8. Conclusions and perspectives

Rheumatoid arthritis sub-types

The main aim of the research presented in this thesis was to find and characterize relevant sub-types of rheumatoid arthritis patients. Instead of an unsupervised approach for finding such sub-types knowledge from Chinese medicine was used to direct the research. In Chinese medicine diagnosis Cold and Heat are key concepts consisting of specific symptom patterns that are used to characterize patients. Consequently, the Cold and or Heat status of patients is very important for choosing the right therapy. In this thesis a systems biology approach was applied to characterize Cold and Heat types of rheumatoid arthritis patients. Multivariate statistical analysis was used to validate the classification of the two patient sub-types.

In Chapter 5 the first biochemical characterization of Cold and Heat RA patients has been presented. From the gene expression profiles measured in CD4+ T-cells, apoptosis turned out to be a key mechanism that is differently regulated in Cold and Heat RA patients. In Heat RA patients apoptosis was found to be stimulated compared to Cold RA patients, in which more apoptosis resistance genes were expressed in the particular subset of T-cells. In Chapter 7 a second study is presented in which Cold and Heat RA patients were characterized, this time with a more extensive LC-MS analysis of urine and plasma samples. This study reveals higher levels of 11 acylcarnitines in the urine of Heat RA patients compared to Cold RA patients. These findings suggest that Cold RA patients have less muscle mass and/or a more pronounced muscle breakdown than RA Heat patients. Carnitine homeostasis has been related to fatigue in chronic fatigue patients and might also be related to fatigue experience in RA, which is a major issue for RA patients. It would be interesting to study whether fatigue is particularly related to one of the Cold or Heat RA sub-types.

Additionally, it was found that levels of DHEAs in Heat RA patients were higher than in Cold RA patients suggesting that the Cold RA group has a more suppressed HPA-axis function. Decreased HPA-axis function is associated with an inadequate response to stress factors. It is hypothesized (Cutolo 2000) that this inadequate response can lead to autoimmune and inflammatory disorders.

In Chinese medicine Cold and Heat RA patients are treated very differently. The results described in Chapter 5 and 7 indicate substantial biochemical differences between the two

types of patients. A text mining study (Li 2007) shows that diseases related to Cold according to Chinese medicine theory are more related to hormone function disturbances and Heat related diseases to immune function disturbances, which is in agreement with our findings. It is hypothesized that Cold RA patients might benefit more from hormone based therapies (such as prednisone) and Heat RA patients from immune based therapies (such as anti-TNF agents).

Recently a new study was started but not yet completed to study the response of Cold and Heat RA patients to anti-TNF treatment. Currently 40-50% of the RA patients fail to respond adequately to these drugs, resulting in side effects but no beneficial effects for the patients. The RA patients have been classified in Cold and Heat based on a questionnaire and response is measured at several time points. The ability to predict these differences in response to treatment with one of the most expensive drugs on the market today would have an enormous impact on health care costs.

This unique approach of targeting therapy to sub-groups of patients based on Chinese diagnostic information could be extended in future studies. In the presented work only two general sub-types of RA are explored, which could be expanded with more sub-types. The study presented in Chapter 6 suggests that the key Chinese medicine concepts Internal and External constitute another important source of variation between RA patients. In addition, the more chronic stages of RA could be studied by exploring disturbances on the level of the Chinese concepts for Organ functions. Such studies might reveal certain clearly characterized sub-types of RA patients that can be treated differently.

Systems biology as a bridge between Western and Chinese medicine

The gene expression and metabolomics profiles of rheumatoid arthritis sub-types provide a biochemical view of some Chinese diagnostic principles. This biochemical view firmly grounds Chinese diagnosis in biology and makes acceptance easier. Recently this approach was discussed in a Nature publication (van der Greef 2011). The scientific community is clearly opening up for the integration of different views on health and science. The foundation of this integration is systems thinking and systems biology. Systems thinking is typical of Chinese medicine in which diagnosis and treatment is based on changing patterns of symptoms. Western science is recently rediscovering the principles and usefulness of systems thinking by developing comprehensive measurement platforms, and data analysis

approaches for studying relationships between measurements.

Besides an open mind, analytical platforms are needed that are able to capture a wide variety of types of information of the system. In this thesis, a gene expression study is described in which the expression of all the genes of CD4+ T-cell were measured. Additionally, several untargeted liquid chromatography and gas chromatography mass spectrometry methods were used to measure metabolite levels in urine and plasma. Although the results are promising, the options for measuring the system could be greatly improved. For instance, the interactions between 'compartments' such as the liver, urine, blood, muscle, brain, could reveal compensation mechanisms. Additionally, analytical platforms could be developed to measure less abundant metabolites. Especially the identification of unknown metabolites proves to be a challenge (Kind 2010).

Systems biology science will reveal much more information about a system when measurements in time become a more viable option. Rheumatoid arthritis as an example of a chronic disease, daily rhythms in the symptoms (Cutolo 2005) are well known, and relationships with disturbances of hormonal rhythms are extensively studied (Cutolo 2008). This has for instance resulted in glucocorticoid chronotherapy with optimal medication at 3 am (Cutolo 2012). More understanding of the rhythmicity of biological systems and disturbances of the system will result in new options for optimizing treatment. Several studies in this area of chronobiology are showing the dynamics in metabolite levels during the day (Eckel-Mahan 2012, Bass 2010, Froy 2010). However, it is a challenge to develop stable analytical platforms to measure large series of samples and analyze the huge amount of data resulting from such measurements.

The systems biology bridge can be strengthened by including the rich Chinese theoretical development and experience with symptom patterns. Symptom patterns seem to behave like attractors with stable states that can change to other states when certain bridge symptoms express themselves. It would be interesting to study changes in symptom patterns and changes in metabolomics and other -omics patterns simultaneously. A challenge here is to design an expert system that is able to easily capture relevant symptom information to prevent the use of extensive questionnaires.

Another approach to strengthen the bridge lies in an integration of nonlinear mathematics and complexity theory with biology to improve the analysis of patterns of relationships. Living systems are inherently nonlinear, where the output is usually not proportional to input, and which cannot be described by linear differential equations. Such systems consist of a large

and variable numbers of components, showing a large degree of connectivity (Higgins 2002). Living systems are also energetically open and organizationally closed, but in a state far from equilibrium. There is a constant uptake and incorporation of substances in the body while other substances are excreted. Systems biology needs to move away from only studying linear relationships and should include nonlinear possibilities.

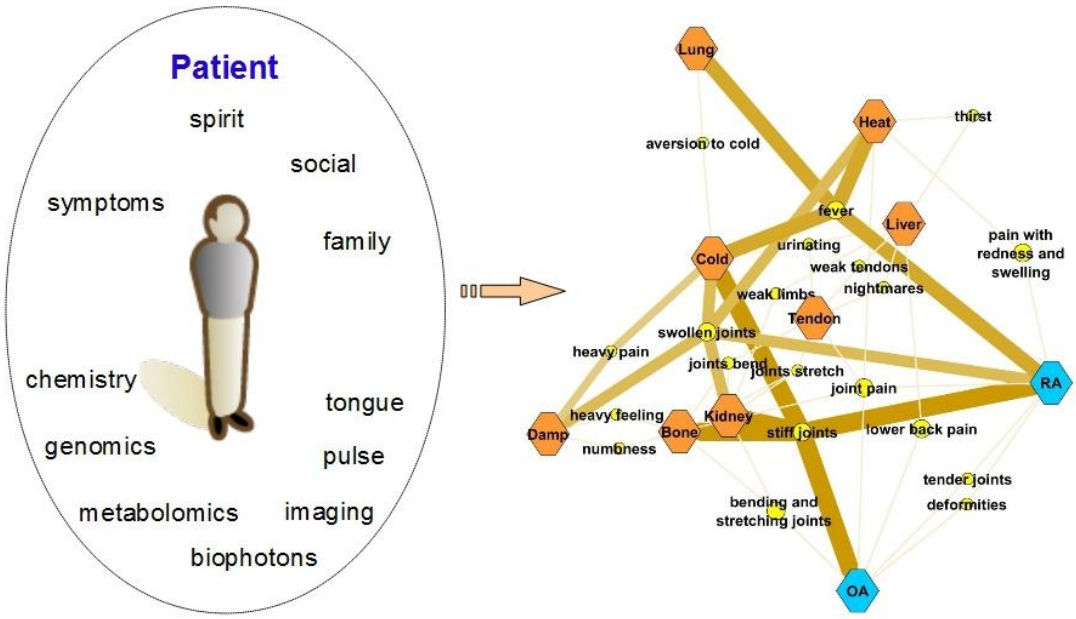

Figure 1. Integration of Chinese and Western concepts of arthritis. Patient information is collected on several levels (left panel). Symptoms which relate Chinese concepts (orange hexagons) with Western concepts (blue hexagons) are visualized in a network (right panel).

Finally, systems biology thrives by good visualization methods that can convey clear messages extracted from the usually huge amount of data. In several studies presented in this thesis, concepts from network theory were applied. In Chapter 2 the relationships between Western and Chinese concepts of arthritis were visually explored (Figure 1). Chapter 5 contains a network view of the genes expressed differently between the Cold RA and Heat RA patients. The relationships between the symptoms used in the questionnaire described in Chapter 6 were also visualized in a network view. Several tools for static network visualization such as Cytoscape (www.cytoscape.org), Pajek (pajek.imfm.si/doku.php?id=pajek) and Grephi (grephi.org) are developing rapidly, however dynamic visualization is much more challenging. New options for visualizing dynamic data were explored in the artist project Aqua Vita (www.theaquavitaproject.com), which used a combination of metabolomics and Chinese diagnosis data. This effort has resulted in an

appealing interactive visualization, novel ideas, and an exhibition with a large amount of public outreach. Bringing disciplines together enhances the creative process and can result in a better embedding of research in the society.

Personalized medicine and health promotion

The introduction of this thesis started out with describing some of the challenges the health care system is facing today. Slowly, scientists and clinicians start to realize that every patient is different and that therefore medicine needs to be developed that is personalized. In this thesis a step was taken in the direction of personalized medicine by characterizing two groups of rheumatoid arthritis patients (Figure 2). Instead of treating all patients in the same manner, a distinction can now be made between the Cold and Heat RA patients. Similarly, two pre-diabetes sub-types based on Chinese diagnosis have been characterized by metabolomics measurements (Wei 2012). A next step might be to find even more subtle sub-types of patients for which treatment can be optimized.

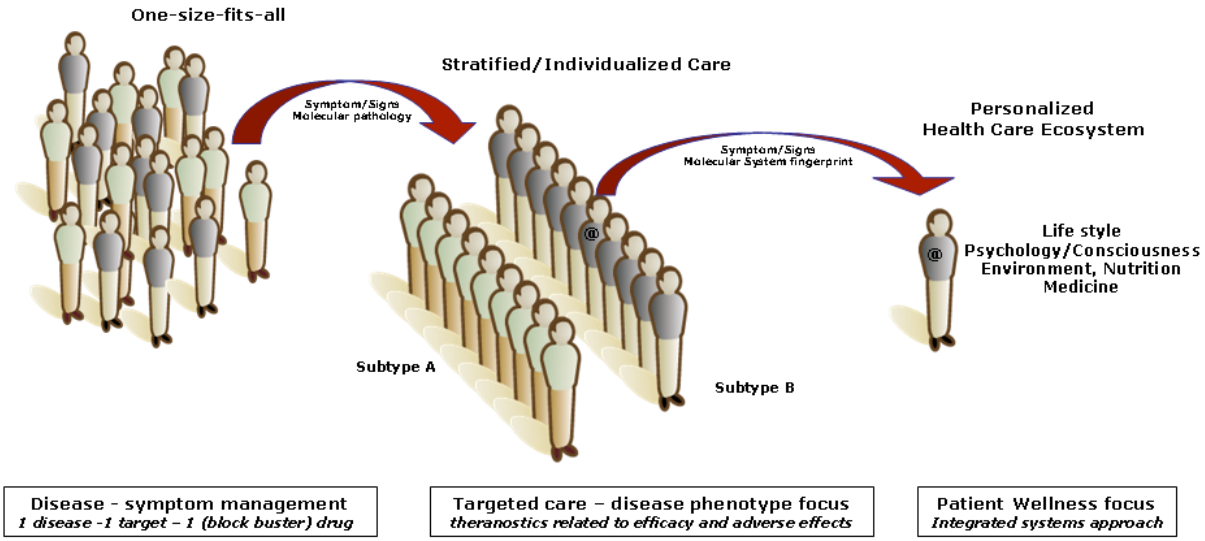

Figure 2. Steps from the current Western 'one-size-fits-all' concept to targeted care and eventually to a personalized health care system.

The development of health promotion strategies will have a major impact on the structure of the health care system and the well-being of people. Chinese medicine is especially developed as a health promotion strategy and contains the knowledge to monitor changes in the healthy state of the body. This knowledge of diagnosis, which includes the development of symptom patterns, is essentially lacking in Western medicine. In this thesis, first steps are

taken to integrate this knowledge with Western systems biology information. Symptom patterns can further direct biomarker research in the area of disease prevention and health promotion. In addition, Chinese medicine includes life-style advice and herbal medicine that are aiming for health promotion and can be used in very early stages of shifting towards an unhealthy state.

A movement towards health promotion invites the question of who is responsible for which part of the health care options. A shift towards a more equal patient-practitioner relationship is already happening and will place the doctor more in the role of a health coach. Health promotion strategies will be much more integrated in daily life by, for example, health apps on mobile phones, courses in stress reduction techniques that people can take, personalized nutrition advice, etc.

As health care will become patient-centered, integrated, preventive and personalized, the role of scientists also needs to change. Science conducted in multidisciplinary teams, including patients, consumers and health care organizations, allows the development of health care products that are actually desired by patients and consumers. The scientist will act as a knowledge organizer and integrator. Discoveries will more easily lead to strategies that can enter clinical practice and reach the consumer. The future health of the society and of the participants therein will emerge from the many interconnected relationships between various actors and the inspiration these relationships will bring about.

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