

THE PATTERN OF ACUTE MYOCARDIAL INFARCTION IN PEOPLE WITH OPPORTUNISTIC INFECTIONS

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Summary: First comparative analysis for some hemocoagulation reactions, immune state of acute myocardial infarction has been done in two groups of patients – with mycoplasma infections and without one. Postinfarction complications in the groups were observed. Atherosclerotic plaques of the patients were also studied on a presence of mycoplasmas and a content of strontium and zinc. A tendency to a mild pattern of acute myocardial infarction was observed in patients with mycoplasma infections. The role of mycoplasmas in atherogenesis and acute myocardial infarction is discussed.

Key words: *Opportunistic infection; Mycoplasmas; Acute myocardial infarction*

Introduction

The causative agents of opportunistic infections are suspected to be some viruses, bacteria and simplest eukaryotes. These are organisms with one common feature – an ability to persist – but of quite different biology. These infections (in particular, mycoplasma infections) were shown to be essential factors in the development of atherosclerosis, plaque destabilizing and acute myocardial infarction (AMI) (13, 14). Infectious agents biasing hemostasis and the vascular system may promote atherogenesis and AMI according to the known immune and biochemical mechanisms of reactivity to infectious agents (18, 27, 30).

The unique biology of a concrete causative agent of the infection as well as individual immunoreactivity seems to determine the pattern of the disease. Mycoplasmas are able to modulate host immunoreactivity and to suppress signal systems responsible for expressive inflammatory processes (23). In this connection, the pattern of AMI in patients with mycoplasma infections is of special interest. We previously reported that a shift in some parameters for hemostasis and immune systems along with the content of some macro- and microelements follows the persistence of mycoplasmas in humans (7). This research was aimed at investigating the influence of mycoplasma infections on the pattern of AMI.

Materials and methods

We studied 76 patients with AMI. Persons were hospitalized for an acute myocardial infarction (with or without electrocardiographic evidence of ST-segment elevation).

A diagnosis of AMI was confirmed according to the clinical, instrumental and laboratory data, including investigations of the most important myocardial markers, – creatine kinase (CK) and its dimeric isoenzyme MB (CK-MB). Twelve-lead electrocardiographic investigations were repeatedly made in patients. The experimental group was divided into two subunits: mycoplasma-infected patients and non-infected ones (54 and 22 patients, respectively). Both subunits did not differ in the AMI localization, age, sex and applied therapy. In general, the experimental group consisted of 57 males (average age – 52.3 yrs) and 19 females (average age – 60.4 yrs). There were 40 control (healthy) patients (average age – 35 yrs). The differences between average ages, sex, smoke status, body-mass index and diabetes mellitus in the experimental and control groups were statistically insignificant. Patients were seen for a follow-up visit at 30 days.

Blood samples were taken to detect mycoplasma infections (in %) and carrier state (in %) of antibodies to some microorganisms associated with persistent infections (including the TORCH group) – cytomegalic virus, rubella, chlamydia, toxoplasma, – features of hemocoagulation system, levels of IgG, IgA, IgM and circulating immune complexes (CIC), concentrations of some macroelements and microelements (strontium, zinc, copper, iron). Frequencies of some AMI complications were monitored as well. The material from atheromatous plaques of coronary arteries of deceased patients was tested for the presence of mycoplasmas; levels of strontium and zinc were also detected.

A polymerase chain reaction (PCR) with use of rDNA oligonucleotide sequences as universal primers for revealing the human-specific mycoplasmas (29) and transmissible

micrography were applied (5). Oligonucleotides were synthesized by the scientific-production company "Litech" (Moscow, Russia). Taq-polymerase ("Litech", Moscow, Russia) was added to the reaction mix before beginning the reaction in concentrations recommended by the producer. The reaction regimen was controlled with the use of "Tercyc" amplifier ("DNA-technology", Moscow, Russia). Electroforetic separation of DNA fragments stained with bromide etidium was performed in 1-2 % agarose gel ("Dia-M", Moscow, Russia) and analysed using a gel-documentation system ("Litech", Moscow, Russia). The presence of antibodies to microorganisms in the blood serum of patients was detected by the enzyme-multiplied immunoassay, a method of separation using the specificity of antibody - antigen binding and quantitation using an enzyme reaction (10). Commercial kits were provided by SPF "Litech" (Russia) and "Vector-Best" (Russia). The contents of macroelements and microelements (iron, copper, zinc, strontium) in clinical material were detected by the atomic absorption spectroscopy (26) using the atomic absorption spectrometer "CA10 mP". Investigation of the serum IgG, IgA, IgM content was performed using radial immunodiffusion (16). The level of the total complement was determined in the reaction of its fixation according to 50 % hemolysis in the standard units CH-50 (29). CIC were revealed by their sedimentation in polyoxyethylene glycol-6000 (15). Calculation of thrombocytes and thrombocyte aggregability were estimated by the "Biola" laser aggregometer (11). Antithrombin III (AT-III) and the activated partial thromboplastin time (aPTT) (a test that measures clotting time in plasma (the liquid portion of blood); it focuses on a specific pathway in the blood clotting process; normal values for aPTT is 25 to 35 seconds) were detected according to Abilgaard (1) and Caen (6), respectively. Transmissive microscopy of atheroma samples was done according to Brown (5), with some modifications. Material was fixed with 2.5 % glutaric aldehyde on a phosphate mixture (pH 7.2), then treated with 1 % osmium oxide during 4 h with an additional 2.5 mM of saccharose. After dehydration in spirit of the ascending concentration, 100 % acetone and oxidopropylene, the material was perfused by epon-812. Thin slices obtained using ultramicrotome LKB-III (Sweden) were contrasted with the saturated water solution of uranyl acetate and then with the solution of zinc citrate.

The data were presented as mean \pm standard deviation. A *p* value of <0.05 was considered significant.

Results

In our study we found that almost half of AMI patients were infected with mycoplasmas (about 52 % vs. 5 % in the control group). Also, in the mycoplasma-infected patients, antibodies to cytomegalic virus, toxoplasma and rubella were detected more often (control group: antibodies to cytomegalic virus and rubella -12.5 % and 2.5 %, respectively,

$p<0.05$), while to chlamidia - more rarely (38.5 and 27.3 %; 26.9* and 13.6 %; 15.4* and 9.3 %, 3.8 and 4.6 %, respectively; $p<0.05$). In the mycoplasma-infected group pathologies in the urogenital system were more frequent in comparison with the non-infected cohort (31.8 % and 15 %, respectively, $p<0.05$).

We detected increased levels of IgG (7.08 ± 1.38 mg/mL; in mycoplasma-free AMI patients: 4.28 ± 1.30 mg/mL), IgM (1.28 ± 0.29 mg/mL; in mycoplasma-free AMI patients: 1.16 ± 0.27 mg/mL, $p<0.05$) and circulating immune complexes (0.049 ± 0.0038 optical units; in mycoplasma-free AMI patients: 0.036 ± 0.0058 optical units, $p<0.05$) as well as a decreased content of total complement (CH50-complement (%)) 38.4 ± 5.59 ; in mycoplasma-free AMI patients: 41.1 ± 8.92 , $p<0.05$; C3-complement (mg/mL) 0.950 ± 0.21 ; in mycoplasma-free AMI patients: 0.963 ± 0.20 , $p<0.05$) were found in the serum of the mycoplasma-infected patients. Thrombophilia was observed (in the mycoplasma-infected patients), as evidenced by the decreased activated partial thromboplastin time (aPTT (s)) 24.3 ± 3.00 ; in mycoplasma-free AMI patients: 41.8 ± 11.20 , $p<0.05$), the increased spontaneous (SPA (s)) 32.0 ± 3.48 ; in mycoplasma-free AMI patients: 20.0 ± 2.20) and induced platelet aggregability (IPA (s)) 24.0 ± 2.20 ; in mycoplasma-free AMI patients: 18.0 ± 0.40 , $p<0.05$) as well as by a tendency to an increased level of fibrinogen (5.02 ± 1.54 g/L; in mycoplasma-free AMI patients: 3.11 ± 0.25 , $p<0.05$).

Also, statistically significant increased levels of strontium and zinc were detected in the atheromas of the mycoplasma-infected patients (12.28 ± 2.53 μ g/mL; 44.6 ± 2.02 μ g/mL; $p<0.05$, respectively) in comparison with the non-infected ones (2.49 ± 0.29 μ g/mL; 24.7 ± 2.71 μ g/mL, $p<0.05$ respectively). In the blood serum of the mycoplasma-infected patients, an increased level of strontium (0.240 ± 0.019 μ g/mL; in mycoplasma-free AMI patients: 0.160 ± 0.014 μ g/mL, $p<0.05$) was observed while the level of zinc (0.557 ± 0.020 μ g/mL; in mycoplasma-free AMI patients: 0.630 ± 0.027 μ g/mL, $p<0.05$) decreased.

Discussion

The different rate of mycoplasma infections among healthy people and AMI patients reflects a high level of distribution of the persistent infection and its possible connection with AMI. As to AB, the immunologic parameter, unlike PCR, is not known to be perfect evidence of the presence of infections in patients at the moment of testing. Thus, we may conclude only that the prevalence of mycoplasma infections in patients with AMI was found.

Chronic mycoplasma infections are usually accompanied by an increase in vascular wall permeability, disturbance in microcirculation, and thrombosis (4). A shift in some parameters for hemostasis and immune system, in the content of some micro- and macroelements follows the persistence of mycoplasmas in humans (27). Indeed, an obvious tendency to thrombophilia was observed in the

mycoplasma-infected patients. These facts could not be explained by the anticoagulant therapy since it was equal in the both groups of patients. The tendency to the shifting hemocoagulation reactions and immune state, as well as the content of strontium in blood serum being similar in mycoplasma infected patients with or without AMI, proved to be similar, but quite differ in comparison with the mycoplasma free patients. The data may reflect peculiarities of the mycoplasma influence on the respective systems, and the cardiovascular problems.

The content of strontium in the blood serum and atherosclerotic plaque features in the mycoplasma-infected patients with AMI seems to be evidence of the involvement of the element into pathogenic reactions induced by these microbes, but the fine mechanisms of the process remain to be clarified. The increased levels of IgG, IgM and circulating immune complexes as well as the decreased content

of total complement found in the mycoplasma-infected patients may be evidence of the immunoreactivity towards infectious agents and of the destruction of its unspecific humoral mechanisms.

The cell wall is absent in mycoplasmas. To create mycoplasma membrane, they need to extract the necessary components from host membranes. Extracting of the component from the host cell membranes promotes destruction of the membrane structure, its antigenic profile and, as a consequence, an arising immune response to the infected cell. In this way, the presence of mycoplasmas in the atherosclerosis plaques (also found in our study) may be a risk factor for destabilizing the plaques. Mycoplasmas may trigger destabilization of the atheroma and the development of AMI according to the known mechanisms of reactivity of nonspecific signal systems towards persistent agents (4, 27) (Fig. 1). It is reasonable to propose active

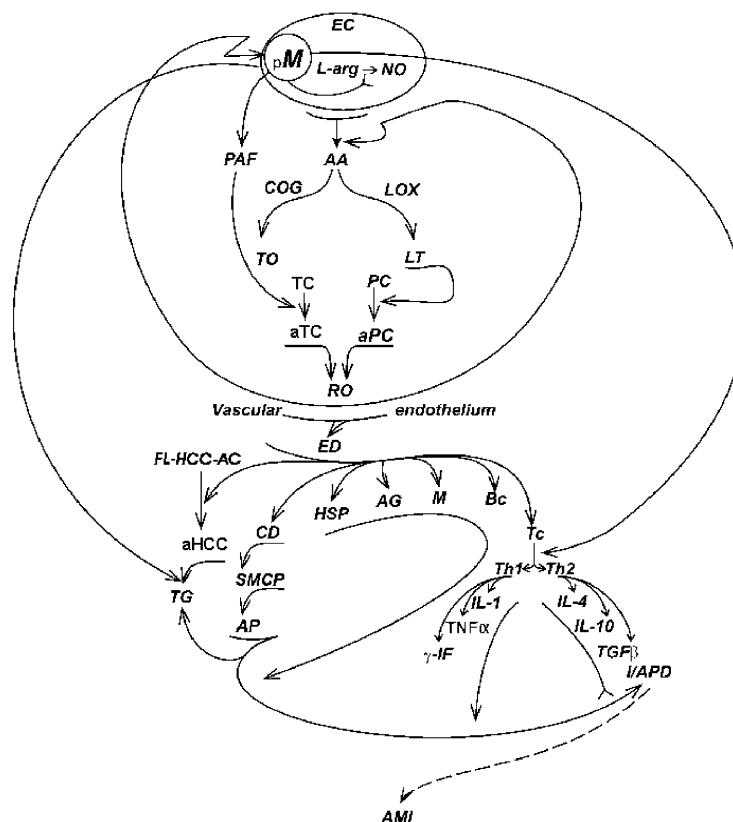


Fig. 1: Scheme of the possible mechanisms for the development of acute myocardial infarction during persistence of mycoplasmas in humans.

Abbreviations: EC - endotheliocyte; pM - persistent mycoplasmas; L-arg - L- arginine; NO - nitric oxide; PAF - platelet activating factor; AA - arachidonic acid; COG - cyclo-oxygenase; LOG - lipoxygenase; TO - thromboxan; LT - leukotriene; TC / aTC - thrombocytes / activated thrombocytes; PC / aPC - phagocytes / activated phagocytes; RO - reactive oxygen; ED - endothelial destruction; HCC / aHCC - hemocoagulation cascade/activation of hemocoagulation cascade; FL - fibrinolysis; AC - anticoagulation; AG - changed antigens of own cells; HSP - heat shock proteins; CD - collagen destruction; SMCP - proliferation of smooth muscle cells; AP - atheromatous plaque; TG - thrombogenesis; M - macrophages; Bc - B-cells; Tc - T-cells (CD4⁺) whose proliferation (Th1 and Th2 producing proinflammatory and anti-inflammatory cytokines) may be induced or inhibited by mycoplasmas; I/APD - inflammation/atheromatous plaque destabilization; AMI - acute myocardial infarction. Solid line corresponds to activation (→) or inhibition (-<) events.

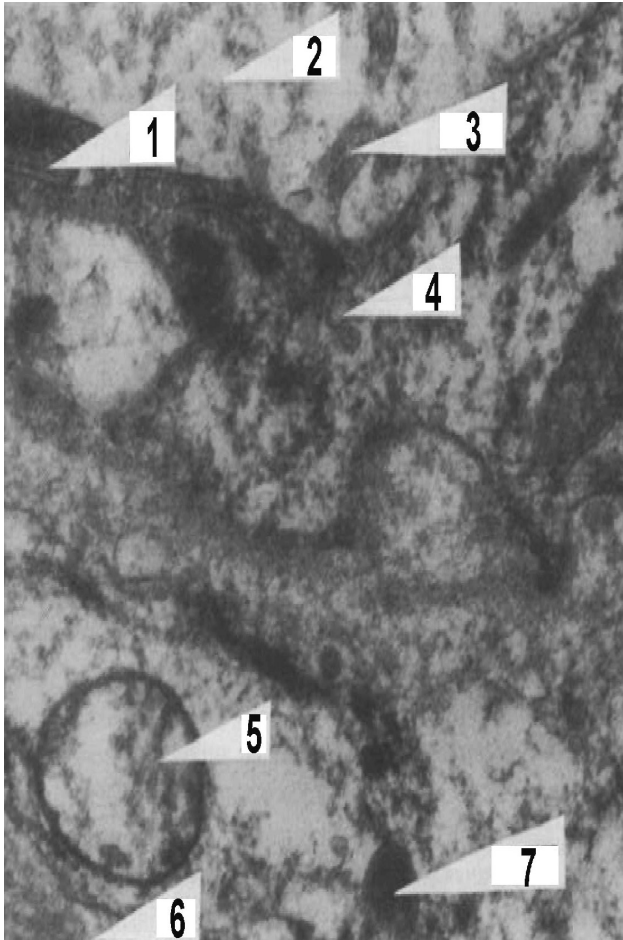


Fig. 2: Transmissive micrograph of atheromatous plaques in patients with persistence of mycoplasmas: 1. desmosome in the area of its contact with endotheliocyte; 2. lumen of the vessel; 3. microvilli; 4. pinocytosis vesicles; 5. mitochondrion; 6. elastin fibers; 7. mycoplasma ultramicroform (UMF).

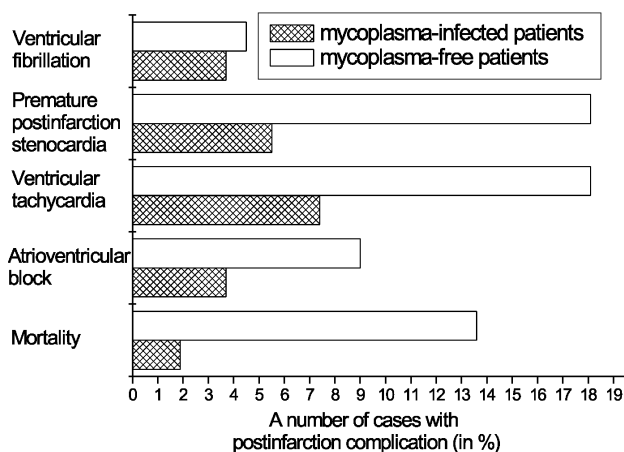


Fig. 3: Frequency of complications in AMI patients (in %).

participation of mycoplasmas in vulnerable plaques by their entering the subendothelial space and creating conditions that favor fat accumulation, dysfunction of the immunological and endothelial response, inflammation and increasing apoptosis, all of which are fundamental ingredients for plaque rupture (13, 14, 21). The bacteria induce depression in the immune response (27), mainly of T cells through possible mechanisms of apoptosis (22) with a reduction in the CD4 T cells, which may be a facilitating mechanism for other infections. Chronic oxidative stress is characteristic of mycoplasma infections (2). Epitopes of oxidized LDL, being antigenic, promote the production of inflammatory reactions in the artery wall, suggesting a possible autoimmune reaction (30, 37). In this connection, antioxidant agents may be reasonable for preventing complications of atherosclerosis (9, 24).

Ultramicroforms (UMF) of mycoplasmas are formed through transformation of the vegetative cell forms during unfavorable growth conditions. UMF are resistant to stress factors and able to revert (8). The dormant state of the bacterium is followed by a shift in its virulence. Our data on transmissive microscopy showed that in the plaque part of the cell population of mycoplasmas they were presented mainly as UMF (microbodies with sizes of 0.2–0.6 μm and their structures corresponding to a vegetative form of mycoplasmas cells as well as ultramicroforms of mycoplasmas (8) were detected among villi at the outer membrane of endotheliocytes and in cytoplasm of macrophages localized in the subendothelial stratum (Fig. 2). Unfavorable growth conditions (including antibiotic therapy) may result in the prevalence of dormant state bacteria (UMF) able to revert into active forms (vegetative cell forms) with disappearing stress factors. Mycoplasmas are able to modulate the reactivity of specific and nonspecific signal pathways of the host organism. That may probably cause the suppression of pathological processes connected with the activation of superoxide dismutase and NO-synthesizing systems producing forms of active oxygen (17, 23). The mycoplasma features provide the phenomenon revealed in our study – a tendency to „mild AMI pattern“ observed in the mycoplasma-infected patients (Fig. 3), but other factors connected with the peculiarities of interrelating the host-parasite signal systems, due to unique biology of the microbe and individual immunoreactivity, can not be excluded. In this connection, an „AMI pattern“ arising on the background of other (non-mycoplasma) persistent infections is of special interest and requires further investigation.

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