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INFECTIOUS ETIOLOGY IN ATHEROSCLEROSIS – FROM HYPOTHESIS TO CERTAINTY ?

Doina Azoicăi[1], Luminița Smaranda Iancu[2]

1. "Gr. T. Popa" University of Medicine and Pharmacy Iaşi; 2. Institute of Public Health Iaşi

Abstract. Experimental pathological studies and clinical surveys made in order to estimate the atherogenic role of some pathogens underlined the intervention of *Cytomegalovirus* (1978), *Chlamydia pneumoniae* (1988) and *Helicobacter pylori* (1994) in the generation and evolution of the specific vascular injuries. A causal relationship between these microorganisms and atherosclerosis though supported by clinical evidence is limited by the encountered difficulties both in the evaluation of infectious case history and microbiological diagnosis as well as the unknown prevalence of antibodies owner in general population. In Romania, few prevalence serological screenings for *C. pneumoniae*, *Cytomegalovirus* and *H. pylori* have been made due to the high costs and difficult methodologies. The validation of this causality-related hypothesis would allow the orientation of the cardiovascular diseases prevention programmes towards the avoidance or neutralization of the influence both the non-infectious and microbial factors.
Key-words: atherosclerosis, risk factor, infection, seroprevalence

Rezumat. Studiile experimentale, anatomo-patologice sau clinice efectuate pentru evaluarea rolului unor agenți patogeni în ateroscleroză au subliniat intervenția *Citomegalovirusului* (1978), *Chlamydia pneumoniae* (1988) şi *Helicobacter pylori* (1994) în producerea şi evoluția leziunilor specifice la nivel vascular. Argumentele clinice în favoarea relației de cauzalitate dintre aceste microorganisme şi ateroscleroză sunt prejudiciate de dificultățile întâmpinate în stabilirea antecedentelor infecțioase, a limitelor metodelor de diagnostic microbiologic şi de faptul că prevalența posesorilor de anticorpi în populația generală este incomplet cunoscută. În România screeningurile serologice de evaluare a prevalenței infecțiilor cu *C. pneumoniae*, *Citomegalovirus* şi *Helicobacter pylori* sunt puține datorită costurilor ridicate ale testelor imunologice şi dificultăților metodologice. Confirmarea acestei ipoteze cauzale ar permite orientarea programelor de prevenție în bolile cardiovasculare spre evitarea sau neutralizarea acțiunii, atât a factorilor neinfecțioşi, cît şi a celor microbieni.
Cuvinte-cheie: ateroscleroză, factor de risc, infecție, seroprevalență

The impossibility of explaining the evolution and the pandemic manifestation of the cardiovascular diseases (CVDs) only by mentioning the existence of the "traditional" risk factors (such as hypercholesterolemia, arterial hypertension, smoking or diabetus mellitus) made scientists to appraise this concept putting forward a hypothesis of the involvement of *Chlamydia pneumoniae*, *Cytomegalovirus* and *Helicobacter pylori* in this process (1).

The history of this concept reveals the fact that infectious hypothesis appeared a century ago; but subsequently, was replaced by the

multifactorial etiological model of the non-infectious diseases.

The role of the inflammation in atherosclerosis was underlined for the first time in 1823, when the calcifications found on arteries were compared to inflammatory process located elsewhere (2,3).

In 1859, Vircow explained in a paper published in "Cellular Pathology" that the infection preceded the laying down of the lipids by an irritative stage, inducing local inflammatory phenomena (3).

During the last years of the eighth decade, the experimental studies realized by Gilbert (1889), Crocq (1894), Boinet and Romary validated this new idea of the atherosclerosis etiopathogenesis (1).

Later, in 1908, some american clinicians and physiologists stipulated this hypothesis for the first time, claiming that "four great factors in the causation of atherosclerosis – the normal wear and tear of life, the acute infections, the intoxications (including smoking, diabetes mellitus, obesity), and those combinations of circumstances which keep the blood tension high" (1).

Subsequently, the "germs' theory" in the etiology of atherosclerosis was replaced by the multifactorial model of the non-infections diseases; the only scientists who rejected this replacement were Benson (1931), Jones and Rogers (1948) (4).

Most of the medicine professionals remained fairly skeptical as far as the role of the microorganisms in the etiology of atheroslerosis in not clear.

The studies made by Beneditt (1973) and especially the experimental proofs of Catherine Fabricant (1978) turned this controversial debate into a topical discussion again (2).

By contamination of birds with an avian herpesvirus the scientists underlined for the first time the involvement of the human herpesviruses (i.e. herpes simplex virus I, II and cytomegalovirus) in atherosclerosis.

Ten years later, in 1988, scientists realized that *Chlamydia pneumoniae* could be a new possible cause of atherosclerosis, according to arguments offered by serological evidence (5). In 1994, they found out that *Helicobacter pylori***,** an agent frequently related to gastroduodenal diseases, could also be involved in the appearance of atherosclerosis. At that time, they had reached no pertinent conclusions which might support this causality.

During the last decades, the evolution of the research, focused on athero-sclerosis and on the possibilities to establish a clinical or microbiological diagnosis, clarified different aspects related to the infection's negative effect on the vascular endothelium, the activation of the smooth muscular cells, the appearance of the foam cells or/and the monocytic and lymphocytic infiltration of the intima.

Besides atherogenesis, the infections generate endothelial disfunctions, inflammatory and thrombotic phenomena, which favour the instability of the atheroma and might produce acute cardiovascular troubles.

Scientists have also underlined the fact that the systemic effects of the infection represented by the atherothrombotic

phenomena were associated with the local autoimmune mechanisms through antigenic mimesis (table 1) (1,4).

A review of the studies published in this field indicates that this topic was very stimulating for the scientific debates (6).

The experimental research, the anatomical, pathological and immunological evaluation, as well as the assessements based on the population related or clinical epidemiology, support or invalidate the atherosclerosis infectious hypothesis (2).

The concept of causality existing between the pathogen and the non-infectious disease requires a solid argumentation based on an algorithm which was initially launched by Henle in 1840. Subsequently, Robert Koch developed this idea and substantiated the causality theory, mentioning three principles: the microorganisms must be present in all cases of the diseases according with the lesions sites; the microorganisms can be isolated in pure culture in vitro; inoculation of the pure culture into animals reproduce the disease and the same species will be discovered in the animal's body (7).

The appearance of the concept of multifactorial pathology made the modern epidemiology to reevaluate the causality criteria and take into consideration the notion of risk factor.

In 1965, Bradford ill suggested that anyone who wanted to check the validity of a hypothesis based on a causal association should check: the force and consistency of the association, its specific character, the temporal relationship, the biological gradient, the biological plausibility, the coherence, the effect of an intervention and the analogy characterizing this association (1).

These stages are pretty difficult to run through; at the same time, it is not easy to prove that *Chlamydia pneumoniae*, *Cytomegalovirus* and *Helicobacter pylori* do not act as "inocent bystanders" in the processes related to atherosclerosis. The force and the consistency of the association (i.e. the first of the above-mentioned criteria) is also very difficult to prove.

The increased prevalence in the general population both atherosclerosis and the infections due to the three pathogens renders difficult the task of assessing the importance of the infections as a risk factor of ischemic arterial injuries. At the same time, the difference existing between various geographical zones, regions and countries plays an important role both in the spreading of cardiovascular diseases and the risk of contamination with microorganisms (2, 7, 8).

Scientists have always appreciated that atherosclerosis was more frequent in the regions where population is characterized by a low social and economic level. Several countries from central part of Europe are characterized by a low rate of ischemic diseases, even they are highly industrialized, whereas in the ex-communist countries where the economic standards are lower, this rate has considerably increased. The analyses of the professionals in geographical epidemiology have pointed out the undeniable importance of the different environmental factors,

whose action is combined with the atherosclerosis individual risk (6). We should also accept the idea of a diverse microbial aggression, according to the country or the population it affects.

Scientists estimate that *Chlamydia pneumoniae* is the common pathogen of all respiratory infections by which an individual can suffer through his life; this pathogen is responsible for 10-15% of the total number of pneumonia, 5-10% of bronchitis cases and 5% of sinusitis or pharyngitis cases. As it is extremely contagious, the markers signalling out the infection caused by *Chlamydia* are present with 10-20% of the world population (9).

Frequent infections caused by *Cytomegalovirus* (CMV) could be re-activated successively because of the pathogen's latency-related features; this fact explains the increased prevalence of the people with anti-CMV antibodies. In some regions, this prevalence amounts to 50-80% or up to 100% (10,11).

The infection due to *H. pylori* is probably the most frequent bacterial infection existing worldwide. In the countries which are not very developed economically, its prevalence can amount to more than 80% and almost 90% with symptomatic cases can soar to 100% with ulcer patients. Serological studies have indicated an increased portage living in the patients entourage (12,13).

In Northern Europe, a prevalence of 5% has been found in children under 3 years; this value increased to 12-52% with adults aged between 35 and 85 years. As for the southern regions, the average prevalence amounts to 60%, varying from one country to another (14).

In Romania, few prevalence screening for *C. pneumoniae*, *Cytomegalovirus* or *H. pylori* have been made due to the high costs and difficult methodologies (10).

Methodological difficulties to assess the causality and technical problems of laboratory diagnosis made the information about these pathogens as risk factor in atherogenesis, very scarce. For *C. pneumoniae*, this relationship is more precisely quantified; the odd ratio (OR) amounts to a value close to 2 [1.83, 95% confidence interval – 1.17-2.85] (13,16). These values are pretty different in *H. pylori* case: 0.5 [95% CI:0.80-1.39] and 1.52 [95% CI:0.99-2.34] (15,16,17).

The past and recent reluctance to admit the possibility of the infectious theory of atherosclerosis is more dramatic given the extraordinary preventive potential that it could offer. A positive result will have little relevance regarding coronary disease primary prevention, particularly considering the lifespan exposure to many chronic infectious diseases. Furthermore, even antibiotics proved to be effective in the secondary prevention of coronary disease, the potential problems of resistances and long-term secondary effects of this therapy need to be carefully evaluated before they become routine medical practice.

Adoption of a broad epidemiologic view of atherosclerosis disease could again bring together the infectious and

the chronic disease paradigms. A synthesis of past and current research may assist in the search for new clues that could provide the answers to old questions (1,18,19).

Table 1. Summary of possible atherogenic effects of infections and available supporting evidence from experimental studies
(after F.J. Nieto, *Infections and atherosclerosis: New clues from an old hypothesis?,* Am J Epidemiol, 2000, 148, 10, 937-948)

Pathophysiologic process	Supporting evidence
Endothelial injury	Viral or bacterial antigen-antibody complexes induce or contribute to endothelial injury. Infected endothelial cells show increased expression of membrane receptors for binding immunocomplexes.
Adherence-migration of leukocytes	CMV* /HSV-1*-infected endothelium shows increased adherence for PMN adhesion associated with replication of CMV in endothelial cells is mediated by ELAM-1* and ICAM-1.*
Foam cell formation	HSV* infection in cultured macrophages and SMC* induces accumulation of cholesterol crystals and altered lipid metabolism, an effect that is prevented by that vaccination. CMV infection of human SMC increases class A scavenger receptor and modified LDL* uptake. Atherogenic effect of infections in animals is increased when combined with hypercholesterolemic diet.
SMC proliferation	Monoclonal character of cells in atherosclerotic plaque. CMV infection is corelated with *p53* accumulation in excessive proliferating SMCs associated with restenosis post-angioplasty. CMV blocks apoptosis of fibroblasts and endothelial cells, an effect that seems to be associated with abnormal cytoplasmic accumulation of *p53*.
Procoagulation	CMV infection produces a depletion of vWF* of cultured endothelial cells. HSV- and *Chlamydia*-infected endothelial cells have procoagulant properties that depend on plasma and tissue coagulation factors.
Inflammation	*Chlamydia pneumoniae* increases lymphocyte proliferative repsonse, particularly in subjects with CHD*. CMV and *C. pneumoniae* replicate in human endothelial cells, macrophages, and SMC, and trigger the cytokine pathway. CMV and *C. pneumoniae* infection in plaque tissue is associated with the degree of inflammatory changes. Infections are associated with acute phase reactants.

* CMV, cytomegalovirus; HSV-1, herpes simplex virus type 1; PMN, polymorpho-nuclear leukocytes; ELAM-1, endothelial leukocyte adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; HSV-herpes simplex virus; SMC-smooth muscle cells; LDL-low density lipoprotein; vWF-von

Willebrand factor; CHD-coronary heart disease.

REFERENCES

1. Nieto F.J.: *Infections and atherosclerosis: new clues from an old hypothesis;* Am. J. Epidemiol., 1998, 148, 10, 937-949
2. Capron L: *Virus et athérosclérose;* Rev. Prat., 1990, 40, 24, 2227-2233
3. Ross R.: *Atherosclerosis - an inflammatory disease;* N. Engl. J. Med.; 1999, 340, 2, 115-123
4. Viard J.P.: *Infection et autoimmunité;* Lettre Infect., 1992, VII, 14, 457-466
5. Ouchi K.: *Chlamydia pneumoniae and atherosclerosis;* Jpn. J. Infect. Dis.;1999, 52, 223-227
6. Mihalache D., Azoicăi D.: *Herpesvirusuri,* edit. Cautes, Iasi, 2000
7. Péchère J.C., Alfaudari S.: *Toutes les maladies sont-elle infectieuses?* Lettre Infect, 1998, 13, 8, 371-374
8. *** *New concepts in the role of intracellular pathogens in chronic diseases;* CMI, 1998, 4, S_4, 1-39
9. Hammerschlag M.R.: *Chlamydia pneumoniae and atherosclerosis: what we know and what we don't know;* Pediatr.Infect.Dis.J., 2000, 19, 73-76
10. Azoicăi D., Ivan A., Perjoiu M., Iancu L., Bosnea D.: *Cytomegalovirus infections and risk of atherosclerosis seroepidemiological reasons ;*Rev. Rom. Cardiol., 1999, IX ,1, 44-47
11. Iancu L., Azoicăi D., Apetrei C.: *The significations of Cytomegalovirus infections on children; possible new correlation between this infections and atherosclerosis;* J. Med. Prev., Iaşi, 1997, 5, 1, 49-55
12. Guiset M., Caton T., Rey P. et al.: *Infection à Helicobacter pylori dans les pays en developement;* Med. Trop., 1997, 57, 77-82
13. Wald N.J., Law M.R., Morris J.K. et al.: *Helicobacter pylori infection and mortality from ischaemic heart disease: negative rezult from a large prospective study*; BMJ, 1997, 315, 1199-1201
14. Broutet N: *Prevalence actuelle de l'infection à H. pylori et tendances evolutives en Europe*; Lettre Infect. 2000, XV, S_3, 28-29
15. Broutet N., Pellicano R.: *Helicobacter pylori et pathologies cardiovasculaires*; Lettre Infect., 1999, XIV, S_1, 3, 32-37
16. Danesh J., Peto R.: *Risk factors for coronary heart disease and infection with Helicobacter pylori: meta-anaysis of 18 studies;* BMJ, 1998, 316, 1130-1132
17. Danesh J., Youngman L., Clark S. et al.: *Helicobacter pylori infection and early onset myocardial infarction: case-control and sibling pairs study;* BMJ, 1999, 319, 1157-1162
18. Kaski J.C., Smith D.A.: *Chlamydia pneumoniae and coronary artery disease: antibiotic trials;* J. Europ. Cardiol., 1999, 57, 15-16
19. Strachan D.P., Carrington D., Mendall M.A. et al.: *Ch. pneumoniae serology to mortality and incidence of ischaemic heart disease over 13 years in the Caerphilly prospective heart disease study;* BMJ, 1999, 318, 1035-1040