Coll. Antropol. **34** (2010) 2: 437–445
Original scientific paper

A View at the Future – A Dynamical, Protocol-based and Computationally Intensive Approach in Cardiovascular Risk Assessment

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A B S T R A C T

Knowledge of cardiovascular risk factors is increasing. At the same time, risk estimation becomes more and more difficult. The need for a more comprehensive, but more individually based approach is evident. To achieve this aim, we propose a systems biology approach in cardiovascular risk assessment. This means that a large amount of health data, describing many aspects of the health-status of patients, is collected and computed and the results are compared with existing knowledge. Finally, a clinical model is created, which can be the first step in ongoing research protocol, aimed at assessing cardiovascular risk. By using this approach, all potentially relevant risk factors can be identified on a small sample. Moreover, risk groups can be more specifically defined, based on the »natural« clustering of data, according to their predictive load. We tested this possibility on an example of hyperhomocysteinemia which is a well-known complex cardiovascular risk factor.

Key words: *cardiovascular risk assessment, new approach, systems biology, systematic health data record, computation, protocol-based prediction*

Introduction

Prediction of cardiovascular diseases (CVD) is based on the risk factor paradigm. Classical risk factors, including: hypertension, cigarette smoking, dyslipidemia, obesity and diabetes, have long been established[1,2]. In addition to these classical risk factors, evidence suggests the existence of new factors, including: low-grade inflammation, chronic latent infections, e.g. with *Cytomegalovirus* or *Helicobacter pylori*, hypercoagulability, hyperhomocysteinemia (elevated serum concentrations of amino acid homocysteine), or increased oxidative stress[3,4]. It has also been established that complex psychological and socioeconomic factors strongly influence risk and prognosis in CVD[5,6].

The addition of these »new« factors can be used for stratifying the risk of CVD more accurately. In this regard, there are indications that most coronary events occur in persons with risk factor values in the middle of the distribution, rather than at extremes[7–9]. This may be a consequence of the fact that risk factors are heterogeneously distributed in the population. Hence, the same risk factors do not show the same effect in different population groups[3,9]. The problem is, however, that large, multi-centric and long-term studies are needed to prove the validity of these »new« factors in cardiovascular risk assessment.

As knowledge of the risk factors and mechanisms involved in the development of CVD increases, the preparation of risk estimation tables and scores becomes more and more difficult[10]. Two contradictory, but also complementary, demands are evident: the need for a more comprehensive and at the same time a more individually based approach[11]. Thus, for example, early strategies used single risk factors to assess the relative risk of severe events: myocardial infarction, sudden cardiac death and stroke. On the contrary, the currently recommended method is a multiple point score system based on the use of several major risk factors for determination of a 10-year absolute risk of severe events[1,10–12]. However, even

Received for publication January 10, 2009

this new system is deficient in classifying complex clinical conditions, such as diabetes mellitus, or the metabolic syndrome, or in verifying the subclinical form of atherosclerotic disease, or positive family history for CVD[11–13].

In general, a more definite answer is needed on the question of how many parameters (and which ones) should be measured to adequately cover subjects at increased risk for severe cardiovascular events. The challenge will also be to determine if more than one specific subgroup of patients at increased risk for CVD can be identified in the population.

The aim of this study is to show that collecting health data systematically, describing many aspects of the health-status of patients, and use of computer-based techniques for data analysis could enable identification of all potentially relevant risk factors for CVD even on a small sample, avoiding the need for multiple, large-scale studies. We tested this possibility on a model of hyperhomocysteinemia.

Hyperhomocysteinemia as a cardiovascular and common ageing disease risk factor

Hyperhomocysteinemia has recently been recognised as an important cardiovascular risk factor[14]. Moreover, based on intensive basic and clinical research, mild hyperhomocysteinemia (>12 mM) has been accepted as a common risk factor for the development of many age-related phenotypes, including: atherosclerosis, thrombosis, depression, neurodegenerative diseases and cancer[15]. In addition, it is assumed to be due to many underlying causes, all connected with increasing age, such as: B-vitamin deficiency, chronic gastritis, chronic renal impairment, thyroid gland dysfunction and other chronic conditions, as well as medical treatments[16,17].

Thus, hyperhomocysteinemia, alone, represents a complex trait[18].

Materials and Methods

Study population

The study was conducted in a general practice, situated in an urban area (the city of Osijek, approximately 90 000 inhabitants), in the eastern part of Croatia, characterised by a high prevalence of cardiovascular and other chronic disorders, higher than the average for Croatia[6,19]. Approximately 1600 patients, registered on the list of one family doctor, were used as the population sample. Two thirds of the patients were aged 50 years or more.

A total number of 93 patients, 35 male and 58 female, 50–89 years old (median 69), out of 150 individuals requiring the influenza vaccine in the season 2003/2004, gave their consent and were enrolled in the study. They were all patients having chronic medical conditions. The study protocol was approved by the local ethics committee.

Systems biology approach

We used a systems biology approach[21]. In contrast to the classical, reductionist methodology approach, this approach is not strongly hypothesis-driven, but is based on the use of research protocol. This means that the basic information is gathered from publications. The model is created on the basis of these pieces of information and tested by experiment, or computer-based simulation (a model-building approach). The results are then used to make corrections to the model[22,23].

Contrary to the reductionist methodology approach, where only a few recognisable variables can be evaluated – a systems biology approach is an analysis of how all components in a biological system interact to determine a particular phenotype. This requires integration of a large amount of heterogenous data. In this way, much hidden information can be revealed[22,23].

Dataset

Based on a systems methodology approach, we determined the health status of the subjects according to several aspects, systematically. For this purpose, a total number of 52 parameters were collected, including: age and sex, frequently used drugs, diagnoses of the main groups of chronic diseases, anthropometric measurements, cognitive impairment test and a set of haematological and biochemical tests[24].

Blood tests were chosen on the basis of two criteria: 1) to determine the main age-related pathogenetic changes and 2) to be available in a real health care system setting. Based on these criteria, we performed blood tests to determine: inflammation, nutritional status, metabolic status, latent infections (*Helicobacter pylori* and *Cytomegalovirus*), chronic renal impairment and neuroendocrine status.

Our intention was not to use all possible factors connected with the ageing process, but to assess the principles and possibilities of a systems biology approach in solving different medical problems.

Testing the method on the model of hyperhomocysteinemia

Since many factors in our dataset had previously been identified in published papers as being associated with hyperhomocysteinemia, we used a model of hyperhomocysteinemia to check the accuracy of the applied method to extract relevant information from the data.

For that purpose, we made minor corrections to the primarily performed dataset, by changing some of the data. The final number of the data was 43 (Table 1 and 2).

We then defined the aims of the study.

– Since hyperhomocysteinemia has been identified as a cardiovascular risk factor having multiple connections with a wide range of age-related disorders, the role of this model was to indicate that a wider background of common ageing disorders should be considered when assessing the total cardiovascular risk.

TABLE 1
QUALITATIVE DATA USED TO DESCRIBE THE HEALTH STATUS OF SUBJECTS

Parameter	Positive cases N (%)	Diagnosis making criteria
Hypertension	77 (82.80)	Medical records Blood pressure checkup
Diabetes mellitus	23 (24.73)	Medical records According to OGTT @ in cases with borderline fasting glucose values
Drugs/statins	18 (19.35)	Medical records
Drugs/nitrous oxide	12 (12.90)	Medical records
Drugs/oral antidiabetic drugs	10 (10.80)	Medical records
Cardiovascular diseases (myocardial infarction, angina pectoris, history of revascularization, stroke, transitory ischemic cerebral event, peripheral vascular disease)	24 (25.81)	Medical records followed by clinical examinations and according to cardiologist and vascular surgeon report
Gastroduodenal disorders (gastritis, ulcer)	32 (34.41)	Medical records, cases proved by gastro-duodenoscopy
Chronic urinary tract disorders (chronic cystitis in women, symptoms of prostatism in men)	51 (55.43)	Medical records, clinical criteria, prostat hypertrophy confirmed by ultrasound
Chronic obstructive pulmonary disease	13 (13.98)	Positive spirometry
Allergy (rhinitis, asthma)	9 (9.68)	Symptoms, treatment with antiinflammatory drugs, positive spirometry
Severe osteoarthritis	23 (25.00)	Severe symptoms or frequent use of nonsteroid antiinflammatory drugs
Malignancy	14 (15.05)	Currently not under active treatment
Osteoporosis	30 (40.00)	Medical records, DEXA×
Neuropsychiatric disorders (anxiety/depression, Parkinson's disease, cognitive impairments)	53 (57.00)	Medical records, use of drugs with psychotropic action, MMSE*

° OGTT (Oral Glucose Tolerance test)
× DEXA (Dual-Energy-X-ray-Absorptiometry), the golden standard to diagnose osteoporosis
In 10 cases the data were missing
*Mini Mental State Examination Score (standard screening test on cognitive impairment)

- Another aim was to show that the same dataset, with only minimal corrections, can be used for solving several research tasks. That assumption was based on the observation that common ageing diseases result from complex interactions between multiple genetic variants and environmental factors and that these factors are shared between related disorders[25,26].
- The main aim of this study was to create a clinical model which represents all elements of the networked reaction producing hyperhomocysteinemia.
- Association studies linking hyperhomocysteinemia and diabetes mellitus have achieved conflicting results.This question is expected to be solved by the creation of a clinical model of hyperhomocysteinemia[27].

Blood sampling

Blood samples for haematological and biochemical analyses were collected from each subject. Haematological analyses were carried out on fresh blood samples, while sera for biochemical analyses were separated by centrifugation and stored at –40°C until assayed. All blood tests were performed in the Central Biochemical Laboratory of Osijek University Hospital Center, using standard techniques (Table 2).

For the main purpose of this study, homocysteine concentrations were determined. Its median estimated value was 11.8 mol/L. This was very close to the cut-off value of 12 mol/L, found to be associated with increased risk for cardiovascular disease and all-cause mortality[14].

Data Mining methodology

Data Mining is an advanced computer-based technique, operating on the principles of searching for recognisable patterns and structures in the data[28,29].

In this study, algorithms of the ILLM (Inductive Learning by Logic Minimization) system, developed in the Laboratory for information Systems, Department of Electronics, »Rudjer Bošković« Institute, Zagreb, were applied[30].

The main characteristics of Inductive Learning methods are that parameters describing patients (called attributes) can acquire any of three types of values, includ-

TABLE 2
NUMERICAL DATA USED TO DESCRIBE THE HEALTH STATUS OF SUBJECTS

Parameter	Min value measured in the sample	Max value measured in the sample	Reference range	Laboratory test used
Fasting glucose	4.6	13.9	4.4–6.4 mmol/L	UV Photometric test with hexokinase
HBA_{1C}	2.89	10.06	4.0–6.4%	Immuno – Turbidimetric test
Total cholesterol	3	8.9	<5.0 mmol/L	Photometric test with cholesterol-oxidase (CHOD-PAP method)
Triglycerides	0.5	9.3	<1.7 mmol/L	Photometric test with glycerophosphate-oxidase (GPO-PAP method)
HDL-cholesterol	0.87	2.53	<1.0 mmol/L for M <1.2 mmol/L for F	Direct method with modified polyethylenglycol and α-cyclodextrane-sulphate
Body mass index	20.24	43.1	<20 underweight 20–25 normal weight 26–30 overweight 31–40 obesity >40 severe obesity	kg/m^2
Waist/hip ratio	0.76	1.11	M to 1.0 F to 0.8	with centimetre
Triceps skinfold thickness	20.24	50.0	(50–65 years) M 8–23 mm F 10–35 mm	with caliper
MMSE	14	30	Max »Score« 30 < 24 positive on dementia	
WBC count	4.06	9.93	$3.4–10.0\times10^9$/L	Electronic blood cell counter
Neutrophils %	28	73.3	44.0–72.0%	Light microscopy, Pappenheimer staining
Eosinophils %	0.3	14.3	1.0–7.0%	Light microscopy, Pappenheimer staining
Monocytes %	3.6	15.7	2–12%	Light microscopy, Pappenheimer staining
Lymphocytes %	18.4	57.7	20–46%	Light microscopy, Pappenheimer staining
CRP	0.8	24.5	to 5.0 mg/L	Immuno – Turbidimetric test
RBC count	2.63	5.37	$4.34–5.72\times10^{12}$/L	Electronic blood cell counter
Hemoglobin	91	167	138–175 g/L	Electronic blood cell counter
Serum albumin	33.1	53	35–52 g/L	Photometric test with bromocresol-green
Creatinine clearance	0.72	3.21	1.6–2.94 mL/s/1.73m^2	Estimated from serum creatinine and 24^h urine creatinine Enzymatic PAP method
Homocysteine	5	25.9	5.0–15.0 μmol/L	Radioimmunoassay
Vitamin B_{12}	97.8	885.6	128–648 pmol/L	Radioimmunoassay
Folic acid	6.5	43.9	6–39 mmol/L	Radioimmunoassay
Cortisol in the morning	180.3	812.1	154–638 nmol/L	Radioimmunoassay
Prolactin in the morning	14.57	838.18	M 65.7 – 439.8 F 76.3 – 400.7 mIU/L	Radioimmunoassay
TSH	0.024	22.7	0.46–4.68 Ul/mL	Radioimmunoassay
FT_3	4.35	7.96	4.26–8.10 pmol/L	Radioimmunoassay
FT_4	8.92	18.9	10–28.2 pmol/L	Radioimmunoassay

M = males, F = females

ing nominal, numerical and categorical. One attribute is chosen as the object of the analysis (target attribute) and its value is arbitrarily defined, using specific criteria. All other attributes are used as the input. Patients are randomly grouped, depending on whether they fulfill the criteria of the target attribute value or not[28,30].

Data Mining modelling

All of 93 patients, participating in the study, were divided into two subgroups, depending on whether their homocysteine levels were above (46 patients) or below (47 patients) the median value. Using Data Mining methodology for comparing the data, the cluster of parameters, most appropriately associated with hyperhomocysteinemia, was extracted from the dataset.

For selected parameters, we repeated the algorithm application procedure on the dataset (the second-step selection procedure). In this way, we gained better insight into the clinical context for a particular parameter and came into how selected parameters are interconnected.

By searching through the databases, interactions between extracted parameters were meaningfully explained. Finally, the networked reaction involving hyperhomocysteinemia (the clinical model) was created.

Results

The result of the ILLM algorithm application on the dataset is represented in the form of a pattern consisting of 6 parameters, expressed with their cut-off values (Basic model, Table 3). These are parameters indicating: folic acid and vitamin B_{12} deficiency (parameters 1 and 2), chronic renal impairment (parameter 3), normal (not elevated) fasting glucose and HbA_{1c} (parameters 4 and 5) and hyperprolactinemia (parameter 6).

TABLE 3
THE RESULTS OF DATA MINING ANALYSIS

Basic model
»Descriptors of elevated homocysteine levels« (>11.8 mmol/L)
Folic acid ≤ 17.75 nmol/L
Vitamin B_{12}≤ 225.50 pmol/L
Creatinine clearance≤ 1.69 mL/s/1.73m^2
Fasting glucose≤ 6.25 mmol/L
HbA_{1c}≤ 4.11%
Prolactin >90.24 mIU/L
Additional model 1
»Descriptors of decreased vitamin B_{12} levels« (≤ 225.50 pmol/L)
Homocysteine >12.05 mmol/L
Age> 67.5 years
Monocyte % >8.35
Triceps skinfold thickness ≤ 31.50 mm
Gastroduodenal disorders = yes
Folic acid ≤ 17.00 nmol/L
Additional model 2
»Descriptors of low creatinine clearance« (<1.69 mL/s/1.73m^2)
Serum albumin≤ 47.15 g/L
Fasting glucose≤ 5.65 mmol/L
Homocysteine >12.50 mmol/L
Age> 67.5 years
Neuropsychiatric disorders = yes
f T_3≤ 5.36 pmol/L

When we analysed the results of the second-step selection procedures, performed separately for each of the selected parameters, we realised that the results of the two of these procedures can add value to the information already obtained by the first-step selection procedure. These are the results of the ILLM algorithm application on the parameters indicating vitamin B_{12} deficiency and chronic renal impairment (Additional models 1 and 2, Table 3).

The results of Additional model 1 provide closer insight of the pathogenetic background associated with vitamin B_{12} deficiency, including also its subclinical form (starting at the value of 225.50 pmol/L).

Two parameters, extracted in this model, homocysteine (>12.05 mol/L) and folic acid (≤17.00 nmol/L), overlapping with the data in Basic model, confirm that correlation does exist between hyperhomocysteinemia and folic acid and vitamin B_{12} deficiency.

Other parameters selected in this model provide information about the clinical conditions associated with B-vitamin deficiency and hyperhomocysteinemia. These conditions include: advanced age (>67.5), malnutrition (indicated by decreased triceps skinfold thickness), chronic gastroduodenal disorders, as well as increased mononuclear leukocyte activity, indicating immune system dysfunction (parameter »monocyte %>8.35«).

The results of Additional model 2 provide information about disorders associated with chronic renal impairment – the main clinical condition causing hyperhomocysteinemia. These disorders include: older age (>67.5 years), neuropsychiatric disorders, glucose metabolism impairment (indicated by fasting glucose values ≤5.65 mmol/L), inflammation/malnutrition (indicated by serum albumin concentrations ≤47.15 g/L) and thyroid gland hormone deficiency (indicated by fT_3 concentrations ≤ 5.36 pmol/L).

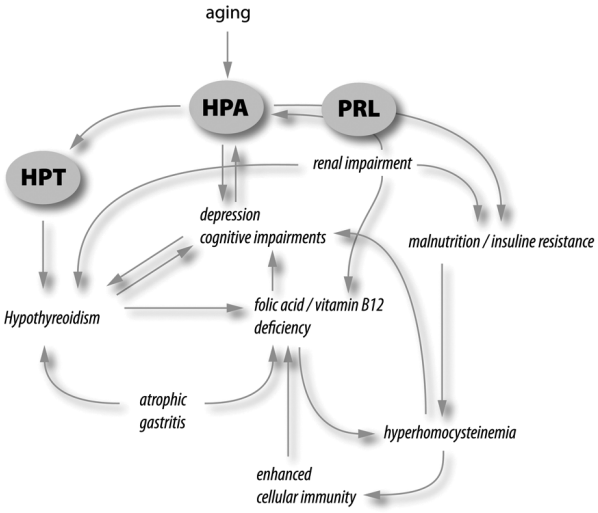

Fig. 1. »The pattern of disorders« involving hyperhomocysteinemia; HPT – the hypothalamus-pituitary-thyroid axis, HPA – the hypothalamus-pituitary-adrenal axis, PRL – prolactin.

Discussion

Based on a clinical model of hyperhomocysteinemia (Figure 1), we observed that a large degree of overlapping does exist between the results obtained in this study and previously existing knowledge. Consequently, the accuracy of the method applied in the study to identify all relevant factors in a large set of data was confirmed.

In general, the construction of a clinical model may be important when deciding which parameters should be collected to investigate a particular clinical feature.

A description of the clinical model – disorders associated with hyperhomocysteinemia

1. B-vitamin deficiency

Parameters selected in Basic model and Additional model 1 (Table 3) indicate that correlation does exist between hyperhomocysteinemia and vitamin B_{12} and folic acid deficiency. These results are in accordance with evidence suggesting that B-vitamin deficiency is the main cause of mild hyperhomocysteinemia[13,31].

The exact mechanism, linking these disorders within the same background, is the metabolic cycle of the amino acid methionine. This is an essential biochemical reaction, a donor of methyl-groups, necessary for maintaining certain vital biological processes[32,33]. Folic acid and vitamin B_{12} are regulators, while the amino acid homocysteine is a by-product in this cycle. Hence, B-vitamin deficiency, even in its subclinical form, can lead to impairment of this cycle and, subsequently, to hyperhomocysteinemia[32,33] (Figure 1).

This is connected with the development of disorders arising from impaired methylation reactions, such as: DNA damage, genome instability, impaired cell proliferation and insufficient neurotransmitter synthesis[32,33]. These are all intermediate mechanisms during the course of the development of diseases, such as atherosclerosis, neurodegenerative diseases and cancer[14,15,33–35]. In addition, hyperhomocysteinemia may independently contribute to the pathogenesis of these diseases, through mechanisms such as: increased oxydative stress, impaired lipid metabolism and activation of inflammatory and apoptotic cell death pathways[36,37].

2. Clinical conditions

Based on the results of Additional models, information on clinical conditions, causes of B-vitamin deficiency and hyperhomocysteinemia, are provided. These conditions are: chronic gastroduodenal disorders (implicated by the results in Additional model 1, Table 3) and chronic renal impairment (elaborated in Additional model 2, Table 3).

2.1. Gastroduodenal disorders

The parameters »gastroduodenal disorders« and »age >67.5 years« (Additional model 1, Table 3), are likely to implicate the nutritional reasons for B-vitamin deficiency in elderly subjects (Figure 1). Decreased absorption of these vitamins, due to chronic gastritis, has long been accepted as an explanation for the frequent occurrence of B-vitamin deficiency in older people[16,38].

Protein malnutrition, indicated by the parameters »triceps skinfold thickness ≤31.5« (Additional model 1, Table 3) and »serum albumin ≤47.15« (Additional model 2, Table 3) may be the common mechanism, shared between chronic gastroduodenal disorders and chronic renal impairment, associated with B-vitamin deficiency and hyperhomocysteinemia.

2.2. Chronic renal impairment

In this study, chronic renal impairment, implicated by the parameter »creatinine clearance ≤1.69« (Basic model, Table 3), was recognised as the main clinical condition leading to hyperhomocysteinemia[39–42]. Proposed mechanisms include: decreased homocysteine clearance, B-vitamin deficiency, increased oxydative stress and protein malnutrition[39–44]. Recently, the unique »malnutrition-inflammation-insulin resistance« syndrome has been identified in a large number of people with chronic renal impairment[45,46].

Our results are likely to implicate the elements of this syndrome. Thus, the parameter »serum albumin ≤47.15« (Additional model 2, Table 3) may be a correlate for malnutrition and inflammation. Parameters »fasting glucose ≤6.25« and HbA1c ≤4.11%« (Basic model, Table 3) and the parameter »fasting glucose ≤5.65« (Additional model 2, Table 3) may indicate insulin resistance, typically in patients with chronic renal impairment characterised by normal (not elevated) fasting blood glucose and glycosylated hemoglobin[45,46] (Figure 1).

In addition, this may explain the fact, assumed as controversial in published papers, that homocysteine serum concentrations do not usually increase in diabetes mellitus – a condition characterised by hyperglycemia[27].

2.3. Neuroendocrine and neuropsychiatric disorders

Based on our results, it can be concluded that neuroendocrine and neuropsychiatric disorders are also associated with hyperhomocysteinemia (parameter »prolactin >90.24«, Basic model, Table 3) and that these disorders are frequently found in patients with impaired renal function (parameters »neuropsychiatric disorders=yes« and »fT3≤5.36«, Additional model 2, Table 3).

Indeed, there is a large body of evidence supporting these results. For example, multiple endocrine disorders, including alterations in the hypothalamus-pituitary-adrenal axis, the hypothalamus-pituitary-thyroid axis, as well as in prolactin and thyroid gland hormone metabolism, can be found in persons with chronic renal impairment[47,48] (Figure 1).

Thyroid gland hormone hypofunction and hyperhomocysteinemia, both disorders frequently occurring in patients with chronic renal impairment, can be associated, each of them separately, with mental and cognitive impairments[34,35,49] (Figure 1).

Hyperprolactinemia may be a result of decreased clearance of the hormone prolactin, due to chronic renal impairment. Alternatively, it may reflect age-related brain degenerative processes, especially those connected with changes in the hypothalamus and the pituitary gland – the central point of the neuroendocrine control[50,51]. It has been found that these patients, compared to other older people, more frequently suffer from anxiety and mood disorders[52]. This may, in our results, be indicated by the parameter »neuropsychiatric disorders« (Additional model 2, Table 3).

Furthermore, evidence suggests that hyperprolactinemia may contribute to the development of insulin resistance and protein malnutrition[53].

Our results could indicate complex relationships existing between all of these disorders. This is based on the following parameters: »prolactin«, »low creatinine clearance«, »neuropsychiatric disorders«, »fasting glucose« and »serum albumin« (Basic model and Additional model 2, Table 3) (Figure 1).

2.4. Chronic autoimmune thyroiditis

There could be another neuroendocrine disorder, »hidden« in our results. This is hypothyroidism, based on chronic autoimmune thyroiditis. In this regard, evidence suggests that this disorder usually appears in co-morbidity with chronic gastritis, both disorders contributing to hyperhomocysteinemia by different mechanisms[17,49,54,55] (Figure 1).

2.5. The immune system impairment

Evidence strongly suggests that chronic activation and proliferation of immunocomponent cells, during the course of chronic ageing diseases, such as chronic gastritis and neurodegenerative and vascular diseases, mentioned here, may result in increased consumption of B-vitamins, leading to their deficiency and, subsequently, to hyperhomocysteinemia[56–60].

Our results are likely to support this proposed scenario. This is based on the parameter »monocyte %>8.35«, indicating increased mononuclear leukocyte activity (Additional model 1, Table 3) (Figure 1).

3. Hyperhomocysteinemia – a part of the network reaction

Clearly, by comparing the results with information gathered from the databases, we could realise, in a step by step analysis, that all these disorders, involving hyperhomocysteinemia, are mutually interconnected, sharing common pathways and operating through a highly integrated network (Figure 1). This is precisely what the systems biology theory proposes – functional integration of the elements of which the system is composed.

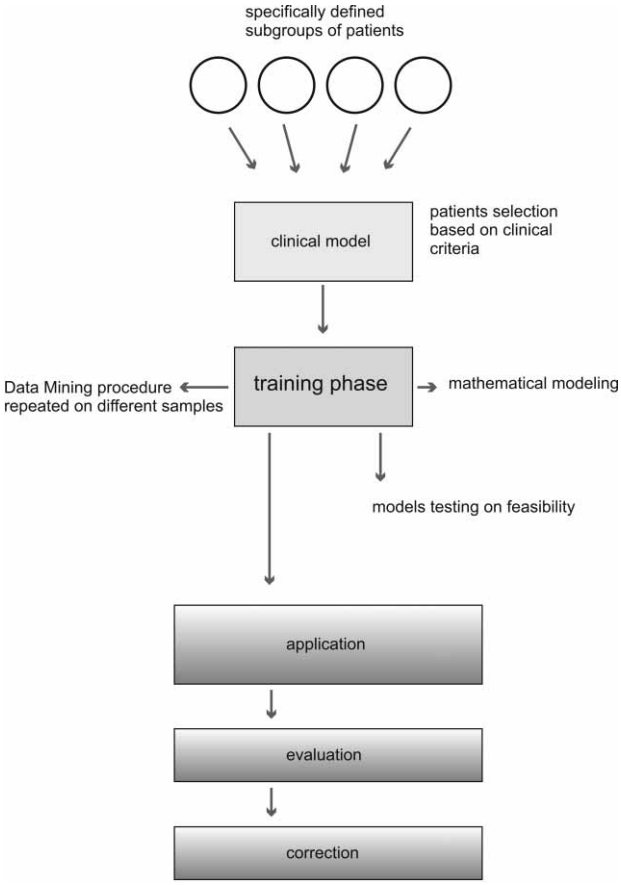

Fig. 2. Computer-based research protocol.

A view at the future – a dynamical, protocol-based and computationally intensive approach in cardiovascular risk assessment

Functional integration of the elements in the system is the reason why is it possible to identify almost all relevant risk factors (predictors) of the medical problem under investigation, based on only one single examination and on a much smaller sample, than by performing large comparative studies. This is possible by using a systems biology approach, during which a large number of data, describing many aspects of the health-status of patients, are collected and computed. The results must be compared with the existing knowledge.

Moreover, by making minimal corrections to the data, the same dataset can be used for solving different tasks.

The main aim of this study was the construction of a clinical model. It may help physicians in patient selection, based on the clinical criteria. In this way, some hidden and previously unrecognised factors or relationships can be identified.

However, the clinical model may only be the first step of research protocol used for assessing cardiovascular risk, or investigation of some other phenomena related to chronic ageing diseases (Figure 2).

During the training phase of the protocol, the challenge will be to repeat the same procedure of parameter selection on other samples, in order to answer whether the same factors are extracted when the sample is changed. This will be valuable information in the process of predictive mathematical modelling (Figure 2). Namely, experience gained so far indicate that only multiple parameters, jointly, can accurately predict a particular event, or clinical outcome[61]. Different types of data are used, including socioeconomic and environmental factors, data from population statistics, clinical and biochemical data, or information collected from genomics, proteomics, or other molecular biology fields[25,61,62].

Moreover, many mechanisms or variables, not easily detectable in clinical examinations, can be identified and included in the estimation of risk.

An important phase of the protocol is the testing of models on feasibility, by means of their predictive accuracy, economical justification and availability of the data in real-life situations (Figure 2).

Through the ongoing process of application and evaluation, models and parameters can further be corrected (Figure 2).

On-line health data record and computer programme experts working as a part of medical teams, will be needed to implement such an approach in clinical practice[63].

This will be a dynamical, protocol-based and computationally intensive approach in cardiovascular risk assessment[64].

In this way, parameters are selected in a cluster on the basis of their predictive load, reflecting their »natural clustering« (functional gathering) within the common network. Consequently, parameters are adjusted to specifically describe a particular, specifically defined, group of patients (Figure 2). This can for example, be a group of patients with early onset of myocardial infarction (MI), or a group of older patients with MI, or a group of patients with MI and (or without) diabetes mellitus, etc. The realisation of such an approach will be close to the concept of personalised medicine which has always been the goal of preventive medicine[65].

Acknowledgements

We would like to express our grateful thanks to Dr. Dragan Gamberger, Head of Laboratory for Information Systems, Institute »Ruđer Bošković«, Zagreb, for his efforts and time spent in performing Data Mining models, necessary for data processing.

REFERENCES

1. KANNEL W B, JAMA, 275 (1996) 1571. — 2. REDDY K S, YUSUF S, Circulation, 97 (1998) 596. — 3. KULLO I J, GAU G T, TAJIK A J, Mayo Clinic Proc, 75 (2000) 369. — 4. MAJNARIĆ LJ, EBLING Z, MARTINIS M, VITALE B, Period Biol, 107(2) (2005) 239. — 5. ENGSTROM G, JERNTROP I, PESSAH-RASMUSSEN H, HEDBLAD B, BERGLUND G, JANZON L, Stroke, 32 (2001) 1098. — 6. EBLING B, MAJNARIĆ-TRTICA LJ, GMAJNIĆ R, EBLING Z, VRANJEŠ Ž, Coll Antropol, 31 (2) (2007) 441. — 7. BUCHANAN A V, WEISS K M, FULLERTON S M, Int J Epidemiol, 35 (2006) 562. — 8. HELLER R F, CHINN S, PEDOE H D, ROSE G, BMJ, 288 (1984) 1409. — 9. ROCKHILL B, Epidemiology, 16 (2005) 124. — 10. International Atherosclerosis Society, Harmonized Clinical Guidelines on Prevention of Atherosclerotic Vascular Disease, Executive summary (International Atherosclerosis Society Office, Houston (TX), 2003). — 11. COOPER J A, MILLER G J, HUMPHRIES S E, Atherosclerosis, 181 (2005) 93. — 12. The International Task Force for Prevention of Coronary Heart Disease, PROCAM study, Design and Principal Results, accessed 15.09.2008. Available from: URL: http://www.chd-taskforce.com/pdf/sk_procam_01.pdf. — 13. MEIGS J B, D'AGOSTINO R B, WILSON P W F, CUPPLES L A, NATHAN D M, SINGER D E, Diabetes, 46 (1997) 1595. — 14. WALD D S, LAW M, MORRIS J K, BMJ, 325 (2002) 1202. — 15. WU LL, WU J T, Clin Chim Acta, 322 (2002) 21. — 16. SIPPONEN P, LAXEN F, HUOTARI K, HARKONEN M, Scand J Gastroenterol, 12 (2003) 1209. — 17. DIEKMAN M J, VAN DER PUT N M, BLOM H J, TIJSSEN J G, WIERSINGA W M, Clin Endocrinol (Oxf), 54 (2001) 197. — 18. MAJNARIĆ LJ, VITALE B, MARTINIS M, EBLING Z, A new avenue for assessing cardiovascular risk – homocysteine as a risk factor. In: Atherosclerosis. Supplements. Abstracts. (77th Congress of the European Atherosclerosis Society, Istanbul, 2008). — 19. MAJNARIĆ TRTICA LJ, STRNAD M, GMAJNIĆ R, EBLING B, EBLING Z, MARKOVIĆ I, ŠAMIJA M, Coll Antropol, 32 (3) (2008) 709. — 20. MAJNARIĆ TRTICA LJ, VITALE B, MARTINIS M, Non-linear approach in establishing influenza vaccine priority groups, Elsevier Editorial System for Vaccine™, Manuscript Draft, accessed 24.09.2009. Available from: URL: http://ees.elsevier.com/jvac/default.asp. — 21. Systems biology, accessed 19.01.2009. Available from: URL: http://en.wikipedia.org/wiki/Systems_biology. — 22. ADEREM A, Cell, 121 (4) (2005) 511. — 23. IRIS F, Biological modeling in the discovery and validation of cognitive dysfunctions biomarkers. In: TURCK W C (Eds) Biomarkers for psychiatric disorders (Springer, Munich, 2008). — 24. Minimental state examination, accessed 15.09.2008. Available from: URL: http://en.wikipedia.org/wiki/Mini-mental_state_examination. — 25. CASAS J P, COOPER J, MILLER G J, HINGORANI A D, HUMPHRIES S E, Ann Hum Genet, 70 (2006) 145. — 26. YANG Q, KHOURY M J, FRIEMAN J M, LITTLE J, FLANDERS W D, Int J Epidemiol, 34 (5) (2005) 1129. — 27. MAZZA A, BOSSONE E, MAZZA F, DISTANTE A, Nutr Metab Cardiovasc Dis, 15 (2) (2005) 118. — 28. Algorithms: the basic methods. In: WITTEN IH, FRANK E (Eds) Data Mining. Practical Machine Learning Tools and Techniques (Elsevier, Morgan Kaufmann Publishers, San Francisco, 2005). — 29. From Data Mining to Knowledge Discovery in Databases, accessed 23.07.2008. Available from: URL: http://www.kdnuggets.com/gpspubs/aimag-kdd-overview-1996-Fayyad.pdf. — 30. GAMBERGER D, ŠMUC T, Data Mining Server. Zagreb (Croatia): Institute Ruđer Bošković. Laboratory for Information Systems 2001, accessed 19.01.2009. Available from: URL: http://dms.irb.hr/. — 31. CLARKE R, ARMITAGE J, Semin Thromb Hemost, 26 (2000) 341. — 32. SELHUB J, Ann Rev Med, 19 (1999) 217. — 33. McCULLY K S, Am J Pathol, 56 (1969) 111. — 34. REUTENS S, SACHDEV P, Int J Geriatr Psychiatry, 17 (2002) 859. — 35. SCOTT T M, TUCKER K L, BHADELIA A, BENJAMIN B, PATZ S, BHADELIA R, LIEBSON E, PRICE L L, GRIFFITH J, ROSENBERG I, FOLSTEIN M F, Am J Geriatr Psychiatry, 12 (2004) 631. — 36. UNGVARI Z, CSISZAR A, EDWARDS J G, KAMINSKI P M, WOLIN M S, KALEY G, KOLLER A, Arteriscler Thromb Vasc Biol, 23 (2003) 418. — 37. ZHANG C, CAI Y, ADACHI M T, OSHIRO S, ASO T, KAUFMAN R J, KITAJIMA S, J Biol Chem, 276 (2001) 35867. — 38. CLARKE R, GRIMLEY E J, SCHNEEDE J, Age Aging, 33 (2004) 34. — 39. LANDRAY M J, THAMBYRAJAH J, McGLYNN F J, JONES H J, BAIGENT C, KENDALL M J, TOWNEND J N, WHEELER D C, Am J Kidney Dis, 38 (2001) 537. — 40. SULIMAN M E, QURESHI A R, BARANY P, STENVINKEL P, FILHO J C, ANDERSTAM B, HEIMBURGER O, LINDHOLM B, BERGSTROM J, Kidney Int, 57 (2000) 1727. — 41. LOVČIĆ V, KES P, REINER Ž, Acta Med Croatica, 60 (2006) 21. — 42. LOVČIĆ V, KES P, REINER Ž, KUŠEC V, Acta Med Croatica, 60 (2006) 201. — 43. VAN GULDENER C, ROBINSON K, Semin Thromb Hemost, 26 (3) (2000) 313. — 44. SENGUPTA S, WEHBE C, MAJORS AK, KETTERER M E, DiBELLO P M, JACOBSEN D W, J Biol Chem, 276 (2001) 896. — 45. STENVINKEL P, Contrib Nephrol, 149 (2005) 185. — 46. PECOITS-FILHO R, LINDHOLM B, STENVINKEL P, Nephrol Dial Trans-

plant, 17 (Suppl 11) (2002) 28. — 47. LUGER A, LANG I, KOVARIK J, STUMMVOLL H K, TEMPL H, Am J Kidney Dis, 9 (1987) 51. — 48. KUTLAY S, ATLI T, KOSEOGULLARI O, NERGIZOGLU G, DUMAN N, GULLU S, Artif Organs, 29 (2005) 329. — 49. CARTA M G, LOVISELLI A, HARDOY M C, BMC Psychiatry, 18 (2004) 25. — 50. FERRARI E, CRAVELLO L, MUZZONI B, CASAROTTI D, PALTRO M, SOLERTE S B, FIORAVANTI M, CUZZONI G, PONTIGGIA B, MAGRI F, Eur J Endocrinol, 144 (2001) 319. — 51. SONINO N, NAVARRINI C, RUINI C, FALLO F, BOSCARO M, FAVA G A, Eur J Endocrinol, 151 (2004) 61. — 52. MAGRI F, CRAVELLO L, BARILI L, SARRA S, CINCHETTI W, SALMOIRAGHI F, MICALE G, FERRARI E, Aging Clin Exp Res, 18 (2006) 167. — 53. TUZCU A, BAHCECI M, DURSUN M, TURGUT C, BAHCECI S, J Endocrinol Invest, 26 (2003) 341. — 54. CENTANNI M, MARIGNANI M, GARGANO L, CORLETO V D, CASINI A, DELLE FAVE G, ANDREOLI M, ANNIBALE B, Arch Intern Med, 159 (1999) 1726. — 55. NESS-ABRAMOF R, NABRISKI D A, BRAVERMAN L E, SHILO L, WEISS E, RESHEF T, SHAPIRO M S, SHENKMAN L, Am J Med Sci, 332 (2006) 119. — 56. SCHROECKSNADEL K, FRICK B, WIRLEITNER B, WINKLER C, SCHENNACH H, FUCHS D, Curr Pharmaceutic Biotech, 5 (2004) 107. — 57. WIDNER B, LEBLHUBER F, FRICK B, LAICH A, ARTNER-DWORZAK E, FUCHS D, J Neural Transm, 109 (2002) 1445. — 58. FUCHS D, JAEGER M, WIDNER B, WIRLEITNER B, ARTNER-DWORZAK E, LEBLHUBER F, Clin Chem Lab Med, 39 (2001) 691. — 59. WICK G, SCHETT G, AMBERGER A, KLEINDIENST R, XU Q, Immunol Today, 16 (1) (1995) 27. — 60. PEEK R M, MILLER G G, THAM KYI T, PEREZ-PEREZ G I, ZHAO X, ATHERTON J C, Lab Invest, 71 (12) 760. — 61. PEARCE N, Am J Public Health, 86 (1996) 678. — 62. KHOURY M J, Genet Med, 5 (2003) 261. — 63. MAJNARIĆ-TRTICA LJ, VITALE B, MARTINIS M, Period Biol, 110(1) (2008) 45. — 64. MAJNARIĆ TRTICA LJ, VITALE B, KOVAČIĆ L, MARTINIS M, Period Biol, 111(1) (2009) 5. — 65. ASPINALL M G, HAMERMESH R G, Harv Bus Rev, 85 (2007) 108.

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POGLED U BUDUĆNOST: DINAMIČKI PRISTUP PROCJENE KARDIOVASKULARNOG RIZIKA, TEMELJEN NA PROTOKU I UPOTREBI KOMPJUTERA

S A Ž E T A K

Znanje o kardiovaskularnim čimbenicima rizika se stalno povećava. Shodno tome, postupak procjene kardiovaskularnog rizika postaje sve kompliciraniji. Javlja se zahtjev za što obuhvatnijim, ali i što više individualiziranim pristupom. Kao način postizanja toga cilja, za procjenu kardiovaskularnog rizika predlažemo pristup s aspekta sustavne biologije. To znači potrebu prikupljanja velikog broja podataka, da bi se opisalo zdravstveno stanje pacijenata. Te podatke treba obraditi naprednim komjuterskim metodama i usporediti s postojećim znanjem. Konačni cilj toga postupka je kreiranje kliničkog modela, što je tek prvi korak cikličkog protokola koji za krajnji cilj ima procjenu kardiovaskularnog rizika. Takvim pristupom bi se mogli identificirati svi potencijalno važni čimbenici rizika na malom uzorku ispitanika. Štoviše, zbog »prirodnog« grupiranja podataka, na temelju njihove prediktivne vrijednosti, na taj način bi se mogle specifičnije definirati rizične skupine pacijenata. Takvu mogućnost smo testirali na primijeru hiperhomocisteinemije, što je poznati kompleksni čimbenik povećanog kardiovaskularnog rizika.