RĪGA STRADIŅŠ UNIVERSITY

DEPARTMENT OF NEUROLOGY & NEUROSURGERY

Viktorija Ķēniņa

Doctoral Thesis

RELATION OF HYPERHOMOCYSTEINEMIA, *CHLAMYDOPHILA PNEUMONIAE* AND CYTOMEGALOVIRUS WITH CEREBRAL INFARCTION, IT SUBTYPES AND STROKE RISK FACTORS

For acquisition the Medical Sciences Ph D

Speciality – Neurology

Riga, 2011

Doctoral work has been performed at:

Rīga Stradiņš University

Paula Stradiņa Clinical University Hospital, Neurology Clinic

Doctoral work supervisors:

Rīga Stradiņš University, Department of Neurology & Neurosurgery

Chair, Associate *Professor* **Andrejs Millers** *MD, PhD*

Rīga Stradiņš University, Department of Neurology & Neurosurgery

Docent **Elvīra Smeltere,** *MD, Phd*

Official reviewers:

Associate Professor **Ilona Hartmane**, *MD, PhD*

Associate Professor **Igors Aksiks**, *MD, PhD Habil*

Professor **Aija Žileviča,** *MD, PhD Habil*

Doctoral work scientific consultants:

Docent **Angelika Krūmiņa,** *MD, PhD*

Professor **Juta Kroiča**, *MD, PhD*

Chairperson, Promotion Board in Internal Medicine:

Professor **Ludmila Vīksna,** *MD, PhD Habil*

Secretary, Promotion Board:

Professor **Maija Eglīte,** *MD, PhD Habil*

Table of Contents

Introduction

Actuality

Best of all any disease is characterized by three parameters, which reflect the nature of the disease – the disease incidence, disability and mortality. In case of cerebral infarction (CI) these figures continue to remain poor, although there are many significant achievements in the stroke prevention and treatment. Yearly 15 million people fall ill by a stroke (World Health Organization 2005). That is one of the main causes of mortality, dementia and disability (1; 2). Inability after stroke is much more important problem than mortality, since it forms substantial additional costs in the budget for health and social care, and as often as not isolates patients from the public.

As one of the possible solutions of this problem could be effective and versatile prophylaxis, which is focused on the adjustment of risk factors. Leading cause of cerebral infarction is atherothrombosis, which could dominate in 50% causes of cerebral infarction (3; 4; 5). Lot of research is devoted to atherosclerosis and atherothrombosis stroke risk factors. Well-known are classical (old) modified risk factors – arterial hypertension, diabetes, dyslipidemia, obesity and sedentary lifestyle, smoking and excessive alcohol consumption. Although many of the above mentioned risk factors are sufficiently explored, and is possible to achieve adequate control over them, the stroke incidence is not tending to diminish. It gives reason to believe that yet not all stroke risk factors are identified and, therefore, we do not always have an access to effective and targeted prophylaxis. We also can not precisely say which of the risk factors causes process of atherosclerosis, but which only stimulates the progression. Also is unclear, why in some stroke patients are not found any of the known risk factors. Still we need to find answers on these questions.

Hyperhomocysteinemia and infection are relatively “new” risk factors for cerebral infarction. Although hyperhomocyteinemia today is marked out as a risk factor for cerebral infarction, there are some studies which do not confirm the link between homocysteine (Hcy) and ischemic stroke (6; 7). It is unclear whether heightened Hcy is a major risk factor for all stroke subtypes, whether an accompanying coronary heart disease indicates more severe hyperhomocysteinemia, whether there is a correlation between Hcy and other stroke risk factors. In contrast to Hcy, the infection is less studied risk factor for cerebral infarction, which is connected with endothelia dysfunction and lipid metabolism disorders (8). Some infections are common in the population, which, on the one hand, do not allow to name them as a specific risk factor, but, however, does not exclude its role in combination with other risk factors (e.g., diabetes mellitus). It is unclear whether exposure to infection induces origin of atherosclerosis or

only stimulates progression of the disease. Studying groups of patients and looking for clinical evidences for theory of infection explicit correlation between any microorganism and atherosclerosis more was marked in patients with cardiovascular problems. In stroke patients, in contrast to cardiovascular patients, characteristic is diversity of etiological factors.

Today worldwide in progress are researches that will help to respond to vague questions, and will let to find effective preventive measures to control consequential stroke risk factors.

Formulation of the problem and the novelty

In Latvia from stroke (ischemic and hemorrhagic) yearly die 230/100 000 people aged 35 to 74 years, which is one of the worst rates in Europe (9). The incidence of CI in Latvia, taking into account only the number of patients hospitalized in 2004 was up to 284/100 000 inhabitants, which in comparison to European countries is sufficiently high score (10). Therefore the problem of the reduction of the frequency of stroke is so topical today. Identification of the stroke risk factors, awareness and analysis of interaction can significantly improve the prevention of CI and is one of the main directions in campaign against a stroke around the world. Till now in Latvia mainly have been studied "conventional" risk factors of CI (10). However, in the literature increasingly emerge reports that, despite to treatment of "conventional" modifiable risk factors and good enough control, stroke and mortality rates do not tend to decay. Consequently, the world is searching for and exploring new CI risk factors, which isolated or in combination with "conventional" risk factors can give an answer to the questions concerning effective prevention of stroke. Having regard to stroke incidence, mortality and disability rates in our country, the study of CI "novel" risk factors may be one of the prior research directions in Latvia. Analyzing hyperhomocysteinemia and in literature most frequently, in relation to stroke, mentioned infection agents - *Chlamydophila pneumoniae (C. pneumoniae)* and cytomegalovirus (CMV) seroprevalence, we made only the first step in research of CI "novel" risk factors. In Latvia the following analysis of the risk factors for CI patients was done for the first time.

Work aim

To clarify connection of hyperhomocysteinemia and seroprevalence of microorganisms (*C. pneumoniae* and CMV) with cerebral infarction, its subtypes, and other stroke risk factors to supplement data on incidence of stroke risk factors and to perfect secondary prophylaxis of CI for patients in Latvia.

Work tasks

1. To determine the incidence of hyperhomocysteinemia and average level of Hcy in patients with CI and in control group, relation of hyperhomocysteinemia to different subtypes of CI and other stroke risk factors.

2. To determine the incidence of IgG antibodies to *C. pneumoniae* in patients with CI and in control group, relation of the seroprevalence of microorganisms to different CI subtypes, and other strike risk factors.

3. To determine the incidence of IgG antibodies to CMV to CI patients and in control group, relation of CMV seroprevalence to different CI subtypes, and other stroke risk factors.

4. To assess the need for detection of Hcy and IgG to *C.pneumoniae* and CMV in clinical practice.

Work hypothesis

Hyperhomocysteinemia is a major risk factor for stroke, with prevalence of incidence in the CI group with atherothrombotic genesis.

C. pneumoniae and CMV seroprevalence is more frequent in CI patients, if compared with control group, especially in atherothrombotic CI subgroup.

Hyperhomocysteinemia and IgG antibodies to *C.pneumoniae* and CMV have correlation with other CI risk factors, which, probably, potentiate their activity.

Determination of the level of Hcy could be recommended as a routine examination for CI patients with the aim to improve secondary prevention of the stroke. Necessary are the further studies, which would clarify the need of determining seroprevalence of microorganisms in the clinical practice.

Doctoral thesis structure and author's personal contribution

Doctoral thesis is written in Latvian. Parts of the doctoral thesis: Introduction, List of Literature, Materials & Methods, Results, Discussion, Conclusions and References. The work consists of 104 pages, including 23 tables and 26 figures.

Author has independently performed data analysis concerning the stroke patients, previously filling in specially developed questionnaire, has compiled, systematized and analyzed patients' clinical data using medical records and information gained from participants in the study and their relatives. Using duplex scanning method the author herself medically assessed brachycephal blood vessels, as well as participated in the process of treatment of patients involved in the study.

Ethical issues

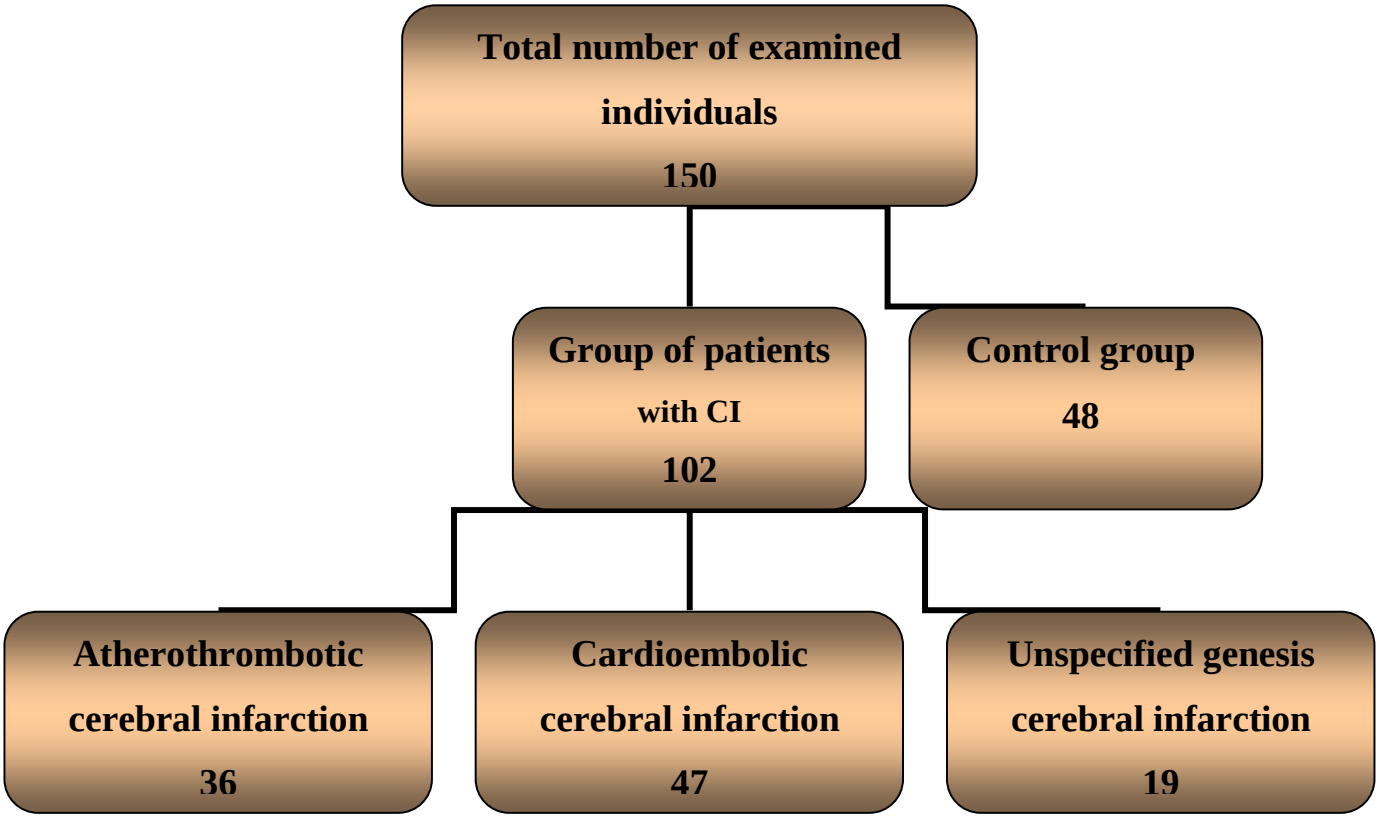
For the realization of doctoral thesis was received consent from the Riga Stradins University Ethics Committee. In the work were applied standard laboratory tests at the P.Stardina Clinical University Hospital's Laboratory.

1. Materials and Methods

1.1. *Clinical part*

1.1.1. Selection of patients and control group

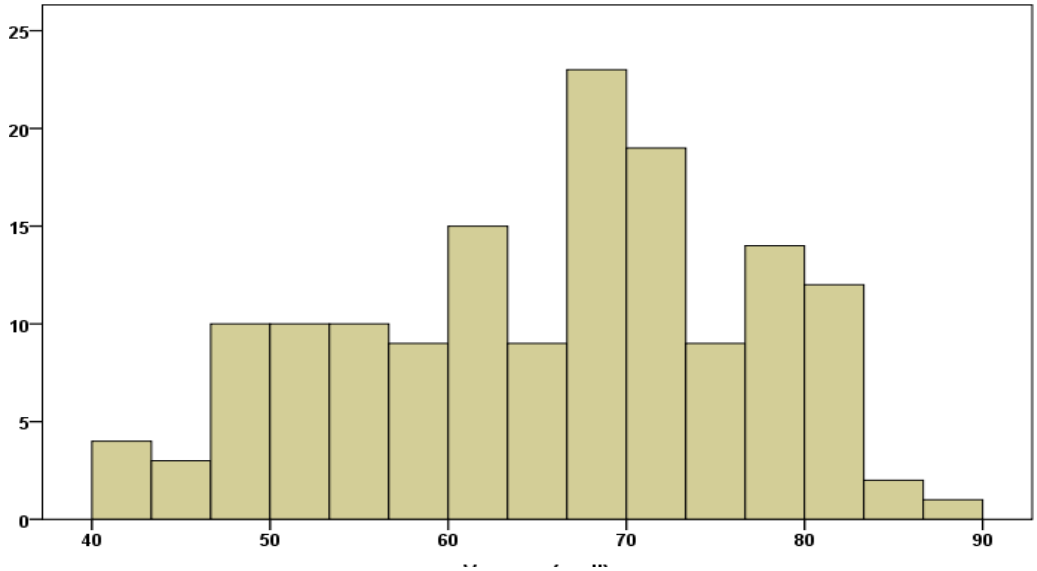
The study is done at the P.Stradina Clinical University Hospital's Neurology Clinics during the period from October 2007 to March 2009. The study has prospective nature, and participated by 150 individuals (1. Figure).



1. Figure. **Division of examined individuals in groups**

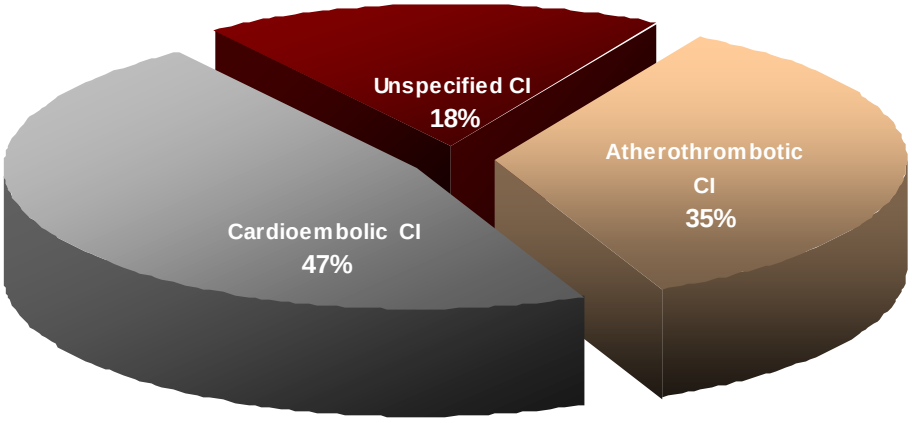
In the study core group were involved 102 patients, of which 61 were male and 41 female aged from 42 to 89 years, mean age 65,8 $\pm$ 10,9 years. In the control group were 48 people, of which 26 were male and 22 female aged from 42 to 81 years, mean age 64,3 $\pm$ 11,8 years. Division of the patients by age histogram is shown in 2. Figure. Division of patients in age

groups by independent-sample test statistically credibly do not differ: (t = 0,806; p = 0,422). Also division of patients in gender groups statistically credibly do not differ: (χ^2 = 0,426; df = 1; p = 0,514). Analyzing the social status of participants in the study was stated that 27,3% (41 patients) were working patients, 15,4% (23 patients) nonworking patients and 57,3% (86 patients) - pensioners.



2. Figure. **Division of the patients by age histogram**

The group of patients involved in the study, in turn, was divided into three subgroups according to the TOAST criteria: atherothrombotic genesis CI (36 patients or 35,3%), cardioembolic genesis CI (47 patients or 46,8%) and unspecified genesis CI (19 patients or 18,6%). Division of the patients in subgroups is shown in 3. Figure.



3. Figure. **Division of patients in subgroups according to the TOAST criteria**

Mean age of the patients in atherothrombotic genesis stroke subgroup is 63,19 ± 11,3 year, in cardioembolic genesis subgroup - 69,9±8,8 years, unspecified genesis subgroup - 60,7±11,9 years. According to the analysis of variance (ANOVA) mean age of the patients in subgroups differed statistically credibly (F = 4,631; p = 0,004). Division of patients in the subgroups by age and gender is shown in 1. Table and 2. Table.

1. Table. **Age of CI patients according to the subtype of stroke**

Subtypes of Cerebral infarction	**Number of patients**	**Mean age**	**Standard deviation**	**Conventional interval of 95% borders**	
				upper	**lower**
Atherothrombotc	36	63,2	11,3	59,4	67,0
Cardioembolic	47	69,9	8,8	67,4	72,5
Unspecified genesis	19	60,7	11,9	54,9	66,4

2. Table. **Gender of CI patients according to the subtype of stroke**

Subtype of cerebral infarction	Gender	
	Male	Female
Atherothrombotic	28 (77,8%)	8 (22,2%)
Cardioembolic	21 (44,6%)	26 (45,4%)
Unspecified genesis	12 (63,2%)	7 (36,8%)

The control group comprised patients who were treated at the P.Stradina Clinical University Hospital's Neurology Clinics, mostly with spinal un-inflammatory illnesses.

1.1.2. Population's inclusion and exclusion criteria

Inclusion criteria:

- acute primary or secondary cerebral infarction;
- genesis of atherothrombotic, cardioembolic or unspecified cerebral infarction.

Exclusion criteria:

- cerebral infarction due to other pathologies;
- cerebral infarction due to the small blood vessel diseases;
- chronic inflammatory diseases in anamnesis;
- oncology diseases in anamnesis;
- pathology of thyroid gland (hypothireosis);
- disorders of renal function (creatine in blood > 113 μmol/l);
- patients taking medicaments affecting S-adenozilmetionin metabolism (Methotrexate, Carbomazepine, Phenitoin, Anticonvulsants, etc.).

1.1.3. Control group's inclusion and exclusion criteria

Inclusion criteria:

- no data concerning the cerebral infarction;

Exclusion criteria:

- chronic inflammatory diseases in anamnesis;
- diseases, which confirmed as associated with hyperhomocysteinemia (multiple sclerosis, Alzheimer diseases, depressions, schizophrenia etc.)
- oncology diseases in anamnesis;
- disorders of renal functions (creatine in blood > 113 μmol/l);
- patients taking medicaments affecting S-adenozilmetionin metabolism (Methotrexate, Carbomazepine, Phenitoin, Nitric Oxide, Anticonvulsants, etc.);
- pathology of thyroid gland (hypothireosis).

1.1.4. Review of the Questionnaire

Data concerning all patients was analyzed by specially designed questionnaire with characterization of patients' neurological condition applying modified Rankine scale before and after stroke. In the questionnaire is analyzed localization of the stroke using computer tomography (CT) and magnetic resonance (MR) data to specify localization of the stroke. In 72 patients (70,6%) ischemia was localized in ACM basin, in 28 patients (27,5%) in VB basin, in 1 patient (1%) in ACA basin and in 1 patient (1%) in border basin.

First-time CI was found in 60 patients, repeated episode in 52 patients.

Incidence of classical risk factors was defined in both patients' and control groups.

Arterial hypertension was defined if:

- systolic pressure is over 140 mmHg, diastolic – over 90 mmHg;
- in the patients' anamnesis data is mentioned previously found arterial hypertension;
- patient regular takes antihypertensive therapy.

Diabetes mellitus is defined if:

- level of glucose on an empty stomach is over 126 mg/dl (5,8 mmol/l);
- in the patients' anamnesis data is mentioned previously found diabetes mellitus;
- patient take insulin or per oral glucose reducing therapy.

Dyslipidemia is defined if:

- total cholesterol ≤ 6.0 mmol/l;
- triglycerides < 2.0 mmol/l;
- low density lipoproteins ≤ 3.3 mmol/l.

According to WHO criteria the body mass index above 25 kg/m^2, is considered to be increased.

The stenosis as impressive in brachycephal blood vessels is considered stenosis over 60%. The blood flow velocity was evaluated by duplex scanning of brachycephal blood vessels with high-class ultrasound apparatus Philips 3110.

Other than classical risk factors, in addition to all the patients were analyzed indicators that were related to the CI pathogenesis and prognosis – amount of white blood cells (WBC) and level of fibrinogens in the blood, as well as additional measurements (thickness of the common carotoid artery *intima – media* complex), reflecting the prevalence of atherosclerosis in the process.

According to the P.Stradina Clinical University Hospital's Laboratory reference interval as increased WBC amount was considered score above 10 x 10^9/l, as increased fibrinogen – score above 3,6g/l.

Intima–media complex was measured by duplex scanning of brachycephal blood vessels with high-class ultrasound apparatus 33i. Measurement was done on both sides of the common carotid artery's bifurcation areas. As thickened *intima – media* complex was considered thickness over 0,9 mm, thickness over 1,3 mm was considered as pustule.

1.2. Laboratory part

In both patients' and control groups was determined IgG to *Chlamydophila pneumoniae,* CMV and level of Hcy in the blood.

To determine Hcy was used IMMULITE 2000 test, what is solid phase hemiluminiscent immunofermentative test designed for qualitative determination of L-homocysteine in plasma and serum. Test is based on two cycles – release of linked Hcy, its conversion into S-adenozil-L-homocysteine (SAH) and immunocorrection. Used for the test antibodies are highly specific for Hcy. As heightened Hcy value is considered level over 15 μmol/l.

To determine IgG *C.pneumoniae* was used Novagnost ™ (Germany) ELISA system, as positive result was considered 8 IU/ml. The test method was half-qualitative and qualitative. Method's specificity is 91,7%, sensitivity 90,2%.

To determine IgG cytomegalovirus was used ADALTIS (Italy) ELISA system, for qualitative and quantitative determination of IgG antibodies in plasma and serum. As positive to anti CMV IgG antibodies were considered samples with concentration over 0,5 IU/ml. Sensitivity of the method ≥ 98%.

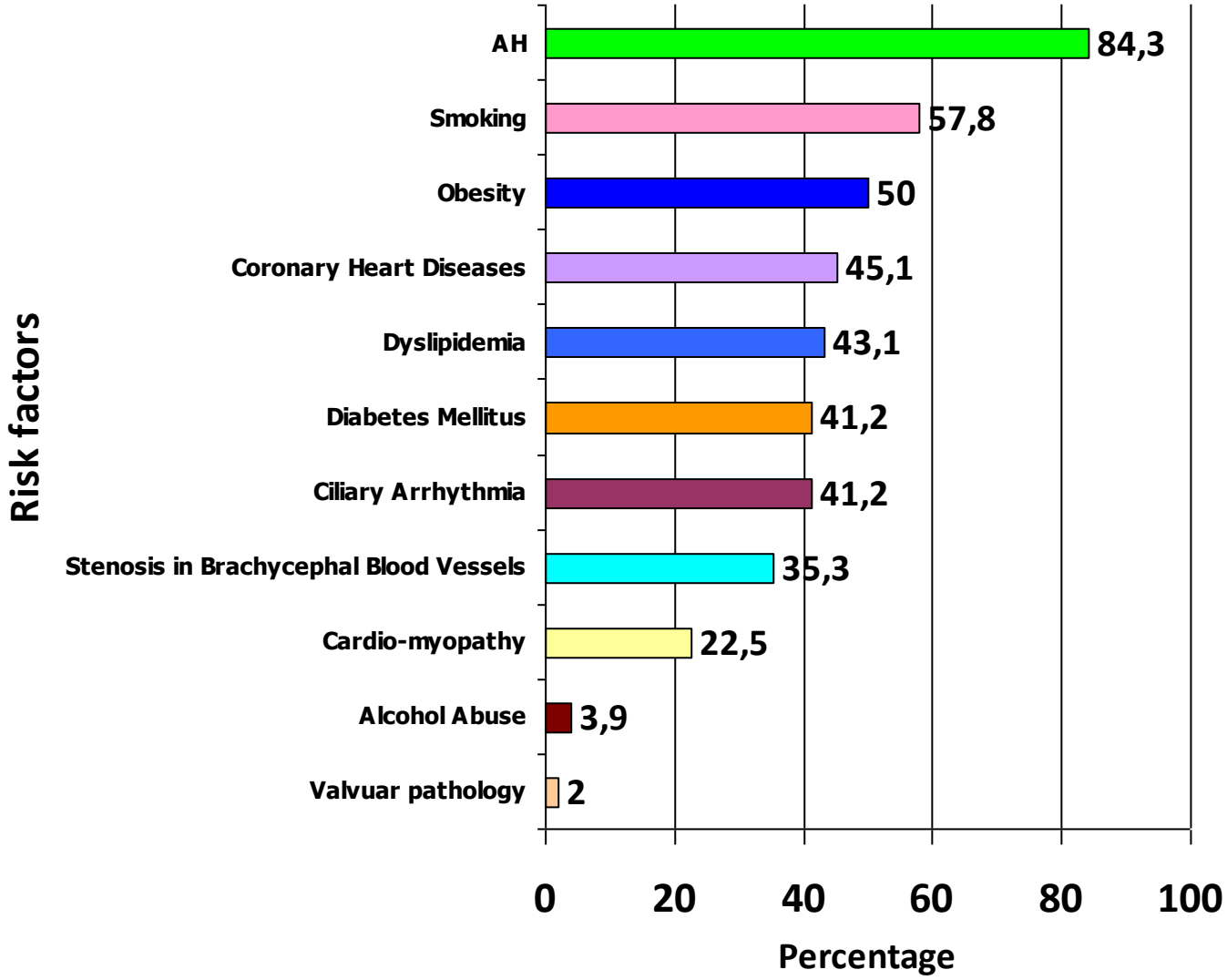
2.3. Data statistical analysis

Processing and analysis of the obtained data was done at the Rīga Stradiņs University Department of Physics in cooperation with Professor U. Teibe. Data were registered in standard forms from which they were converted into electronic format. Statistical data analysis was done with standard statistical data processing program (SPSS for Windows 16.0; SPSS Inc.), using descriptive and analytical statistical methods. For comparison of the average were used analysis of variance (ANOVA) and t-test. Incidence was expresses as a percentage using multi-factor (or also r x c) incidence tables. Score differences in the indices specific weight were tested with Pirson χ^2 and Fisher tests in program „Statcalc - exe", but mutual relations between the rates were evaluated using Pirson's correlation module.

Differences were accepted as plausible, if ≤0,05.

2. Results and Analysis

The incidence of CI risk factors in the groups of patients is shown in 4. Figure



4. Figure. **Incidence of stroke risk factors in patients with cerebral infarction**

Arterial hypertension (AH) as a cause of classic risk factor was present in 86 out of 102 patients (84,3%). Least frequently occurring risk factor – valvuar pathology was found in only 2 patients out of 102.

In the control group was analyzed incidence of the stroke risk factors. As in the patients, most common risk factor was AH. However, the incidence of AH, compared with the patients, was significantly less frequent – in 18 out of 48 participants (37,5%). The second most common risk factor – smoking was found in 7 out of 48 participants (14,6%). The next most common risk factor – dyslipidemia was found in 6 out of 48 participants (12,5%), but increased body mass index – in 5 out of 48 participants (10%). Diabetes mellitus (DM) and relevant stenosis in brachycephal blood vessels – only in 3 out of 48 participants (6%), coronary heart disease and auricle fibrillation – only in 1 out of 48 participants (4%). As a risk factor for any control group member was not identified alcohol abuse and valvuar pathology.

Incidence of leucocytosis and heightened fibrinogene level in patients and in members from control group is shown in 3. Table.

3. Table. **Incidence of leucocytosis and heightened fibrinogene level in patients and in members from control group**

	Patients group	**Control group**
Lecocytosis	29 (28%)	3 (6%)
Heightened fibrinogene level	85 (83%)	2 (4%)

In the group of patients, leucocytosis and heightened fibrinogene level was found significantly more often, which partly reflects the pathogenetic mechanisms of CI development.

Thicken *intima-media* complex was found in a majority of patients with CI, and only in a third of the control group participants (4. Table). In addition, relevant stenoses (> 60%) in the group of patients with thicken *intima-media* complex was found in 35 cases and only in 3 cases in the control group.

4. Table. **Incidence of thicken *intima-media* complex in patients and control groups**

	Patients group (n=102)	**Control group (n=48)**
Thicken *intima- media* complex	85 (83%)	18 (37,5%)

Analyzing the incidence of risk factors, dyslipidemia was analyzed in details, depending on the type of deviations in lipidogramme. In searching the link between hyperhomocysteine, seroprevalence of microorganisms and risk factors correlation analysis with each subtype of lipoprotein can help to understand the mechanism of action and role of Hcy and infectious agents in the process of atherosclerosis.

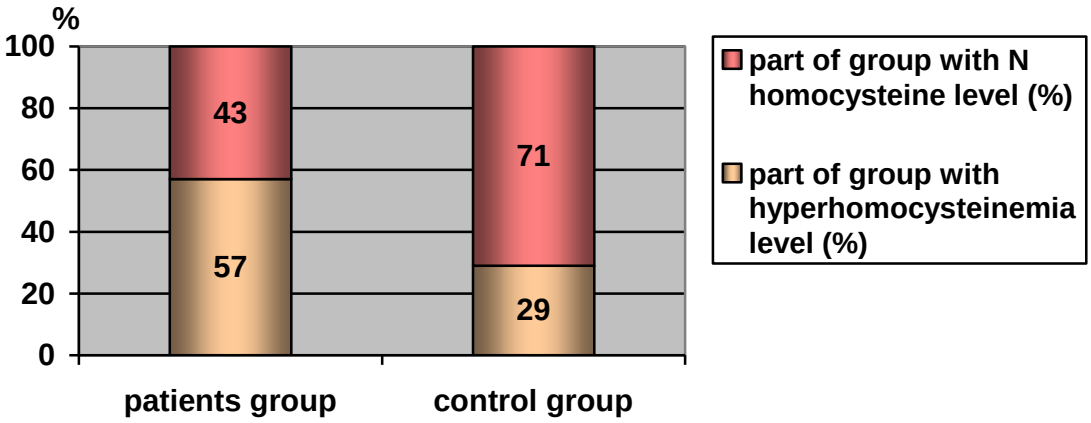
The results of distribution is as follows: heightened level of total cholesterol was found in 46 patients, heightened level of triglycerides – in 15 patients, heightened level of low density lipoproteins – in 47 patients.

2.1. *Hyperhomocysteinemia as cerebral infarction risk factor*

2.1.1. Clinical review

Mean Hcy level in patients (N = 102) was 16,3 ± 6,8 μmol/l but in the control group (N = 48) 12,8 ±4,9 μmol/l, which are statistically credibly differed (t = 3,26; p = 0,001).

Hyperhomocysteinemia was found in 58 patients from 102 and in 14 from 48 from the control group participants, respectively 57% and 29%, what according to chi-square test statistically credibly differed (χ_2 = 10,915; df = 2; p = 0,004) (see 5. Figure).



5

5. Figure. **Incidence of Hyperhomocysteinemia (%) in the patients and control group**

In patients over 60 years of age average Hcy level was higher than in patients up to 60 years of age (see 4. Table), as well in female the average Hcy level was higher than in male (p= 0.21) (see 5.Table).

4. Table. **Level of Homocysteine in different age groups of patients with CI**

Homocysteine (µmol/l)	**Age (years)**	**Number of patients**	**Average index of homocysteine**	**Standard deviation**
	<60	46	14,2	5,5
	>60	104	15,8	6,8

5. Table. **Level of Homocysteine in different gender groups of patients with CI**

Homocysteine (µmol/l)	**Gender**	**Number of patients**	**Average index of homocysteine**	**Standard deviation**
	Male	87	14,5	5,7
	Female	63	15,7	6,4

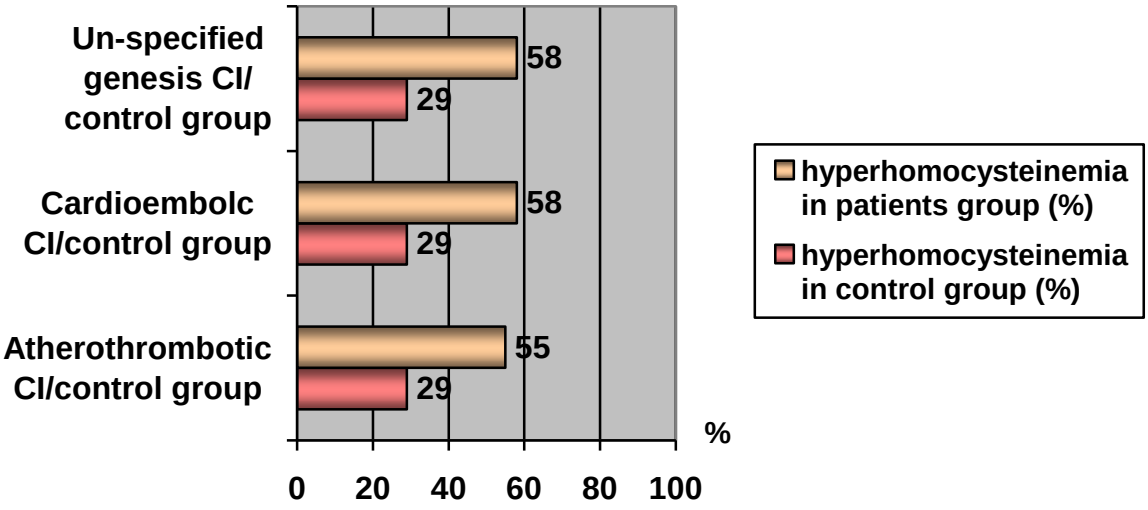
Mean Hcy level in patients with atherothrombotic CI was 17,3 ± 9 μmol/l, in patients with cardioembolic CI – 16,1 ± 5,8 μmol/l, inpatients with non-specified genesis CI – 15,4 ± 3,6 μmol/l, what according to analysis of variance statistically credibly do not differ (F = 3,957; p>0,05) (see 6. Table).

6. Table. **Level of homocysteine depending on CI subtypes**

Cerebral infarction subtype	Number of patients	Average index of homocysteine	Standard deviation
Atherothrombotic	36	17,3	9,0
Cardioembolic	47	16,1	5,8
Non-specified genesis	19	15,4	3,6

2.1.2. Incidence of hyperhomocysteine in patients with different subtypes of cerebral infarction

Analyzing hyperhomocyteinemia in each subgroup, in cases of atherothrombotic genesis CI hyperhomocysteinemia was found in 20 patients from 36, what in comparison to the control group statistically credibly differed (χ^2 = 5,95; p = 0,015), in turn, cardioembolic genesis CI hyperhomocysteinemiz was found in 28 patients from 47, what in comparison to the control group statistically credibly differed (χ^2 = 8,9; p = 0,003), and un-specified genesis CI homocysteinemia was found in 11 patients from 19, what in comparison to the control group statistically credibly differed (χ^2 = 4,8; p = 0,028) (see 6. Figure).



6. Figure. **Incidence of hyperhomocysteinemia in patients with different cerebral infarction subtypes**

2.1.3. Hyperhomocysteinemia and other cerebral infarction risk factors

Analyzing Hcy in patients with coronary heart diseases (CHD) in anamnesis (n = 46) and in patients without (CHD) (n = 56), the mean Hcy level was higher in patients with attendant CHD. In patients with CHD also hyperhomocysteinemia was more common than in group without CHD (accordingly 60,9 and 53,9%) (see 7. Table).

7. Table. **Mean level of homocysteine (µmol/l) and incidence of hyperhomocysteinemia in patients with coronary heart disease and without it**

	Patients with CHD (n = 46)	**Patients without CHD (n=56)**	**p**
Mean level of homocysteine (µmol/l)	16,7±6,2	14,7±6,5	0,082
Incidence of hyperhomocysteinemia (%)	28 (60,9%)	30 (53,6%)	0,46

Analyzing Hcy in patients with diabetes mellitus (DM) in anamnesis (n = 42) and in patients without DM (n = 60), the mean level of Hcy was higher in patients with attendant diabetes mellitus. In patients with DM homocysteinemia was more incident than in group without DM (accordingly 71,4 and 46,7%) (see 8. Table).

8. Table. **Mean level of homocysteine (µmol/l) and incidence of hyperhomocysteinemia in patients with and without diabetes mellitus**

	Patients with CD (n=42)	**Patients without CD (n=60)**	**p**
Mean level of homocysteine (µmol/l)	17,5±7,7	14,4±5,7	0,008
Incidence of hyperhomocysteinemia (%)	30 (71,4%)	28 (46,7%)	0,01

Analyzing the hyperhomocysteine and *C. pneumonia* seroprevalences' possible relationship, statistically credible correlation was not found. In the hyperhomocysteine group seroprevalence to *C. pneumonia* was group in 37 patients from 58, but in group with normal level of Hcy – 27 from 44 (p = 0,9). For other risk factor results of analysis were similar, without prevalence of incidence of hyperhomocysteinemia and differences in the mean Hcy level between the groups.

DM is the only CI classical risk factor with whom was found statistically credible correlation (r = 0,224; p = 0,026).

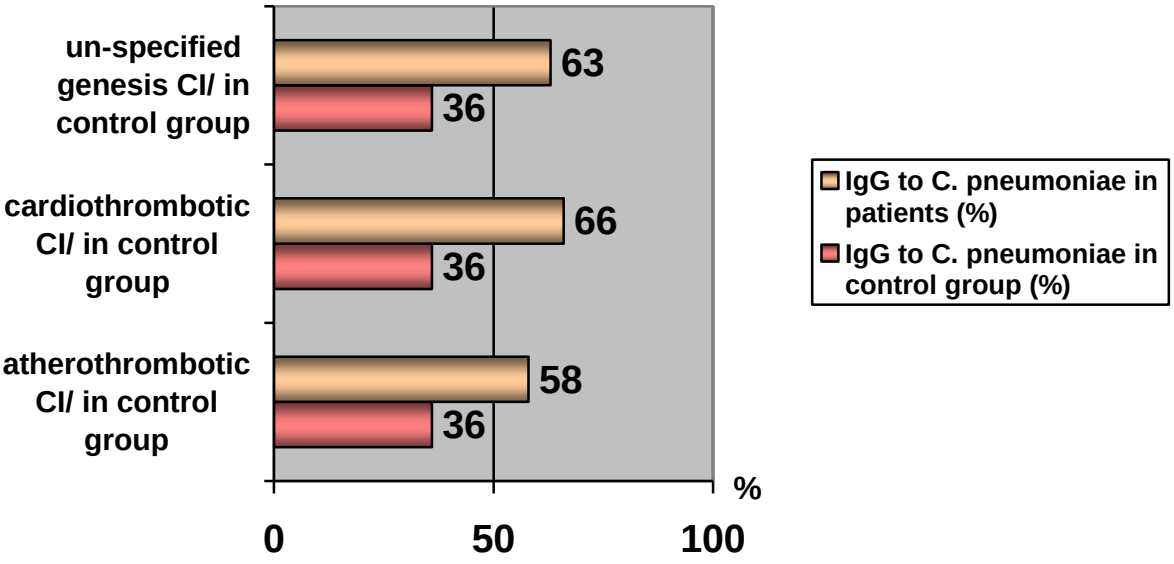
2.2. Relation of Chlamydophila pneumonia and Citomegalovirus with cerebral infarction

2.2.1. Incidence of IgG to *Chlamydophila pneumonia* in patients with cerebral infarction and in control group

IgG antibodies to *C. pneumonia* were found in 64 patients from 106 (62,7%) and in 17 participants from 48 (35,4%) in the control group. Division of patients in the groups statistically credibly differed (χ_2 = 9,8; df = 1; p = 0,002).

2.2.2. Incidence of IgG to *Chlamydophila pneumonia* in patients with different subtype of cerebral infarction

Analyzing seroprevalence of *C. pneumonia* in each subgroup, in case of atherothrombotic genesis CI, positive *C. pneumonia* antibodies were found in 21 patients from 36, which in comparison with the control group statistically credibly differ (χ_2 = 4,36; p = 0,037). In the cardioembolic genesis CI group the positive antibodies to *C. pneumonia* were found in 31 patients from 47, which in comparison with the control group also statistically credibly differ (χ_2 = 8,86; p = 0,003). Un-specified genesis CI positive antibodies to *C. pneumonia* were found in 12 patients from 19, what in comparison with the control group statistically credibly differ (χ_2 = 4,27; p = 0,039) (see 7. Figure).



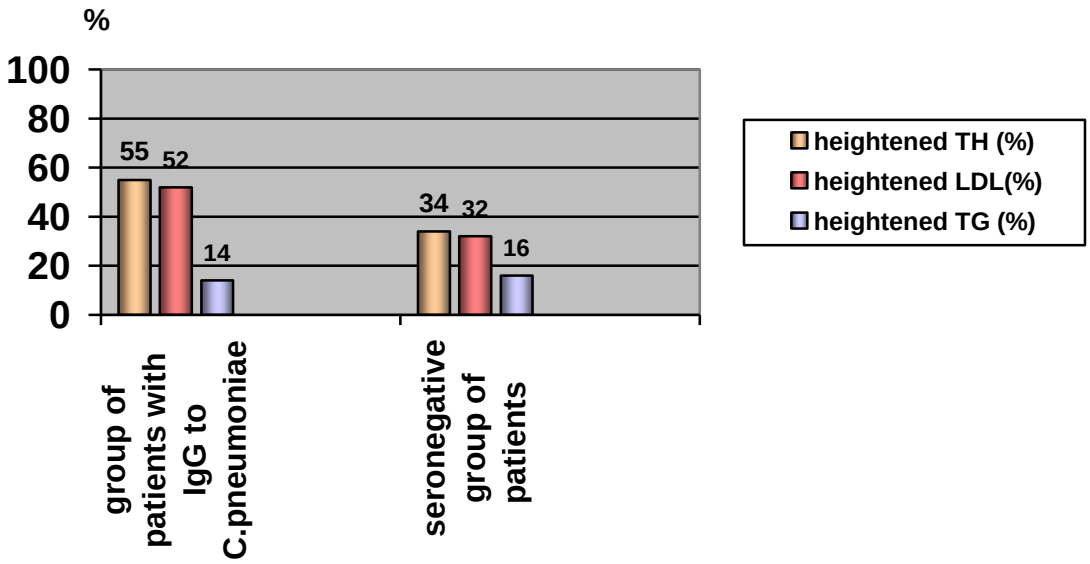
7. Figure. **Incidence of *C. pneumoniae* seroprevalence in patients with different subtypes of cerebral infarction**

2.2.3. Relation of *Chlamydophila pneumoniae* seroprevalenes with cerebral infarction risk factors

Considering the *C.pneumoniae* atherogenic properties was analyzed profile of lipids in patients with positive antibodies to microorganism.

From 64 patients with IgG to *C.pneumoniae*:

- heightened TH level was found in 33 patients, but in seronegative group of patients (n=38) – in 13 patients (χ^2=2,90; p=**0,089**);
- heightened LDL level was found in 35 patients, but in seronegative group of patients (n=38) – in 12 patients (χ^2=5,12; p=**0,024**);
- heightened TG level was found in 9 patients, but in seronegative group of patients (n=38) – in 6 patients (χ^2=0,06; p=**0,8**) (see 8. Figure).

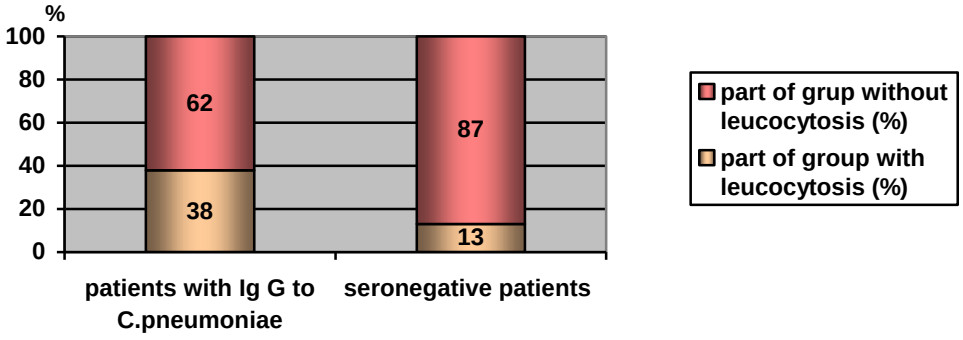


8. Figure. **Lipid profile analysis in patients with IgG to *C.pneumoniae* and in seronegative patients**

Analyzing incidence of IgG to *C. pneumoniae* and its relation with the other risk factors, the differences in statistically credible correlation and in incidence between the groups with or without risk factor were not found.

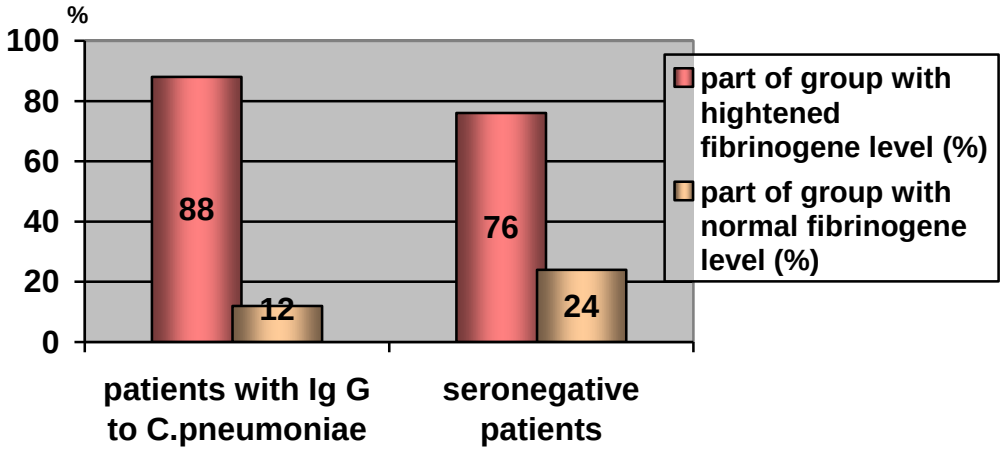
Also was analyzed possible relation of *C. pneumoniae* seroposivity with leucocytosis, level of the fibrinogen in blood and thickness of *intima-media* complex.

Leucocytosis entering the study was found in 24 patients from 64 with „+"IgG to *C.pneumoniae* and in 5 from 38 with „-" IgG (χ^2 = 6,94; **p = 0,008**) (see 9. Figure).



9. Figure. **Number of patients (%) with and without leucocytosis in the group with IgG to *C.pneumoniae* and in the group of seronegative patients**

Heightened level of fibrinogen was found in 56 patients from 64 with IgG to *C. pneumoniae* and in 29 from 38 without IgG (χ^2 =2,15; **p = 0,14**) (see 10. Figure).



10. Figure. **Number of patients (%) with and without heightened fibrinogen in the group with IgG to *C. pneumoniae* and in the group of seronegative patients**

Analyzing a group with IgG antibodies to *C. pneumoniae* statistically credible correlation was found with the indicator of inflammation – leucocytosis (r = 0,258; p = 0,009), positive antibodies to *Chlamidophila pneumoniae* and LDL (r = 0,221; p = 0,026). Correlation with the other stroke risk factors was not founded.

2.2.4. Incidence of IgG to CMV in patients with cerebral infarction

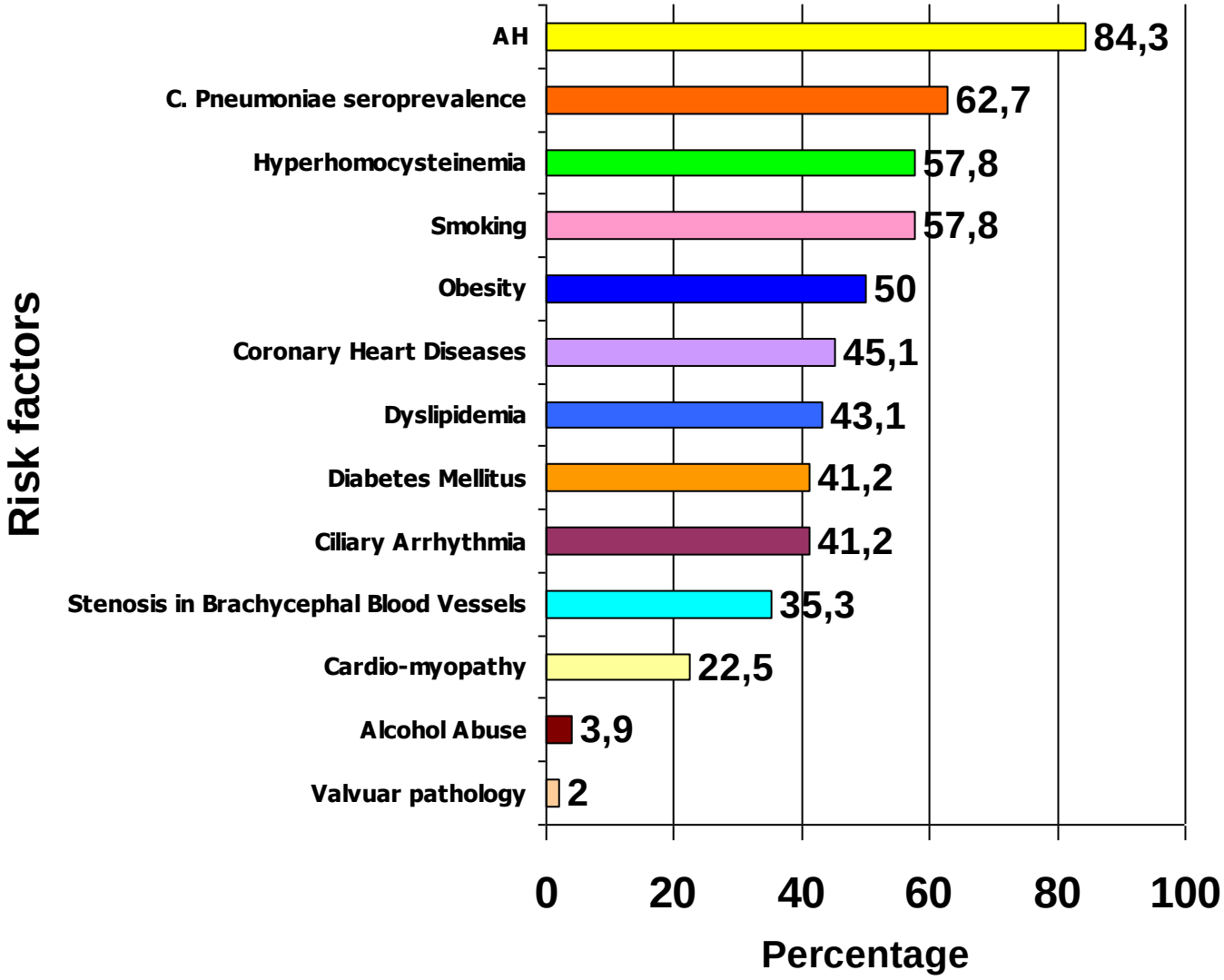
IgG antibodies to CMV were found in 97 patients from 102 (95%) and in all members of the control group. The results between these groups statistically credibly did not differ (χ^2 = 2,43; p = 0,12).

2.2.5. Relation of CMV seroprevalence with the other cerebral infarction risk factors

Analyzing the patients with IgG antibodies to CMV statistically credible correlation was found with the indicator of inflammation – leucocytosis (r = 0,282 p = 0,004), positive antibodies to CMV and CHD (r = 0,221; p = 0,026). Correlation with the other stroke risk factors was not founded.

Characterization of the patients in groups according to the incidence of stroke risk factors, considering the analyzed risk factors is shown in 11. Figure.

11. Figure. The incidence of risk factors



Discussion

Aim of the Doctoral Thesis was to study seroprevalence of the hyperhomocysteinemia, *C. pneumoniae* and as relatively new cerebral infarction risk factors, assessing the significance of each factor in the Latvian population. In the study seroprevalence of Hcy, *C pneumoniae* and CMV were examined not only as isolated and independent risk factors, but also was examined the interaction of these factors, as well as interaction with other CI risk factors. In order to improve access to the problem of secondary prevention of the stroke patients important was to determine, which of the above-mentioned risk factors for the CI subtypes (atherothrombotic or cardioembolic) is of more importance.

Hyperhomocysteinemia is one of the relatively new and modified CI risk factors. In the present study average Hcy level was higher in the group of patients compared with the control group (p = 0,001). Also incidence of hyperhomocysteinemia was higher in the group of patients (p=0,004). Considering that both the results were with statistically credible difference, could come to a conclusion – the study has demonstrated that hyperhomocysteinemia is CI risk factor also for the Latvian inhabitants. In patients older than 60 years Hcy average was higher than in patients at the age to 60 years (p=0,17), as well as in female Hcy average was higher than in male (p = 0,21).

Analyzing sources of literature where are described epidemiological nature researches related to hyperhomocysteinemia and CI, appeared that the results in various researches widely differ, what mainly is explained by their design and the ethnic features of population. However, most studies (11; 12; 13; 14; 15) show a positive correlation between hyperhomocysteinemia and stroke, which was also confirmed in this work. Age and gender are two factors that affect homocysteine. Deficiency of folates, vitamins B 6, B 12 and deterioration of renal functions in the elderly are the factors that alter Hcy level. With that, in our study, is explained increase of Hcy level in the elderly patients.

It is known that Hcy level grows rapidly in the post-menopausal period, and in women this period is faster than in men. That happens due to the hormonal changes – strongly negative correlation between the levels of estradiol and Hy in women in post-menopausal period (16; 17). Data of our study reflects this fact.

Some authors stress that an increased Hey level potentiates athersclerosis directly in the large blood vessels (18; 19; 20; 21) so causing atherothrombotic genesis CI. Other authors take an opposite view that ka hyperhomocysteinemia is an important risk factor directly for cardioembolic stroke (22). In our study incidence of hyperhomocysteinemia is statistically credibly higher in the group of patients, if comparede with the control group, and as well in all CI subtype groups separately. This supports the conclusion that the Hcy is an important risk factor for both atherothrombotic and cardioembolic stroke, which is related to presence of atherosclerosis in most of cardioembolic CI patients. It should be noted that in this study in group with atherothrombotic CI mean Hcy level is higher than in groups with other subtypes. However, the average Hcy rates between CI subtype groups did not differ statistically credibly, what allows to think about the importance of atherothrombosis un atherosclerosis process in all stroke subtypes and confirms effect of hyperhomocysteinemia on the blood coagulation system.

Although the Hcy is defined as an independent stroke risk factor (23; 24), there are severl studies suggesting relation of hyperhomocysteinemia with other vascular risk factors, such as hypertension, diabetes mellitus, smoking, etc. (25; 26). Analyzing the patients with DM and without it, in our study was concluded that the diabetes is the only vascular risk factor for whom has been found statistically credible correlation with hyperhomocysteinemia (p=0,026). In patients with DM the incidence of hyperhomocysteinemia as well as the average of Hcy was higher than in patients without DM. DM is a disease that affects kidney function, is associated with elevated albumin excretion and often combines with pernicious anaemia. All these factors affect the level of Hcy, increasing it. Depending on the stage of the disease, renal function, vitamin status, and diabetes treatment tactic also changes the level of Hcy in the blood. In view of this tactics, it can be concluded that the timely and correct treatment of diabetes helps to protect for more the patient from hyperhomocysteinemia and its progression.

Separately were analyzed patients with hyperhomocysteinemia and CDH, hyperhomocysteinemia and *C.pneumoniae* seroprevalence. In both groups was not stated strong correlation between the risk factors, although the average Hcy level in both CDH group and *C. pneumoniae* group was higher.

From 20 to 40% of the population are smokers, 20% from them regularly abuse alcohol, what influence the vitamin status in the body. Many individuals several times by day use caffeine-containing drinks (e.g., coffee), in the population sufficiently prevalent are gastrointestinal tract, thyroid and kidney diseases. Also should be remembered genetically determined hyperhomocysteinemia, which, depending on gene mutation and ethnic features in the population, could be found in up to 40%.

Taking into account the above mentioned facts, it can be concluded that each case of hyperhomocysteinemia more likely is multifactorial. Knowledge of the facts, which could potentially affect the Hcy level raising it and making correction in it, should be started with changes in the lifestyle and diet. In some cases lifestyle adjustments and diet is noy sufficiently effective way to achieve normal Hcy level, and should be started drug therapy. In this work was not specified a possible aetiology for each patient but, considering a fact that over 90% of the studied hyperhomocysteinemia patients had mild hyperhomocysteinemia, it can be concluded that increased Hcy level is based on unhealthy lifestyle and vitamin deficiency, which is quite easily adjustable.

In issued in year 2009 guidelines concerning secondary prevention of I and transitory ischemic attacks, which are based on the American and European guidelines is recommended prescription of multivitamins with adequate dose of vitamin B6 (1,7 mg / per day), B12 (2,4 μg / per day), folic acid (400 μg / per day) for patients with hyperhomocysteinemia (>10 μmol/l) and had in anamnesis CI or TIL [27]. According to the P. Stradiņa Clinical University Hospital's data from about 500 patients, who underwent treatment cure in year 2009 at the Neurological clinic with diagnosis – CI, to two was set Hcy level and to another three patients was recommended to determine it ambulatory. None of the patients after having had CI was recommended to use multivitamins for secondary prophylaxis.

Taking into account the national economic situation and the study data, as well as identification of several factors having an influence on Hcy in patients involved in the present study, should be considered prescription of vitamin therapy to all patients after CI, paying special attention to patients with DM and CHD. It should be noted that folic aid and vitamin B containing drugs together with healthy lifestyle is relatively cheap, safe and effective way, as to help to improve results of secondary prevention for stroke patients. (28)

For the first time possible relation between *C.pneumoniae* seroprevalence and stroke was found in year 1988 during the serological research in Finland. Thence already more than 500 articles are devoted to relation of microorganisms with atherosclerosis and stroke. In our study, analyzing *C.pneumoniae* seroprevalence in patients and in control group was found statistically credible difference between the groups. IgG antibodies in the group of patients were met in 62,7%, bet in the control group – only in 35,4% (p=0,002). In addition, should be noted that the incidence of seroprevalence in each CI subtype group was higher in comparison to the control group. This fact indicates the involvement of atherosclerotic process in each of CI subtypes, as directly with it is connected importance of *C.pneumoniae*. If we compare the results with data in literature, it can be concluded that the incidence in the group of patients comply with other results that are generated in the researches, while showings in the control groups, according to

sources in literature, vary from 15 to 60%. Such diversity of the results can be explained both with differences in the methods used to determine chlamydia and criteria to select the population, and ethnic characteristics.

In terms of Chlamydia relation to other CI risk factors, a little more emphasis should be put on dyslipidaemia. It is known that lipids are one of the dominant stands in the research of atherosclerosis. Naturally there are a lot of different lipids and fats with their role and importance. It is clear that they are necessary both in the process of the body's cell structure and in maintaining the energy function. From the blood cholesterol to the cells is delivered by LDL, which is used as a transporter. According to the most popular theory, the lipids (LDL) infiltrate arterial wall and penetrate in intima, thereby stimulating the development of atherosclerosis. There are also other theories which declare that the production of lipids in atheroma is a complex process and chlamydias can actively participate in it. As already mentioned, chlamydia is necessarily intracellular microorganism, which no only uses the host's metabolic processes, but also, to survive, requires host's food and lipids. The way in which microorganisms use host's lipids – modification of used substance. A cell infected with chlamydia, LDL no longer fulfills its functions, but changes into chlamydia "maid". *C pneumoniae* blocks the normal cellular metabolism, bringing the cholesterol to Golgi complex. Then cholesterol is used both as a building material for chlamydia vacuoles – elementary particles cell walls, and as a food. Only when the chamydia leaves a cell, used cholesterol offloads, but unfortunately, in the form of cholesterol crystal which can not be used to fulfill cell's required functions.

In the doctoral thesis were analyzed the lipid profile in patients with and without proved IgG antibodies to microorganisms. The results showed that increased LDL level more frequently was found in the *C. pneumoniae* seropositive patients (p=0,024). It leads once again to think about the interaction of risk factors in the human body and the role of lipids in atherosclerotic process.

Correlation of the infection with inflammatory parameters is another interesting question, which need to be discuss more detailed. It is known that there are a number of markers and mediators indicating acute or chronic inflammation process, or body's immune response to it. Several of them (C reactive protein, fibrinogen etc.) have been extensively studied in the atherosclerosis and stroke patients. In the study number of leucocytes and level of fibrinogen were analyzed as the simple routine analyses that are readily available at the clinics already on the Receiving-room stage. Results of the study showed statistically credible IgG antibodies to *C.pneumoniae* correlation with leucocytosis (p=0,008), but negative for chlamydia and fibrinogen correlation (p=0,14). The leucocytes (macrophages and lymphocytes) have an important role in the development process of atherosclerosis. The number of leucocytes

correlates with progression of atherosclerosis (29) (30), CHD (31) (32) un stroke (33) (34). The macrophages and T-lymphocytes are visible in atheromas even at the early stage of disease, indicating to the immune reaction as an underlying mechanism in the process of atherosclerosis. Is the chronic infection one of the factors that potentiates atherogenesis and what leucocytosis reflects by? Having regard to the data of our study could be thought about such a mechanism in the group of *C. pneumoniae* seropositive patients.

The heightened level of fibrinogen is an independent CI risk factor. (35) (36) the level of fibrinogen increases after the stroke, and it is attributed with the repeated risk of cerebrovascular accident. It is proved that in patients with CI and accompanying infection the level of fibrinogen is higher than in those with not detected accompanying infection (37).

Cytomegalovirus infection is widespread in the population, thus it can not be considered as a specific risk factor. Literature data on CMV infection and stroke is controversial. There are studies confirming the relation of CMV with CI, but most of the researches emphasize the role of CMV infection only in correlation with other vascular risk factors, noting the correlation with increase in viral antibodies' titre. Similar results were reported in our patients.

In the Doctoral thesis research in 93% of all patients and 100% of the control group subjects were found positive to MV antibodies. Such results indicate that the antibodies to CMV could not be considered as the possible CI risk factor. In literature cytomegalovirus is associated with proliferation of blood vessel smooth muscularity (38) and increased thickness of *intima-media* complex (39). In the performed research was found correlation between the CMV IgG antibodies and thickness of *intima-media* complex ($r=0.221$, $p=0.026$). Literature data about the role of cytomegalovirus in combination with other risk factors, such as coronary heart disease (40) and leucocytosis (41), was also confirmed in our work. In the study was found connection between the IgG antibodies to and leucocytosis ($p = 0{,}004$), coronary heart diseases ($p = 0.022$).

Modern research is still unable to give an unequivocal answer about the role of infection in the aetiology of stroke. Exist luck of researches involving a large number of patients, also there are no consensus on the study design and criteria for selecting the patients and control group. CI classification criteria is not unified in all studies. Serious matter is choice of the laboratory diagnostic methods used for the identification of microorganisms and determination of the presence of infection.

Health of the population, rational usage of the state budget and human's life quality – issues which actuality requires to press on searching an effective stroke's problem settlement. However, must be resolved several problems to confirm the infection's theory and its role in the etiology of stroke. Each new study is another step that can help to put together a complex mosaic of challenges and find answers to the given questions.

In the course of this study we have been able to confirm the role of hyperhomocysteinemia in the CI development process in the Latvian population, which are comparable to similar studies in other European countries and in the world, drawing conclusions. In the chapter concerning the seropositivity of microorganisms the obtained data concerning the seroprevalence make possible to compare the status of population with the results of similar studies in other countries, and is a serious ground for continuation of the study related with *Chlamydophila pneumonia* demonstration in native preparations, which is an expensive and laborious process.

Conclusions

1. Hyperhomocyteinemia is an important risk factor for atherotrombotic, cardioembolic and unspecified genesis cerebral infarction subtypes.

Correlation of the hyperhomocysteinemia with the diabetes mellitus suggests it as a disease which affects the level of homocysteine.

2. Incidence of *C.pneumoniae* seroprevalence is higher in the groups of patients with cerebral infarction, which confirms its role in the pathogenesis of stroke for all subtypes of cerebral infarction.

Correlation of *C.pneumoniae* with dyslipidemia (increased level of low-density lipoproteins) and amount of leukocytes specifies the atherogenic properties of microorganism.

3. No difference was showed in the analysis of the incidence of IgG to CMV neither in patients nor in control groups.

4. Hcy determination can be recommended as a routine examination for patients with cerebral infarction and diabetes mellitus.

.

Practical Recommendations

1. Detection of Hcy level can improve the prognostic value of „classical" stroke developmental risk factors.

2. Correction of hyperhomocysteinemia and other risk factors of stroke can redice the risk of cerebral infarction.

3. Determination of *C. Pneumoniae* seroprevalence in the patient's goup with high risk of cerebral infarction can help in selection of optimal prophylactic treatment (with a preference to anti - Chlamydia medication).

List of literature

1. Murray CJL, Lopez AD: Global mortality, disability and the contribution of risk factors: Global burden of disease study. Lancet 1997.-349:1436-1442 p.

2. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ: Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 2006; 367:1747-1757.

3. Sandercock PA, Warlow CP, Jones LN, Starkey IR. Predisposing factors for cerebral infarction: the Oxfordshire community stroke project. Br. Med. J. 1989.- 298:75-80p.

4. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtype of cerebral infarction. Lancet 1991.- 337:1521-6p.

5. Schulz UG, Rothwell PM. Differences in vascular risk factors between etiological subtypes of ischemic stroke: importance of population – based studies. Stroke 2003.- 34:2052-9p.

6. Alfthan G. et al. Relation of serum homocysteine and lipoprotein concentrations of atherosclerotic disease in prospective Finnish population based study. Atherosclerosis 1994.-106:9-19p.

7. Verhoef P et al. A prospective study of plasma homocysteine and risk of ischemic stroke. Stroke 1994.- 25:1924-30p.

8. Libby P, Egan D, Skarlatos S: Roles of infectious agent in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation* 1997.- 96:4095-4103p.

9. Sarti C, Rastenyte D, Cepaitis Z, Tuomilehto J: International trends in mortality from Stroke, 1968 – 1994. 2000.- 31:1588-1601p.

10. E. Miglane, G. Eniņa, B. Tilgale. Risk factors and some clinical factors in various subtypes of cerebral infarction. Atherosclerosis Supplements, 2004.-5/4:35p.

11. Bostom AG, Rosenberg IH, Silbershatz H, et al: Nonfasting plazma total homocysteine levels and stroke incidence in elderly persons: The Flamingham Study. Ann Intern Med. 1999.-131:352p.

12. Bots ML, Launer LJ, Lindemans J,et al: Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. Arch Intern Med. 1999.- 159:38p.

13. Perry IJ. et al. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995.-346:1395-8p.

14. Ridker PM et al. Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 1999.-281:1817-21p.

15. Aronow WS et al. Increased plasma homocysteine i san independent predictor of new atherotrombotic bramin infarction in older persons. Am J Cardiology 2000.-86:585-6p.

16. Bush D et al. Estrogen emplacement reverses endothelial dysfunction in postmenopausal women. Am J Med 1998;104:552-558

17. Giri S et al. Oral estrogen improves serum lipids, homocysteine and fibrinolysis in elderly men. Atherosclerosis1998; 137:359-366

18. Yoo, J.H., Chung, C.S. & Kang, S.S. Relation of plasma homocysteine to cerebral infarction and cerebral atherosclerosis. *Stroke*, 1998; 29, 2478-2483.

19. Spence, J.D., Malinow, M.R., Barnett, P.A., Marian, A.J., Freeman, D. & Hegele, R.A. Plasma homocysteine concentration, but not MTHFR genotype, is associated with variation in carotid plaque area. *Stroke*, 1999; 30, 969-973.

20. Pezzini, A., Grassi, M., Del Zotto, E., Assanelli, D., Archetti, S., Negrini, R., Caimi, L. & Padovani, A. Interaction of homocysteine and conventional predisposing factors on risk of ischemic stroke in young people: consistency in phenotype-disease analysis and genotype-disease analysis. *J. Neurol. Neurosurg. Psychiatry*, 2006; 77, 1150-1156.

21. Yokote, H., Shiraishi, A., Shintani, S. & Shiigai, T. Acute multiple brain infarction in large-artery atherosclerosis is associated with hyperhomocysteinemia. *Acta Neurol. Scand*., 2007; 116, 243-247.

22. Nida Tascilar et.al.: Hyperhomocysteinemia as an Independent Risk Factor for Cardioembolic Stroke in the Turkish Population. The Tohoku J. Exp. Med.2009; 218, 293-300

23. Stehouwer CD, Weijenberg MP, Vanden BerghM. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly parsons. *Arterioscler Thromb Vascul Biol.* 1998; 18: 1895–1901.

24. Adunsky A, Weitzman A, Fleissig Y. The relation of plasma total homocysteine levels to prevent cardiovascular disease in older patients with ischemic stroke. *Ageing (Milano)* 2000; 12: 48–52.

25. Munishi MN, Stone A, Fink L.Hyperhomocysteinemia following a methionine load in patients with non-insulin dependent diabetes mellitus and macrovascular disease. *Metabolism* 1996; 45: 133–135.

26. Adunsky A, Weitzman A, Fleissig Y. The relation of plasma total homocysteine levels to prevent cardiovascular disease in older patients with ischemic stroke. *Ageing (Milano)* 2000; 12: 48–52.

27. G. Eniņa, A. Millers, B. Tilgale. Cerebrāla infarkta un transitoras išēmiskas lēkmes sekundārās profilakses vadlinijas. 2009; 50.

28. Walds DS et al. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis BMJ 2002; 325:1202-1212.

29. Elkind MS, Cheng J, Boden-Albala B, Paik MC, Sacco RL. Elevated white blood cell count and carotid plaque thickness: the Northern Manhattan Stroke Study. *Stroke.* 2001; 32: 842–849

30. Salonen R, Salonen JT. Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. *Atherosclerosis.* 1990; 81: 33–40.

31. Yarnell JWG, Baker IA, Sweetnam PM, et al. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. *Circulation.* 1991; 83: 836–844

32. Kannel WB, Anderson K, Wilson PW. White blood cell count and cardiovascular disease: insights from the Framingham Study. *JAMA.* 1992; 267: 1253–1256

33. Grau AJ, Boddy AW, Dukovic DA, Buggle F, Lichy C, Brandt T, Hacke W; CAPRIE Investigators. Leukocyte count as an independent predictor of recurrent ischemic events. *Stroke.* 2004; 35: 1147–1152

34. Prentice RL, Szatrowski TP, Kato H, Mason MW. Leukocyte counts and cerebrovascular disease. *J Chronic Dis.* 1982; 35: 703–714.

35. Yarnell Jwg, Baker IA, Sweernam PM, et al: Fibrinogen, viscosity, and white blood cell count are major risk factor for ischemic heart disease. Circulation 83:836,1991

36. Qizilbash N; Fibrinogen and cerebrovascular disease. Eur Heart J 16 (Suppl A):42,1995

37. Americo SF et al: Immunohematologic characteristic of infection-associated cerebral infarction. Stroke 22:1004, 1991

38. Epstein, SE., Zhou, YF., Zhu, J. Infection and atherosclerosis: emerging mechanistic paradigms. *Circulation*, 1999; 100, 20–28.

39. Nieto, FJ., Adam, E., Sorlie, P., Farzadegan, H., Melnick, JL., Comstock, GW., Szklo, M. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation*.1996; 94, 922–927

40. Amarenco, P., Cohen, A., Tzourio, C *et al*. Atherosclerosis disease of the aortic arch and the risk of ischemic stroke. *New Eng.l J. Med.*, 1994; 331, 1474- 1479.

41. Grau, AJ., Weimar, C., Buggle, F., et al. (2001) Risk factors, outcome, and treatment in subtypes of ischemic stroke: the German stroke data bank. *Stroke*2001; 32, 2559-2566

Publications

Articles in peer-reviewed journals:

1. **V Ķēniņa**, P. Auce, Z. Priede, A. Millers, E. Smeltere. Homocysteine, Atherothrombosis, and Stroke. Seminars in Neurology 2009, 3 (41) p139-143
2. **V. Ķēniņa**, P.Auce, Z. Priede, I. Irbe, L. Vainšteina, E. Smeltere, A. Millers. (2010) Cytomegalovirus chronic infection as a risk factor for stroke: a prospective study. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences. 64:3, 133-136
3. **V. Ķēniņa**, P. Auce, Z. Priede, L. Vainšteine, I. Irbe, A Millers. Hyperhomocysteinemia as a Risk Factor for Stroke: a Prospective Study. // Collection of Scientific Papers.- RSU, Riga, 2010.- p.79 – 82.
4. **V. Ķēniņa**, Z. Priede, P. Auce, N. Sūna, A. Millers. Carotid artery stenosis correlation with hyperhomocysteinemia in stroke patient group: a prospective study.//Acta Chirurgica Latviensis 2010, (10/2) p-39-41
5. Z. Priede, **V. Ķēniņa**, E. Miglāne, A. Millers,E. Pūcīte, M. Radziņa S-100 proteīns kā cerebrāla infarkta plašuma un iznākuma prognostisks marķieris// Collection of Scientific Papers.- RSU, Riga, 2011. **Accepted** for publication

Co-author of the Monograph:

1. **V. Ķēniņa,** O. Sabeļņikovs, A. Millers „Neirointensīvās terapijas principi akūta cerebrāla infarkta gadījumā" sadaļa grāmatai „Klīniskā anesteziolo ģija un intensīvā terapija" edited by I. Vanags and A. Sondore. Riga, National publishers, 2008, 1039.-1047.p.

Abstracts & Posters at the International Congresses:

1 L. Vainšteine, A. Millers, E. Smeltere, **V. Ķēniņa**, Z. Priede, I. Irbe. hyperhomocysteinemia as a risk factor of stroke.// 6th Baltic Congress of Neurology 2009, Abstracts, **Poster,** Vilnius, 2009:45

2. P. Auce, A. Millers, E. Smeltere ,L. Vainšteine, E. Smeltere, **V. Ķēniņa**, I.Irbe. C. Pneumoniae chronic infection as a risk factor of stroke//6th Baltic Congress of Neurology 2009, Abstracts**, Poster,** Vilnius, 2009:45

3. **V. Ķēniņa**, Z. Priede, A. Millers, G. Baltgaile. Association between increased carotid intima-media thickness and cytomegalovirus seropositivity in stroke patients. **Abstarct** for 15th Meeting of the European Society of Neurosonology and Cerebral Hemodynamics
4. **V. Ķēniņa**, P. Auce, Z. Priede, L. Vainšteine, I. Irbe, A. Millers. Hyperhomocysteinemia association with diabetes mellitus and coronary heart disease in the patients' group with stroke. **Poster presentation** XIX European Stroke Conference

Abstracts & Posters at the Latvian Conferences:

1. A.Millers, **V.Ķēniņa**, Z.Priede. Homocisteīns un hroniska infekcija kā ateroģenēzi stimulējošie faktori. // RSU Year 2008 Annual Scientific Conference Abstracts, **Poster,** Riga, 2008 : 132p.

2. Millers, E. Smeltere, **V. Ķēniņa**, L. Vainšteine, I. Irbe. C. Pneumoniae hroniska infekcija kā akūta cerebrāla infarkta riska faktors, tās korelācija ar insulta subtipu// RSU Year 2009 Annual Scientific Conference Abstracts, **Poster,** Riga, 2009

3. V. Ķēniņa, P. Auce, Z. Priede, L. Vainšteine, I. Irbe, A Millers. Lipīdu profīla analīze pacientiem ar akūto cerebrālo infarktu un pozitīvām IgG antivielām pret *C. pneumoniae*// RSU Year 2010 Annual Scientific Conference Abstracts, **Poster,** Riga, 2010

Nomenclature & Abbreviations

Abbreviation	Explanation in English
χ^2	Chi-square test value
HDL	High –density lipoprotein
ANOVA	Analysis of Variance
C.pneumoniae	*Chlamydophila pneumoniae*
CM	Diabetes mellitus
CI	Cerebral infarction
CMV	Cytomegalovirus
CT	Computerised Tomography
df	Degree of freedom
EHO CG	Echocardiography
ELISA	Enzyme ImmunoAssay
F	Fisher value
Hcy	Homocysteine
IgG	Immunoglobulin Class G
IgM	Immunoglobulin Class M
TH	Total cholesterol
CHD	Coronary heart disease
TI 95%	Confidence Interval of 95%
t	*t*-test value
TG	Triglycerides
TOAST	Trial of Org 10172 In Acute Stroke Treatment
LDL	Low-density lipoprotein

Acknowledgments

I render my thanks to Associated Professor **Andrejs Millers** and Docent **Elvīra Smeltere** for the management of my work.

Hearty thanks to Professor **Ināra Logina,** Professor **Juta Kroiča,** Docent **Angelika Krūmiņa** and Docent **Evija Miglāne** for the methodological recommendations.

Thanks for the laboratory part of my work and valuable advice to Head of the P. Stradiņa Clinical University Hospital Centre of Clinical Immunology Dr **Inta Jaunalksne** and Head of the P. Stradiņa Clinical University Hospital Central Laboratory Dr **Arta Balode**.

I thank my colleagues Dr **Pauls Auce**, Dr **Lana Vainšteine**, Dr **Inese Irbe**, Dr **Ildze Krieviņa** for the response, support and helpfulness in the routine work.

Thanks to the RSU Pro-rector for Science, Professor **Iveta Ozolanta** and Scientific Secretary **Ingrīda Kreile** for rendered advices during my doctoral studies.

Thanks to Professor **Uldis Teibe** for methodological recommendations on statistical analysis of the obtained results.