' perceptions of the world? How can one understand the qualia or states of phenomenal consciousness, which are purely intrinsic and not dependent on the causal relationships between the brain and the outside world [44]? Where does the intuitive sense of knowing ('it's on the tip of my tongue') [45] that arises before thought and speech originate? Where do the new 'intuitions' in human history that have the characteristic of representing 'a way of direct knowing that seeps into conscious awareness without the conscious mediation of logic and the rational process' originate [45]? How can one explain human pattern recognition, the key element of expert information processing, whereas three major theories (template matching theories, feature detection theories, and prototype theories) [46] are not able to explain all aspects of pattern recognition? Where does one find in the organism the intermediate level of abstraction that is called the 'moderately abstract conceptual representation' (MACR): a compromise between different abstractions like comparisons in the disciplines of physics and chemistry and concrete, specific problems [14], and that is able to 'produce' all expressions from one pattern and recognize one pattern in all expressions? How can one explain the exceptional skills of some of the 'savants', individuals with IQ levels that are potentially in the normal range but who have developmental disorders (particularly autism), and who demonstrate remarkable abilities or skills. Some savants, for example, are able to successfully perform calculation exercises with lightning speed without any aids or previous education; this makes the characteristic performance so remarkable. There is currently no widely accepted cognitive theory to explain the combination of talent and deficit found in savants [47].

What is the ontological quality of non-verbal meaning? Everybody can experience that there is a difference between the meaning of something, and the thoughts and words one has. One's perception of the meaning first refers to the whole that is experienced, and afterwards shapes the individual components that collectively express the meaning. The occurrence of specific typing errors, for example, shows that "the former" can disturb "the latter." People may have already typed a letter that should appear later in the sentence. Presumably, most people have had a similar experience, where they know exactly what they want to say before they say it. This is even clearer in those moments when a person cannot find the right words, and/or the spoken words do not match precisely what he/she really wants to say. Apparently, one can compare inner words with something that already exists, which is subjectively experienced and has wordless meaning.

All described examples demonstrate scientific questions that are directly related to the ontological question currently approached by the same type of scientific (materialistic and reductionist) thinking. The axiom is that only material substances (and their physical and chemical relationships) are the building blocks of nature. Science, therefore, only searches for material, physical hypotheses and explanations. If something new arises in the development of an organism (form in time or space, qualia, consciousness, intuitions, etc.), it then consequently must also be the result of matter. This also accounts for all patterns (e.g. biological self-organization levels in organisms, and pattern recognition in consciousness) that organize lower-level 'material' (e.g. proteins or processes in organisms, or words, perceptions or thoughts in consciousness). These organizing patterns even possess non-materialistic qualities such as qualia or dynamic structures in space, time and function on several levels of organisms (e.g. the organized movements of biological processes in time).

Looking at both the described ontological unanswered questions and the current reductionist reasoning, it is time for a renewed scientific interest in additional explanations positing non-physical properties for the 'substance' and content of self-organizing patterns or structures in organisms and in consciousness. Science has revealed that, on the one hand, during most of human history, until far into the 19th century, non-reductionist explanations positing non-physical properties were a part of scientific reasoning. Secondly, science has taught people that new theories arise when the old ones appear to be unable to describe and explain all the facts. The several holistic but physicalist theories (e.g. epigenetics and emergence), which have been developed by scientists to explain the described ontology-related 'problems,' are not yet sufficient to describe and explain all the facts.

Therefore scientists, instead of labeling these approaches as unscientific or pseudoscientific [48], should keep an open attitude towards explanations positing alternative ontologies, such as the non-atomistic/holistic explanations used in anthroposophic medicine.

11.5 Synthesis

The latest paradigm in medicine is the evidence-based medicine approach, which has brought a huge change to medicine since it was conceptualized in the early nineties. Since then, a huge amount of money and effort has been invested in providing evidence of the quality, safety, efficacy, effectiveness and cost-effectiveness of, for example, diagnostic procedures and treatments. The goal is to change medicine from expertise-based to evidence-based in order to provide optimal care for the individual patient.

However, after a while new paradigms grow old(er) and the shortcomings and unfulfilled expectations and unsolved problems become clearer. That is the time for new paradigms to arise, which provide new ways of thinking and new ways of solving problems *in addition* to the old paradigms. For example, the genome project brought a huge amount of information, but did not answer all questions on the direct influence of exposure to the environment, e.g. resulting in in-

dividually different disease susceptibilities. As a reaction, scientific attention was directed more towards epigenetics. The same type of paradigmatic shift can be seen in the shift from molecular biology to systems biology.

Health promotion is also a relatively new paradigm that fits in with these other paradigmatic changes. As with all paradigm shifts, new paradigms have to demonstrate that they are able to solve the 'left overs', the problems that could not be solved by the older approaches, while at the same time remaining consistent with the already established evidence. New ways of considering and solving healthcare problems with 'health promotion glasses' will take some time until they have become sufficiently evidence-based, are proven to be important, have become more familiar, and are integrated into healthcare with the fighting disease approach in order to achieve the best of both worlds. This thesis contributes to this development.

11.6 Future perspectives

Three major perspectives can be described based on the results of this thesis: implementation of new research lines, the further development of professional preventive and curative health promotion as a contribution to the further innovation of medicine and healthcare, and the investment in CAM/IM/AM research.

11.6.1 Implementation of new research lines

Based on the results of this thesis, new research lines can be defined and implemented:

1. Development and validation of health promotion strategies:
   a. Further development and validation of the health concept and its working mechanisms.
   b. Designing of preventive and curative health promotion strategies based on the validated health concept.
   c. Testing of safety, efficacy, effectiveness and cost-effectiveness of preventive and curative health promotion strategies for specific indications.
   d. Integration of fighting disease and health promotion strategies for specific indications.
2. Development and validation of pattern analysis methodologies:
   a. Development and testing of statistical methods to explore, model and test patterns such as patterns of immunological elements representing higher organization and epigenetic levels.
   b. The further development, validation and application of trained judgment and the qualitative Goethean phenomenological method as methods to acquire scientific objective knowledge on higher organization levels in science and clinical practice.
3. Development and testing of the contribution of CAM treatment for specific indications to

the innovation of system biology-orientated medicine.
   a. Development and testing of multi-therapy and multi-target treatments.
   b. Diagnostic and therapeutic individualization using pattern recognition methodologies.
4. Optimizing and validation of Citrus/Cydonia comp. treatment of seasonal allergic rhinitis:
   a. Testing of the different workings mechanisms of Citrus and Cydonia *in vivo*.
   b. Optimizing, if necessary, the integration of the different working mechanisms of Citrus and Cydonia in one product *in vivo*.
   c. Designing and execution of pragmatic and explanatory trials.
   d. Studying the extracellular matrix and the effects of the specific subcutaneous route of administration in this extracellular matrix compared to other routes of administration.

11.6.2 Further development of professional preventive and curative health promotion as a contribution to the further innovation of medicine and healthcare

Based on the results of this thesis, new development programs can be defined and implemented:

1. Programmatic development of qualitative effective CAM and IM products and the evidence of safety, efficacy, effectiveness and cost-effectiveness, following the line of development from practice-based evidence to evidence-based practice.
2. Programmatic development of specific healthcare 'products' for specific diseases, integrating the best of both worlds of conventional medicine and CAM into IM 'products'.
3. Programmatic development of specific integrative products for specific diseases, integrating the best of both worlds of the fighting disease and the health promotion approach.
4. Continuous professional support of the development of patient competence to its full potential in all aspects of healthcare.

11.6.3 Investment in integrative medicine research

Based on the results of this thesis and the strategic plans of, for example, the NCCAM [49] in the United States and ZonMW [50] in the Netherlands, the following investments in CAM and IM research are suggested:

1. Advance research on CAM mind and body interventions, practices, and disciplines.
2. Advance research on CAM natural products.
3. Advance research on monitoring and balancing the demands of patients on the one hand and CAM and IM healthcare products on the other hand.
4. Advance research on context factors, healing environments and placebo effects.

5. Increase understanding of the "real-world" patterns and outcomes of CAM use and its integration into health care and health promotion.
6. Improve the capacity of the field to conduct rigorous research.
7. Improve and/or develop methodologies that are in line with ontological and epistemological aspects of IM streams.
8. Invest in cooperation between conventional and IM researchers and healthcare professionals.
9. Develop and disseminate objective, evidence-based information on CAM aims, patient experiences, interventions, safety, efficacy, effectiveness, and cost-effectiveness.

11.7 Final conclusions

The aim of this thesis is to contribute to the further development of the professional curative health promotion approach and its integration with the fighting disease approach in medicine. The specific contributions of this thesis to this development are:

- The development of a more valid concept of health than the WHO concept of 1948, which can serve as a theoretical fundament for preventive and curative health promotion.
- The development of systems biology-orientated, pattern variable biomarkers for the monitoring of health changes.
- The testing of the anthroposophic medicine Citrus/Cydonia comp. in SAR treatment as an example of curative health promotion, demonstrating the safety, moderate efficacy, and specific immunological working mechanism of the medicine.
- The testing of the efficacy, effectiveness and cost-effectiveness of CAM compared to conventional medicine, demonstrating that CAM could be effective and might have significant cost-saving potential.
- The scientific underpinning of the holistic health promotion approach based on the current developments regarding the ontological, epistemological and methodological aspects of the holism-reductionism scientific framework.

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Appendix

Patients whose GP knows complementary medicine tend to have lower costs and live longer

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Abstract

Background: Health economists have largely ignored complementary and alternative medicine (CAM) as an area of research, although both clinical experiences and several empirical studies suggest cost-effectiveness of CAM.

Objective: to explore the cost-effectiveness of CAM compared to conventional medicine.

Methods: A data set from a Dutch health insurer was used containing quarterly information on healthcare costs (care by general practitioner (GP), hospital care, pharmaceutical care, and paramedic care), dates of birth and death, gender and 6-digit postcode of all approximately 150,000 insurees, for the years 2006 - 2009. Data from 1913 conventional GPs were compared to data from 79 GPs with additional CAM training in acupuncture (25), homeopathy (28) and anthroposophic medicine (26).

Results: Patients whose GP has additional CAM training have 0 to 30 percent lower healthcare costs and mortality rates, depending on age groups and type of CAM. The lower costs result from fewer hospital stays and fewer prescription drugs.

Discussion: Since the differences are obtained while controlling for confounders including neighborhood specific fixed effects at a highly detailed level, the lower costs and longer lives are unlikely to be related to differences in socio-economic status. Possible explanations include selection (e.g. people with a low taste for medical interventions might be more likely to choose CAM) and better practices (e.g. less overtreatment, more focus on preventive and curative health promotion) by GPs with knowledge of complementary medicine. More controlled studies (replication studies, research based on more comprehensive data, cost-effectiveness studies on CAM for specific diagnostic categories) are indicated.

Keywords: healthcare costs, life expectancy, complementary medicine.

JEL classification: I11, I12.

Introduction

Complementary and alternative medicine (CAM) has been largely ignored by health economists as an area of research. That fact is possibly related to the low esteem of CAM in the medical profession.

Defining CAM is difficult, because the field is very broad and constantly changing. According to the National Center for Complementary and Alternative Medicine (NCCAM) CAM is a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine [1]. The Cochrane Collaboration definition of complementary medicine is that it includes all such practices and ideas which are outside the domain of conventional medicine in several countries and defined by its users as preventing or treating illness, or promoting health and well being. These practices complement mainstream medicine by satisfying a demand not met by conventional practices and diversifying the conceptual framework of medicine [2].

Patients around the globe are increasingly embracing CAM as a contributor to health. A recent study by the US National Institute of Health shows that 4 out of 10 Americans used some form of CAM in 2007. Another study on Switzerland reported that almost 11% of the population had used one of five CAM streams (anthroposophic medicine, homeopathy, neural therapy, phytotherapy and Traditional Chinese Medicine) in 2002. The CAM doctors in that study treated patients that tended to be younger, female and better educated. These patients also tended to have a favorable attitude towards complementary medicine and to exhibit chronic and more severe forms of disease. The majority of alternative medicine users appear to have chosen CAM mainly because they wish to undergo a certain procedure; additional reasons include desire for more comprehensive treatment, and expectation of fewer side-effects [3]. In a referendum in Switzerland in 2009, two thirds of the voters were in favor of a wider coverage of CAM by public health insurance. In January 2011, based on the positive outcome of a national referendum, the Swiss authorities decided that five main streams of CAM (anthroposophic medicine, homeopathy, neural therapy, phytotherapy and Traditional Chinese Medicine) will be covered by the mandatory health insurance for a period of six years (2012-2017) [4].

In many cases, the effectiveness of CAM has not been proven in clinical trials [5]. However, lack of proof of effectiveness is obviously not the same as proof of ineffectiveness. Clearly, the status of a treatment can change from CAM into conventional medicine once scientific evidence on effectiveness becomes available. Two examples of CAM treatments that have become (more) accepted by conventional medicine are St. John´s wort and acupuncture for specific indications. St. John´s wort, for more than 90 years used in anthroposophic medicine, has become part of the conventional guidelines for the treatment of depression, based on scientific evidence from randomized controlled trials [6]. Hopton and McPherson [7] conclude on the basis of a systematic review of pooled data from meta-analyses that acupuncture is more than a placebo for commonly occurring chronic pain conditions. In addition, in her thesis, van den Berg [8] recently demonstrated positive effects of acupuncture on obstetric health problems (breech presentation). Also Servan-Schreiber [9] presents a series of recent examples of the transition from CAM to conventional medicine in

depression treatment. Some of the methods described by Servan-Schreiber have been practiced for centuries, cannot be patented, and are available at low costs. These findings underscore the fact that methods that are considered CAM today could be effective and have a large cost-savings potential.

Anthroposophic medicine, acupuncture and homeopathy are three main streams of CAM. One of the core features of CAM is its orientation on preventative and curative health promotion as an additional approach to a more conventional fighting disease approach. Anthroposophic medicine is an integrative diagnosis and therapy concept, developed from 1921 onwards and practiced today in over 60 countries. It combines mainstream scientific medicine with Rudolf Steiner's anthroposophy. Anthroposophic medicine considers a human being as a whole entity - body, mind, soul and individuality. It aims to stimulate the self-healing forces of the body, restoring the balance of bodily functions and strengthening the immune system, rather than primarily relieve the symptoms of disease. Specific anthroposophic approaches include anthroposophic medicinal products, massage therapy, art and music therapy, and speech and movement therapies [10].

Homeopathy is a form of alternative medicine, first proposed by the German physician Samuel Hahnemann in 1796, that attempts to treat patients with heavily diluted substances. These substances which cause certain symptoms in healthy individuals are given as the treatment for patients exhibiting similar symptoms. The appropriate homeopathic medicinal product aims to stimulate the body's inherent forces of self-recovery [11].

Acupuncture is one of the main forms of treatment in Traditional Chinese Medicine (TCM). It involves the use of sharp, thin needles that are inserted in the body at very specific points. This process is believed to adjust and alter the body's energy flow into healthier patterns, and is used to treat a wide variety of illnesses and health conditions [12].

In their review, Herman et al. [13] report that some studies indicate that CAM therapies may be considered cost-effective compared to usual care for various conditions: acupuncture for migraine, manual therapy for neck pain, spa therapy for Parkinson's, self-administered stress management for cancer patients undergoing chemotherapy, pre- and post-operative oral nutritional supplementation for lower gastrointestinal tract surgery, biofeedback for patients with 'functional' disorders (eg, irritable bowel syndrome), and guided imagery, relaxation therapy, and potassium rich diet for cardiac patients. A systematic review of randomized clinical trials on the use of so-called Natural Health Products shows evidence of cost effectiveness in relation to postoperative surgery but not with respect to the other conditions assessed [14]. Studer and Busato [15] demonstrated that general practitioners who have completed certified additional training in CAM after obtaining their conventional medical degree (GP-CAMs) (n = 257) with general practitioners who have not (GPs) (n = 174) have equal costs per patient per year, but significantly lower costs per doctor (29%) per year, although GP-CAMs take more time per patient. A NCCAM study in 2007 demonstrated that CAM costs were 11.2% of total out-of-pocket expenditures on health care in the USA [16].

GP care varies between European countries in terms of structure, working methods, and responsibilities. In the Netherlands GPs are the central gatekeepers for reference to the rest of healthcare, like specialists and paramedics. Dutch general practitioners generally receive a quarterly fixed fee per patient plus a fee-for-service per consultation and per drug prescription. There is

no difference between the financial incentives faced by GPs and GP-CAMs. In the Netherlands purchasing basic health insurance is mandatory for all citizens. In addition, citizens are free to purchase supplementary insurance.

Since there is a lack of cost-effectiveness data of CAM in The Netherlands, in this paper, we compare the performance of general practitioners who have completed certified additional training in CAM after obtaining their conventional medical degree (GP-CAMs) with general practitioners who have not (GPs). More specifically, we consider GP-CAMs with additional training in anthroposophic medicine, homeopathy, or acupuncture (about 1 percent of GPs for each of these CAM types).

Methods

Model overview

We analyze costs at the patient level using linear and loglinear regression analysis. While the linear specification is more common, the loglinear specification can be argued to be more appropriate given that costs are nonnegative and cost distributions typically have long tails. Given the large average differences in health and health care needs across age groups, the cost analysis has been performed separately for the age groups 0-24, 25-49, 50-74, 75+. In all cost regressions, the explanatory variables are: gender, age (linear, within each age category), dummies for each quarter, dummies for anthroposophy, homeopathy, and acupuncture, and 6-digit postal code fixed effects

Effects on mortality rates are analyzed using a Logit model with fixed effects at the 4-digit insuree postcode level. Given the relatively low proportion of deaths (less than 3 percent of insurees died during our sampling period) fixed effects at 6-digit insure level are infeasible. To check for robustness against functional form specification we also analyze mortality using fixed effects Linear Probability Models.

Dataset on healthcare costs and demographics

A dataset from health insurer Azivo, active primarily in the city of Hague and its wider vicinity, was used for the analyses. Azivo is a former *Ziekenfonds* (sick fund) founded in 1895. It merged with health insurer Menzis in 2008, but keeps operating as "Azivo" in the the Hague region. Its share in the market for basic and supplementary health insurance in this region is about one quarter.

The data set contains quarterly information on the healthcare costs of all Azivo insurees for the years 2006 up to 2009. In addition, it contains the date of birth of the insuree, date of death (if applicable), gender, and 6-digit postcode of the insuree's residence. For each insuree-quarter combination, information on the costs of four different types of care are available: care by GP,

hospital care, pharmaceutical care, and paramedic care (like physical therapy). The data set does not contain information on the supplementary insurance *status* of insures; the cost information is the sum of expenses covered by both the basic and (if applicable) supplementary health insurance.

General practitioners

The data set also contains the names and addresses of the general practitioners who have patients who are insured by Azivo, which allows us to distinguish between conventional GPs and GP-CAMs. We defined a general practitioner as anthroposophic GP-CAM if his or her name appears in the list of general practitioners with additional training in anthroposophic medicine as provided by their professional association [17]. GP-CAMs with homeopathy [18] and GP-CAMs with acupuncture [19] are defined similarly.

Statistical analyses

Significance of coefficients is tested using *t*-test, with clustering of standard errors at the level of the insuree. Calculations were performed using StataSE 10.0.

Results

Patient demographics

The dataset contained information on 151,952 insurees with a mean age of 38.4 (SD=22.6); 53 percent is female. These patients live in 21902 different 6-digit postal codes.

General practitioners

The dataset contained information from 1992 GPs: 1913 conventional GPs and 79 complementary GPs (GP-CAM) (anthroposophy: 26, homeopathy: 28, acupuncture: 25). The number of patients insured with Azivo is highly unevenly distributed across GPs. For example, 5 out the 26 anthroposofic GPs in the data set account for more than 95 percent of the claims by patients with anthroposofic GP. This is because Azivo has a relatively large market share (about one quarter) in the The Hague region and a very low market share in most other regions. The average number of Azivo patients with these 5 anthroposofic GPs is about 570. The corresponding figures for the other

GP types are 850 (conventional), 150 (homeopathy) and 360 (acupuncture). The differences can be due to variations in the size of the total practice as well as in variations in Azivo's market share across the four groups of patients.

Healthcare costs

The costs of patients with a GP-CAM are 7 percent lower compared to conventional GPs, which amounts to 140 Euros per patient annually. However, this difference in raw means of total costs is significant only for anthroposophic GP-CAMs. The lower total costs result from lower hospital and pharmaceutical costs. Patients with a GP-CAM have slightly higher costs for paramedic care, but this difference is small. When the costs are compared by age group, in absolute terms, the differences are particularly large for patients aged 75 and above with an anthroposophic GP-CAM (more than 1000 Euros on an annual basis) (Table 2).

The analyses also demonstrate large demographic differences between patients with a conventional GP versus patients with GP-CAMs (Table 1). GP-CAMs have a larger fraction of female patients than conventional GPs and fewer patients from disadvantaged neighborhoods. Clearly, the costs differences reported in tables 1 and 2 are partly due to differences in the demographic composition of the various groups of patients, and therefore difficult to interpret.

After controlling for these demographic differences by means of regression analyses we find that for patients in the age group 25 to 49 with a GP-CAM with acupuncture total costs are 66 euro lower per quarter (Table 3, left panel). Secondly, for patients aged 75 and above with an anthroposophic GP-CAM total costs are about 400 Euros lower per quarter. The magnitude of this difference is large, about one third lower. The separate regressions for the costs components show that these lower costs come from lower hospital and lower pharmaceutical costs. The results for the loglinear specification show a somewhat different pattern. Homeopathic GP-CAMs have about 15 percent lower costs in all three age categories below age 75. The lower costs for patients aged 25-49 who have a GP-CAM with acupuncture are found again for the loglinear specification.

It is important to note that 6-digit postal codes in the Netherlands are highly detailed, representing on 16 households on average. Within such a code households are highly homogeneous in terms of socio-economic status. Given that we have controlled for 6-digit postal codes in the regressions, the results are unlikely to be due to differences in socio-economic status.

Health status

In the present data set the only information available on health outcomes is mortality in the years 2006 up to and including 2009. For the population of insurees in our data, the mortality rate was approximately 3 percent. After controlling for demographics (including age) and 4-digit postal codes, we find that patients with a GP-CAM have significantly lower mortality rates (Table 4). For

all three types of CAM the effect is significant for some specifications, but not for all specifications. The magnitude of the effect again varies between 0 and 30 percent.

Discussion

There are four types of explanations for the differences reported in the previous section. First, the differences could be due to selection on unobservables in patients' GP choice. For example, patients who are healthier and more health-conscious, or patients with a strong preference to minimize exposure to medical interventions might be more likely to choose a GP-CAM. In both cases costs will be lower due to lower demand for health care. A standard approach to control for selection on unobservables is to use instrumental variables. A potential instrumental variable (IV) in this case is the distance between a patient's home and the various GPs. However, the distance measures would be perfectly correlated with the 6-digit postal code dummies. As a consequence, this IV would only work if we would control for less detailed neighborhood information, like 4-digit postal codes. However, since socio-economic differences within a 4-digit postal code are typically large, this would not be a credible approach for identifying a causal effect of CAM on costs.

Second, the results could be due to undertreatment by GP-CAMs. In the present data set we were only able to analyze mortality and found that patients with a GP-CAM tend to have lower mortality rates. A number of studies have reported that patients seeking anthroposophic or homeopathic care have longer lasting and more severe health problems than patients in conventional care. At the same time, these patients report fewer adverse side effects of treatments and higher patient satisfaction [e.g., 20, 21]. These findings combined with the results in this study provide some indication that undertreatment by GP-CAMs is unlikely. Firmer conclusions require more data on outcomes.

Thirdly, the results could be due to better practices of CAM due to a stronger focus on preventive and curative health promotion and less overtreatment. For example, a GP-CAM might try a low cost CAM treatment first. As mentioned, the primary professional orientation of CAM doctors is to strengthen the self-healing capacity of the body and the self-management of the patient. This approach is associated with prescribing fewer conventional pharmaceuticals, tests, and operations.

Fourthly, the lower costs could be related to the fact that patients interested in CAM might have higher out-of-pocket expenses since CAM is not included in the basic health insurance package. On the other hand, patients interested in CAM are more likely to buy supplementary insurance that covers CAM. This would imply that the marginal out-of-pocket expenses for these insurees are lower than for insurees with a conventional GP, leading to *more* consumption of healthcare (recall that the Azivo data contain costs covered by basic health insurance plus costs covered by optional supplementary health insurance). Yet, we find that the costs of patients with a GP-CAM are lower. Clarifying the role of out-of-pocket expenses is an empirical issue that requires additional data.

Several studies that compare the health status of patients treated in CAM and in conventional

medicine in primary care settings find that patients treated in CAM practices suffer more often from severe and chronic illnesses (e.g., [20, 21]). This suggests that if we could control for severity and chronicity of illnesses (with additional data), the estimated cost differences might be larger.

Another result of this study is that GP-CAMs have a larger fraction of female patients than conventional GPs and fewer patients from disadvantaged neighborhoods. Similar findings have been reported for the US [22] and for Switzerland [20].

The major limitations of this study concern the limited dataset. First of all the dataset is from only one insurer in one specific Dutch region and the data reflect the behavior of only a small number of GPs with additional training in CAM. This challenges the generalizability of the results. Secondly, the dataset does not cover all the information needed to perform an optimal comparison of cost-effectiveness. Missing information includes costs distinguished by basic and supplementary insurance, out-of-pocket expenses, morbidity, work absence, subjective health, and patient satisfaction.

Consequently, a large number of issues remain for future research. We mention three of them specifically. First, replication studies based on similar data sets are needed to confirm the present results. Secondly, further research is needed to determine to what extent selection on unobservables and causal effects explain the lower costs and lower mortality rates of patients with a GP-CAM. Thirdly, more research is needed with regard to the cost-effectiveness of CAM for specific diagnostic categories.

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Table 1. Descriptive statistics on patients with GPs and GP-CAMs

	GP	*GP-CAMs*		
Costs of health care	Conventional GP	GP-CAM anthroposophy	GP-CAM homeopathy	GP-CAM acupuncture
Total	515[a]	479***	485	480
GP	32	33***	31***	32
Hospital	266	236***	251	235**
Pharmaceutical	209	197*	192	206
Paramedic	9	13***	10**	8*
I*ncidence of costs of health care (0/1)* (per quarter)				
GP	1	1	1	1
Hospital	0.29	0.28***	0.26***	0.30
Pharmaceutical	0.68	0.67***	0.62***	0.65***
Paramedic	0.04	0.06***	0.04**	0.04**
Hosp., Pharma, and/ or Paramedic	0.72	0.71*	0.66***	0.69***
Mortality	0.026	0.021	0.038**	0.025
Insuree characteristics				
Female (fraction)	0.53	0.57***	0.56***	0.54***
Birth year (average)	1969	1970***	1965***	1966***
Disadvantaged neighborhood (fraction)[b]	0.22	0.09***	0.07***	0.04***
Number of Azivo insurees	151,952	3271	1181	1470
Number of GPs	1913	26	28	25

[a] Costs of healthcare are in Euros per quarter; ***, **, * indicate a statistically significant difference with Conventional GP at the 1, 5, 10 percent level, respectively.

[b] Based on a government list of most disadvantaged neighborhoods in the Netherlands ("Vogelaar-wijken"). These neighborhoods are uniquely identified by their 4-digit postal code.

Table 2. Costs of health care; by type of GP and insuree age category

	GPs	GP-CAMs		
Costs of health care (euros per quarter)	Conventional GP	GP-CAM anthroposophy	GP-CAM homeopathy	GP-CAM acupuncture
Age 0-24				
Total	**215a**	**190**	**275**	**191**
GP	26	26	24***	25
Hospital	103	85*	153**	96
Pharmaceutical	77	69	88	62
Paramedic	8	11***	10	8
Age 25-49				
Total	**372**	**418*** **	**286** **	**296*** **
GP	28	31***	25***	26***
Hospital	186	201	156	146**
Pharmaceutical	155	180***	103***	122**
Paramedic	4	7***	3	1***
Age 50-74				
Total	**824**	**752** **	**614*** **	**687*** **
GP	37	39***	35***	35***
Hospital	432	382**	270***	324***
Pharmaceutical	342	311**	294*	317
Paramedic	12	19***	14*	11
Age 75+				
Total	**1337**	**1088** **	**1309**	**1139***
GP	57	57	59	56
Hospital	727	576**	820	595
Pharmaceutical	527	426**	403*	466
Paramedic	27	30	27	21

[a] Costs of healthcare are in Euros per quarter

***, **, * indicate a statistically significant difference with Conventional GP at the 1, 5, 10 percent level, respectively.

Table 3. Effects of complementary care on costs per insuree age category

	Linear			*Loglinear*		
	dummy for GP-CAM anthro-posophy	*dummy for GP-CAM homeo-pathy*	*dummy for GP-CAM acupunc-ture*	*dummy for GP-CAM anthro-posophy*	*dummy for GP-CAM homeo-pathy*	*dummy for GP -CAM acupunc-ture*
Age 0-24						
Total	**6a**	**100**	**-32**	**0.016**	**-0.138****	**-0.052**
GP	1	-2*	1	0.015	-0.043*	0.019
Hospital	3	76	-5	0.064	-0.153*	-0.034
Pharmaceutical	1	25	-27	-0.078*	-0.250***	-0.108
Paramedic	2	0	-1	0.048	-0.006	-0.008
Age 25-49						
Total	**14**	**-50**	**-66***	**0.022**	**-0.160****	**-0.106****
GP	2***	-3***	0	0.030**	-0.045**	-0.004
Hospital	3	4	-47**	0.008	-0.161**	-0.135**
Pharmaceutical	8	-51**	-17	-0.035	-0.365***	-0.136*
Paramedic	1	-1	-2***	0.032	-0.029	-0.060***
Age 50-74						
Total	**63**	**-48**	**-2**	**-0.030**	**-0.153****	**-0.084**
GP	4***	0	0	0.040*	-0.001	0.017
Hospital	60	-121	-64	0.032	-0.145	-0.073
Pharmaceutical	-7	69	61	-0.204***	-0.352***	-0.162
Paramedic	6*	4	1	0.080	0.016	-0.009
Age 75+						
Total	**-405****	**81**	**214**	**-0.130**	**0.077**	**0.184**
GP	-2	6	7	-0.030	0.058	0.111
Hospital	-263**	52	87	-0.029	0.069	0.171
Pharmaceutical	-125*	31	127	-0.169	0.048	0.196
Paramedic	-15	-8	-7	-0.106	-0.085	0.034

a Costs of healthcare are in Euros per quarter. Each row is based on two regressions with either costs (left panel) or the natural logarithm of costs (right panel) as the dependent variable. Explanatory variables are: gender, age (linear, within each age category), dummies for each quarter, dummies for anthroposophy, homeopathy, and acupuncture; the table reports the coefficients on the latter dummies. All regressions control for 6-digit insuree postcode fixed effects; standard errors clustered at the insuree level.

***, **, * indicate a statistically significant difference with Conventional GP at the 1, 5, 10 percent level, respectively.

Table 4. Effects of complementary care on mortality

	dummy for GP-CAM anthroposophy	*dummy for GP-CAM homeopathy*	*dummy for GP-CAM acupuncture*	*Combined*
Logit with fixed effects	0.031	-0.198	-0.333*	-0.128
LPM with fixed effects	-0.005*	-0.004	-0.009**	-0.006***
Women				
Logit with fixed effects	0.034	0.010	-0.203	-0.031
LPM with fixed effects	-0.007*	0.004	-0.008	-0.005*
Men				
Logit with fixed effects	0.020	-0.627*	-0.493	-0.291*
LPM with fixed effects	-0.003	-0.014	-0.013**	-0.008**

Dependent variable: death in 2006, 2007, 2008, or 2009.

The table is based on models with the following explanatory variables: gender, age, dummies for anthroposophy, homeopathy, and acupuncture (dummy for complementary in the last column); the table reports the coefficients on the latter dummies.

LPM regression controls for 4-digit insuree postcode fixed effects.

***, **, * indicate a statistical significance at the 1, 5, 10 percent level, respectively.

Summary

The prognosis of higher life expectancies of both men and women in the Netherlands, often related to chronic diseases and high healthcare costs, the changed role of patients into competent patients, and the increasing interest of patients in complementary medicine worldwide provides the scientific rationale to put more emphasis in healthcare on developing valid, effective, cost-effective and safe strategies that support and enable patients to actively contribute to their own health and disease status in order to promote their health in a preventive and/ or curative manner.

Although much effort has been invested worldwide, a lot of scientific and practical work still has to be done to develop the health promotion approach into one that is evidenced-based and professional like the fighting disease approach. This thesis focuses on some of the conceptual, methodological and empirical issues of developing professional preventive and curative health promotion as a contribution to the further innovation of medicine and healthcare.

Chapter 2 analyzed historic and current health concepts and developed and preliminarily validated the new concept of 'health by self-regulation' by means of analyzing its internal consistency, its accordance with other scientific concepts (e.g. the concepts of epigenetics, systems biology, and emergence) and its accordance with empirical facts. Finally, it demonstrated two examples of integration of the fighting disease and the health promotion approach and discussed the theoretical and practical implications of this new concept.

In Chapter 3, the fundamental scientific discussion of holism versus reductionism was summarized. This chapter demonstrated that there are several arguments against the current reductionist point of view. This provides a scientific opening for a more holistic or systems biology-orientated concept of health and a more holistic or systems biology-orientated methodological approach.

Chapter 4 described a new system's biology-orientated methodological approach in developing immunological biomarkers for monitoring treatment effect in seasonal allergic rhinitis (hay fever) research. Using permuted stepwise regression, pattern variables that reflect immune system functioning on the systems level were computed and tested. Computation was based on a dataset (from a randomized controlled trial comparing two routes of administration) of allergen-specifically induced expression levels of cytokines (IL-1β, IL-5, IL-10, IL-12, IL-13, IL-17, IFN-γ and TNF-α) and symptom severity scores from 22 seasonal allergic rhinitis (SAR) patients measured before and after six weeks of treatment with medicinal products containing Citrus and Cydonia. Further computation and biomarker validation with larger datasets, including data from healthy persons and SAR patients, is indicated.

In Chapter 5, the experiences of 39 Dutch general practitioners with anthroposophic SAR treatment were examined. The results of this survey provide the first practice-based evidence of positive treatment results of Citrus/Cydonia comp. for SAR.

Chapter 6 examined, in two *in vitro* studies, the immunological pathways of the effects of Citrus/Cydonia comp. from a healthy and an allergic donor, respectively. Peripheral blood mono-

nuclear cells (PBMCs) were isolated out of peripheral blood and analyzed *in vitro* after polyclonal stimulation of T-cells. The differentiation capacity and the influence regarding Th1 (IFN-γ) and Th2 (IL-5) cells were examined. Citrus/Cydonia comp. has a selective effect on the differentiation of Th-cells by producing relatively more IL-10 than IL-12. It also seems to have an effect on the induction of regulatory (IL-10 producing) T-cell subsets. It is *in vitro* capable of partly neutralizing the changes, characteristic to allergic rhinitis, regarding the maturation, differentiation, and activity of the immune system. Thus, it was concluded that Citrus/Cydonia comp. can potentially restore the disturbed immune state of rhinitis patients, which essentially could be sufficient to make allergic symptoms disappear permanently.

Chapter 7 examined the effects of the combined product, *Citrus e fructibus/Cydonia e fructibu*s (Citrus/Cydonia), and separate products of Citrus and Cydonia on the immuno-pathological pathways involved in seasonal allergic rhinitis (SAR). Peripheral blood mononuclear cells (PBMCs) from five healthy and five grass pollen-allergic donors were isolated and analyzed *in vitro* after polyclonal and allergen-specific stimulation of T-cells in the presence of the three extracts. The analyses demonstrated acceptable cell survival with no signs of toxicity. Citrus mainly had a selective effect on reducing allergen-specific chronic inflammatory (TNF-α) and Th2 (IL-5) pathway activity; whereas both Cydonia and Citrus/Cydonia mainly affected the induction of the allergen-specific Th1 (IFN-γ) pathway. Citrus and Cydonia demonstrated differential working mechanisms in the treatment of SAR, and the combination product did not demonstrate larger effects than the separate preparations. Hence, it was concluded that further effectiveness and efficacy studies comparing the effects of the products on SAR *in vivo* are required.

Chapter 8 studied a small group of 13 patients with a mean history of hay fever with grass pollen allergy of 9 years' duration, who in previous years used conventional hay fever medication because of the severity of their symptoms during the pollen season. Gencydo injections were administered to 12 patients before the onset of and during the grass pollen season, and in one patient during the grass pollen season only. Nasal and non-nasal hay fever symptom severity, use of rescue medication (antihistamines or corticosteroids), and the subjective experiences of patients were used as outcome measures. It was concluded that there are clear indications that Gencydo treatment is effective in a large subgroup of the research population.

Chapter 9 compared the efficacy and safety of two routes of administration (subcutaneous injections (SI) versus nasal spray (NS)) in a national, randomized, comparative clinical trial with two parallel groups. A total of 23 patients fulfilled the study requirements. After a one- or two-week wash-out period, twenty-three patients were randomized to a six-week treatment period. The outcomes assessed were immunological and symptom severity changes and safety. Immunologic outcome assessments were blinded to group assignment. It was concluded that both routes of administration are safe and demonstrate immunological and clinical effects, with larger inflammatory and innate immunological effects of the NS route and larger allergen-specific clinical effects in the SI group.

Chapter 10 reported a study that was aiming to update the safety status of anthroposophic and homeopathic solutions for injection through a systematic evaluation of the reported adverse drug

reactions (ADRs). ADRs were extracted from the pharmacovigilance databases of eight German anthroposophic and homeopathic manufacturers covering the period of 2000-2009. These eight manufacturers represent, in total, more than 94 percent of the sales of anthroposophic and homeopathic solutions for injection in Germany. Analyzed ADRs included reports in humans only, reports from post-marketing surveillance, literature cases and clinical/safety trials, and spontaneous reports from healthcare professionals and patients. The results suggest an excellent safety profile of solutions for injection as therapeutically applied in anthroposophic medicine and homeopathy, where the overall incidence of ADRs are very rare.

Chapter 11 presented the major findings of this thesis, studying the concept of health, the methodology to monitor (changes in) health states, the effects and safety of Citrus/Cydonia comp. on seasonal allergic rhinitis as an example of curative health promotion, and the cost-effectiveness of integrative medicine in the Netherlands. The theoretical and practical implications of the results of this thesis were discussed and reflected on the scientific fundament of anthroposophic medicine. Future perspectives were also described for the implementation of new research lines, the further development of professional preventive and curative health promotion as a contribution to the further innovation of medicine and healthcare, and the investment in CAM/IM/AM research.

In the Appendix, a cost-effectiveness study is presented in which the possible contribution of integrative medicine to the reduction of healthcare costs was studied. This study compared the performance of general practitioners who have completed certified additional training in CAM after obtaining their conventional medical degree (GP-CAMs) with general practitioners who have not (GPs). Patients whose GP has completed additional CAM training have 0–30 percent lower healthcare costs and mortality rates, depending on age group and the type of CAM. The lower costs result from fewer hospital stays and fewer prescription drugs. Since the differences are obtained while controlling for confounders, including neighborhood-specific fixed effects at a highly detailed level, the lower costs and longer lives are unlikely to be related to differences in socioeconomic status. Possible explanations include selection (e.g. people with low interest in medical interventions might be more likely to choose CAM) and better practices (e.g. less overtreatment, more focus on preventive and curative health promotion) by GPs with knowledge of complementary medicine. More controlled studies (replication studies, research based on more comprehensive data, cost-effectiveness studies on CAM for specific diagnostic categories) are required. These findings highlight the fact that the health-promoting methods that are considered CAM today could be effective and might have significant cost-saving potential.

With the example of Citrus/Cydonia comp. in SAR treatment, this thesis demonstrates the safety, moderate efficacy and specific immunological working mechanism of this medicine, underscoring that CAM could be effective and might have a significant cost-saving potential, and collectively, the validity of the concept of preventive and curative health promotion.

Samenvatting

Door in de gezondheidszorg meer nadruk te gaan leggen op een gezondheidsbevorderende benadering ('health promotion') in aanvulling op een ziektebestrijdende benadering ('fighting disease') kan ingespeeld worden op een aantal belangrijke ontwikkelingen in de gezondheidszorg in Nederland van de laatste decennia: de prognose van een hogere levensverwachting voor zowel mannen als vrouwen in Nederland, de daaraan gerelateerde hogere prevalentie van chronische ziekten en hoge kosten van de gezondheidszorg, de veranderde rol van patiënten in competente patiënten, en de toenemende wereldwijde belangstelling van patiënten voor complementaire vormen van geneeskunde. Het is daarom van belang valide, (kosten)effectieve en veilige strategieën te ontwikkelen die het patiënten mogelijk maken om actief bij te dragen aan hun eigen gezondheid- en ziektetoestand. Daarmee bevorderen zij hun gezondheid op een preventieve en/of curatieve manier. De verwachting is dat dit naast gezondheidswinst tevens een bijdrage levert aan het in de hand houden van de kosten in de gezondheidszorg.

Hoewel er wereldwijd al veel geld en menskracht is geïnvesteerd, moet er nog veel wetenschappelijk werk en werk in de zorgpraktijk verricht worden om de *gezondheidsbevorderende aanpak* te ontwikkelen tot één die even evidence-based en professioneel ontwikkeld is als de *ziektebestrijdende* aanpak. Dit proefschrift richt zich op een aantal conceptuele, methodologische en empirische aspecten van het verder ontwikkelen van professionele preventieve en curatieve gezondheidsbevordering als een bijdrage aan de verdere innovatie van de geneeskunde en de gezondheidszorg.

Hoofdstuk 2 analyseert de definitie van gezondheid van de World Health Organization (WHO) uit 1948 en huidige gezondheidsdefinities en reviseert en presenteert een nieuwe definitie: 'gezondheid door zelfregulatie'. Door de interne consistentie van de definitie te analyseren, en de overeenstemming met andere wetenschappelijke concepten (bijvoorbeeld de concepten van de epigenetica, de systeembiologie en de emergentie) en met empirische feiten vast te stellen, wordt de kwaliteit van de definitie onderzocht. Vervolgens worden aan twee voorbeelden (hooikoorts en chronische, psychologische traumatisering) de mogelijkheden van een integratieve behandeling met ziektebestrijdende en gezondheidbevorderende onderdelen gedemonstreerd. Ten slotte worden theoretische en praktische implicaties van deze nieuwe definitie beschreven.

In hoofdstuk 3 wordt de basale wetenschappelijke discussie over holisme versus reductionisme samengevat. Aannemelijk werd gemaakt dat er een aantal argumenten bestaat tegen het huidige, in de wetenschap gangbare, reductionistische standpunt. Deze argumenten bieden een wetenschappelijke opening voor een meer holistisch, systeembiologisch georiënteerde definitie van gezondheid en een meer holistische, systeembiologie georiënteerde methodologische benadering in de geneeskunde.

Hoofdstuk 4 beschrijft een nieuwe, op de systeembiologie georiënteerde, methodologische benadering bij de ontwikkeling van immunologische biomarkers voor het monitoren van effecten

van de behandeling van seizoensgebonden allergische rhinitis (SAR) (hooikoorts). Met behulp van gepermuteerde stapsgewijze regressie ('permuted stepwise regression') werden patroonvariabelen ontwikkeld en getest die de activiteit van het immuunsysteem op systeemniveau monitoren. Bij de ontwikkeling van de patroonvariabelen is uitgegaan van een dataset uit een gerandomiseerde, gecontroleerde studie waarin twee toedieningswijzen (neusspray en subcutane injecties) van Citrus/ Cydonia comp. werden vergeleken. De SAR gerelateerde cytokineproductie scores (IL-1β, IL-5, IL-10, IL-12, IL-13, IL-17, IFN-γ en TNF-α) en de ernst van de symptoomscores van 22 SAR patiënten werden vóór en na zes weken behandeling vergeleken. Op basis van deze resultaten wordt geconcludeerd dat het geïndiceerd is om patroonvariabelen-biomarkers met behulp van grotere datasets verder te ontwikkelen en te valideren, waarbij de gegevens van SAR patiënten tevens vergeleken moeten worden met de gegevens van gezonde personen.

Hoofdstuk 5 vat de ervaringen van 39 Nederlandse huisartsen samen die een antroposofische SAR-behandeling hebben voorgeschreven. De resultaten van dit onderzoek vormen de eerste practice-based evidence van de positieve resultaten van de behandeling Citrus/Cydonia comp. voor SAR.

In hoofdstuk 6 worden twee *in-vitro* onderzoeken beschreven waarbij de immunologische werkingsmechanismen van de effecten van Citrus/Cydonia comp. op voor SAR relevante cytokineproductie scores in het bloed van een gezonde en een allergische donor zijn geanalyseerd. Mononucleaire cellen werden geïsoleerd uit perifeer bloed (PBMCs) en *in-vitro* geanalyseerd na polyklonale stimulatie van T-cellen. De veiligheid en de effecten op Th1 (IFN-γ) en Th2 (IL-5) cellen werden onderzocht. Citrus/Cydonia comp. heeft een selectief effect op de differentiatie van Th-cellen wat zichtbaar wordt in het produceren van relatief meer IL-10 dan IL-12. Het lijkt ook een effect te hebben op de inductie van regulatoire (IL-10 producerende) T-cel subsets. Het geneesmiddel is in-vitro in staat om gedeeltelijk de veranderingen, kenmerkend voor SAR, ten aanzien van rijping, differentiatie en activiteit van het immuunsysteem, te neutraliseren. De conclusie van het onderzoek is dat Citrus/Cydonia comp. mogelijk de verstoorde toestand van het immuunsysteem van SAR-patiënten zodanig kan herstellen, dat de allergische symptomen permanent verdwijnen.

In hoofdstuk 7 worden de effecten onderzocht van het gecombineerde product, *Citrus e fructibus/ Cydonia e fructibus* (Citrus/Cydonia), en de aparte producten Citrus en Cydonia op de immunologische ´pathways´ van SAR. Perifere bloed mononucleaire cellen (PBMCs) van vijf gezonde en vijf graspollen-allergische donoren werden geïsoleerd en in-vitro geanalyseerd, na toediening van de extracten van de drie producten en aansluitend polyklonale en allergeen-specifieke stimulatie van T-cellen. De analyses toonden acceptabele overlevingscijfers van de immuuncellen, zonder tekenen van toxiciteit. Citrus had vooral een selectief effect op het verminderen van de allergische chronische ontsteking (TNF-α) en de Th2 (IL-5) activiteit. Zowel Cydonia als Citrus/Cydonia hadden vooral invloed op de inductie van de Th1 (IFN-γ) 'pathway'. Citrus en Cydonia blijken verschillende werkingsmechanismen bij de behandeling van SAR te hebben. Bij het combinatieproduct werden geen grotere effecten aangetoond dan bij de afzonderlijke preparaten. Geconcludeerd werd dat verder onderzoek naar effectiviteit en werkingsmechanismen van de afzonderlijke producten en het combinatieproduct bij SAR *in vivo* geïndiceerd is.

In hoofdstuk 8 wordt een groep van 13 patiënten onderzocht met een gemiddelde voorgeschiedenis van 9 jaar hooikoorts (graspollenallergie), die in voorgaande jaren tijdens het pollenseizoen conventionele hooikoortsmedicijnen gebruikten in verband met de ernst van hun symptomen. Gencydo (Citrus/Cydonia comp.) injecties werden toegediend aan 12 patiënten vóór aanvang en tijdens het graspollenseizoen, en bij één patiënt tijdens het graspollenseizoen alleen. De ernst van de nasale en niet-nasale hooikoortssymptomen, het gebruik van 'rescue' medicatie (antihistaminica of corticosteroïden), en de subjectieve ervaringen van patiënten werden gebruikt als uitkomstmaten. Er werd geconcludeerd dat er duidelijke aanwijzingen zijn dat de Gencydo behandeling effectief is in een grote subgroep van de onderzoekspopulatie.

Hoofdstuk 9 beschrijft een onderzoek naar de werkzaamheid en veiligheid van twee toedieningswijzen (subcutane injecties (SI) versus neusspray (NS)) van Citrus/Cydonia comp. in een nationale, gerandomiseerde, vergelijkende klinische trial met twee parallelle groepen. 23 patiënten voldeden aan de inclusie- en exclusiecriteria. Na een één- of tweeweekse washout periode waarin patiënten geen hooikoorts medicatie gebruikten, werden 23 patiënten gerandomiseerd naar een van de twee behandelgroepen voor een behandelperiode van zes weken. Effectmaten waren SAR-gerelateerde immunologische veranderingen, ernst van de SAR-symptomen en veiligheid. De immunologische analyses werden geblindeerd voor de groepstoewijzing uitgevoerd. Geconcludeerd werd dat beide toedieningswijzen veilig zijn en immunologische en klinische effecten geven. Wel zijn er grotere inflammatoire - en 'innate' immunologische effecten bij de NS-groep en grotere allergeen-specifieke en klinische effecten in de SI-groep.

Hoofdstuk 10 beschrijft een onderzoek dat gericht was op het beoordelen van de veiligheid van antroposofische en homeopathische injectievloeistoffen door middel van een systematische evaluatie van de gemelde bijwerkingen (Adverse Drug Reactions, ADR's). ADR´s werden geëxtraheerd uit de farmacovigilantie (geneesmiddelenbewaking) databases van acht Duitse antroposofische en homeopathische fabrikanten over de periode van 2000-2009. Deze acht fabrikanten vertegenwoordigen in totaal bijna 95% van de verkochte antroposofische en homeopathische injectievloeistoffen in Duitsland. De resultaten demonstreren een uitstekend veiligheidsprofiel van therapeutisch toegepaste injectievloeistoffen in de antroposofische geneeskunde en de homeopathie, waarbij de totale incidentie van bijwerkingen geclassificeerd kan worden als zeer zeldzaam.

Hoofdstuk 11 beschrijft de algemene discussie van dit proefschrift. Allereerst werden samenvattend de belangrijkste resultaten van dit proefschrift gepresenteerd: een gereviseerde WHO definitie van gezondheid; een methodologie om veranderingen in gezondheidtoestand te kunnen monitoren met zogenaamde patroonvariabelen op systeemniveau; bewijzen van veiligheid en immunologische en klinische effectiviteit van Citrus/Cydonia comp. op seizoensgebonden allergische rhinitis als een voorbeeld van curatieve gezondheidsbevordering; en een voorbeeld van kosten-effectiviteit van integratieve geneeskunde in Nederland. De theoretische en praktische implicaties van de resultaten van dit proefschrift werden besproken. Tevens werd, vanwege het feit dat Citrus/Cydonia comp. een antroposofisch geneesmiddel is, op het wetenschappelijke fundament van de antroposofische geneeskunde gereflecteerd. De analyse van de methodologische en epistemologische verschuivingen in de gezondheidszorg van een veelal reductionistische naar

een meer holistische benadering laat zien, dat de antroposofische gezondheidszorg steeds meer in overeenstemming komt met de hedendaagse wetenschappelijke methodologieën en concepten. Tot slot zijn scenario's beschreven voor de implementatie van nieuwe onderzoekslijnen, voor de verdere ontwikkeling van professionele preventieve en curatieve gezondheidsbevordering als een bijdrage aan de verdere innovatie van de geneeskunde en gezondheidszorg, en voor de investering in CAM/IM/AG (Complementary & Alternative Medicine, Integrative Medicine, Anthroposophic Medicine) onderzoek.

In de bijlage zijn de resultaten van een kosten-effectiviteitsonderzoek gepresenteerd waarin de mogelijke bijdrage van integratieve geneeskunde (Integrative Medicine) aan de kostenreductie van de gezondheidszorg werd onderzocht. In deze studie werden de praktijken vergeleken van huisartsen die, na het behalen van hun reguliere huisartsopleiding, een gecertificeerde aanvullende opleiding in CAM hadden afgerond, met die van huisartsen die geen aanvullende CAM-opleiding hadden gevolgd. Bij patiënten van wie de huisarts een extra CAM-opleiding heeft afgerond blijkt in de verschillende analyses van 0 tot 30 procent minder gezondheidszorgkosten gemaakt te worden. Bovendien zijn de sterftecijfers significant lager. De leeftijdsgroep en de aard van de CAM-behandeling waren bepalende factoren voor de uiteindelijke kostenbesparing. De lagere kosten zijn het gevolg van minder verblijf in het ziekenhuis en minder voorgeschreven geneesmiddelen. Deze verschillen blijken zeer waarschijnlijk onafhankelijk te zijn van sociaal-economische omstandigheden zoals opleiding en woonsituatie. Mogelijke verklaringen voor de lagere kosten en sterftecijfers zijn selectie (bijvoorbeeld mensen die zo min mogelijk medische interventies willen, kiezen wellicht eerder voor CAM) en kwalitatief beter werkende praktijken (bijvoorbeeld minder overbehandeling en/of meer aandacht voor preventieve en curatieve gezondheidsbevordering) door huisartsen met kennis van complementaire geneeskunde. Meer gecontroleerde studies (replicatie studies, onderzoek op basis van meer uitgebreide gegevens, kosten-effectiviteit studies naar CAM voor specifieke diagnostische categorieën) zijn geïndiceerd.

Met het voorbeeld van Citrus/Cydonia comp. voor de behandeling van hooikoorts laat dit proefschrift de veiligheid, effectiviteit en specifieke immunologische werkingsmechanismen van dit geneesmiddel zien. Aan het voorbeeld wordt tevens duidelijk dat CAM werkzaam *kan* zijn en dat het een belangrijk kostenbesparend potentieel heeft. Het proefschrift als geheel ondersteunt de geldigheid van het concept van preventieve en curatieve gezondheidbevordering.

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Curriculum vitae

Erik W. Baars was born in 1961 in Amsterdam, The Netherlands. For 16½ years he worked as a medical doctor at the Zeylmans van Emmichoven Clinic (Internal Medicine) and the Bernard Lievegoed Clinic (Psychiatry) in Bilthoven, The Netherlands. He has a Master of Science in epidemiology. He has been coordinator and researcher at the Cats-Polm Institute (a non-profit research institute in the filed of chronic traumatization) in Zeist, The Netherlands, and director and senior-researcher Healthcare of the Department of Healthcare & Nutrition of the Louis Bolk Institute, Driebergen, The Netherlands. Since 2007 he is also a part-time Professor (in Dutch: Lector) of Anthroposophic Healthcare at the University of Applied Sciences, Leiden, The Netherlands. His particular research interests include epidemiological and clinical studies, case-studies, health promotion, holism-reductionism, anthroposophic medicine, concept development and methodology development for research and clinical practice. Erik Baars has published ca 140 papers, articles, book chapters and monographs.

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