TEST Engineering & Management

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

Possibility the Co-Infective Participation of Helicobacter Pylori in Dermal Manifestations of Lyme Borreliosis

Bogdanka Andric 1, Milan Jovanovic 1, Milena Đurovic 2, Stefan Kecina 1
1 Clinic for Infectious Disease – Medical Faculty, University of Montenegro, Podgorica Montenegro
2 Clinic for Dermathology – Medical Faculty, University of Montenegro, Podgorica, Montenegro

Article Info
Volume 83
Page Number: 5703 - 5718
Publication Issue:
March - April 2020

Abstract:
At the end of 19th and the beginning of the 20th century, medical science progress contributed pre-optimistic insights that infectious diseases are going to become a past, which only the history and past heroes will remind us on (1). In contrary, in 21st century, infectious diseases enter into their renaissance (2, 3). It is confirmed by modern medical praxis and science. Numerous proves and hypothesis indicate the possibility of an action of different infectious agenses in mutual immunopathogenetic basis of many diseases (4-7). They are responsible for restitution of chronic infections, start up of immune, autoimmune, malignant mechanisms of infections / co-infections. They represent huge and complex diagnostic, differential-diagnostic, therapeutic problems and increasing danger towards human health and life (8-11).

One of the most significant characteristics of borrelia burgdorferi (bb), as a cause of Lyme borreliosis (LB), is the possibility of establishment co-infectious relations with numerous microorganisms from transmissive arthropod zoonosis complex (TAZ) in natural reservoirs, vectors, human infections, as well as capability to reactivate numerous, latent microorganisms within infected organism, based on its own immunological competitiveness (12, 13, 14).

Discovery of correlation between bb. and Helicobacter pylori (Hp), the main cause of chronic and malignant diseases gastrointestinal tract (GIT) and outside GIT, drew the attention of many researchers in the world (15). That encouraged us, in 2002-2017 period of time, to dedicate one segment of examination of LB towards detection of patients who are cases of etiologically simultaneous proven presence and eventually correlation of bb and Hp.

Between 2012 and 2017, on Infective diseases Clinics in Podgorica, 406 patients with etiologically confirmed LB diagnosis were examined, of which 268 patients were detected to have simultaneous presence of Hp, too.

Keywords: *borrelia, helicobacter, co-infections, dermatological manifestation.*

Article History
Article Received*: 24 July 2019*
Revised*: 12 September 2019*
Accepted*: 15 February 2020*
Publication*: 28 March 2020*

I. INTRODUCTION

Lyme borreliosis (LB) exists for thousands of years among mammalian population. In human pathology it is as old as human's relation with animals. The oldest proof is the result of DNA of bb in 5300 years old mail's mummy (Homo tyrolensis) in South Alps (16).

LB is multisystematic infective disease of acute, sub-acute and chronic flux and belongs to a group of transmisive arthropod zoonosis (TAZ). The cause of bb is gram-negative, mobile spirochete. It is transferred by a stitch of a tick from Ixodes species (17), although it is possible via other haematophagous vectors. Until now, 20 different types of bb have been discovered, of which at least 8 are pathogenic for humans. The presence of flagella allows it an active motion and infections of different tissues and organs (18-26). It is categorized in highly invasive microorganisms (11). The proteins of external membrane of bb, from OspA to OspF, represent 63% of total spirochete mass and have important role in its transmission into host's cells by immunological processes of infections. Bb uses different strategies to maintain infections (27-29). Avoiding immune response

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

of the host is enabled by genetic recombinations which lead to the change of superficial protein VlsE, avoiding phagocytosis based on complement with a help of molecules that bond to factor H, and intro-cellular position of the causer (30).

Metabolism of infected cells of the host is limited in a way that bb liberates energetic precursors into the host. Their genome codes protein transport as ABC transporter. Bb also codes enzymes and proteins by using the system of phototransferase. The characteristic genome of bb exists on linear chromosome of 910,725 bases, besides other 17 linear and circular plasmids which combine more than 533,000 bases. As a result of great amount of plasmids, and around 200 different proteins, the genetic organization of bb is specialized, and the causer itself is categorized into a group of very dangerous agenses that destroy immune system (31).

Bb infection is related to wide spectrum of clinical manifestations (1, 2, 32), which include dermal, rheumatologic, cardiovascular and other diseases of: spleen, muscles, eyes, liver. It could lead to hearing damage and psychological disorders; transplacental transmission, which is proven in human population, to fetopathy, embryopathy, and fetal death (33). One of the basic characteristics of LB is a presentation with relatively small number of specific, and wide spectrum of nonspecific clinical manifestations, and because of that it got epithet “big imitator”. On 20% of patients the disease goes asymptomatic (34). LB is the world’s most frequent disease from TAZ group. In Europe, on an annual basis, it is registered in around 85000 cases, while in USA that number is between 1500 to 2000 cases, with endemic character in 15 countries in the USA (3, 35). Modern researches show that LB is registered more frequently in co-infective forms than as independent nosological entity. By researches on 3000 cases of chronic LB in Europe, co-infections are proven on 50% of examinees, of which 30% were proven multietiological basis of co-infections (36).

First medical publications about some manifestations of LB as different diseases with an unclear etiology originate from Europe at the end of 19th and the beginning of 20th century. Modern history of LB begins in 1975 and epidemics with rheumatic form of LB in Lyme area (Connecticut, USA), by which it was named , and got its place in a group of “new” infective diseases. The initial researches of Professor Steere and his team of experts from the Yale University represented the beginning of large and intensive research work, which has provided LB a legitimacy of specific nosological entity from 1977. The common name LB was adopted in 1985 at Wien Congress and it obtained all clinical manifestations of this disease (14).

Morphological characteristics of Helicobacter pillory (Hp) were described over 100 years ago by W. Javorski from Krakow. Isolated and cultivated in 1982 (37), it was considered as usual stomach bacteria, and after thirty years of its discovery it drew an attention of modern medical practice and science, because of its immunopathogen potential and possibility of wide spectrum of involvement in dangerous human infections / co-infections, malignant and other diseases, in or out of digestive tract (38). Except in gastric mucosa, it has been found in dental deposits, wounds in oral cavity, saliva and feces, various organs: skin (chronic urticaria, Morgellons dermatosis), Cardiovascular system (ischemic heart disease), hematological (anemia), immunologic (immune thrombocytopenia), lungs, eye, hepatobiliary tract (39). In CNS it can cause low intensity inflammation, connected to many neurodegenerative diseases (40). Confirmations of these discoveries are extremely difficult, but significant , as they could change diagnostic and therapy approach towards numerous diseases. Today, hp is categorized among most significant and dangerous infective agenses (41). In 1994 WHO proclaimed it as one of the leading carcinogen causer (41, 42). For now, there are 35 different species of Helicobacter genus known and only some of them are pathogenic for humans (H.pyllory, H.suis, H. bozozeronii, H. salomonis) (43).

Epidemiology researches proved world population Hp infection , 20-50% in developed countries, and progress up to 80% in undeveloped and developing countries. Prevalence of Hp infection depends on numerous factors. High prevalence of Hp infection is present in children population, but also in population of adults. It is more common among persons who attended preschool or childcare institutions during the childhood. (44).

While bb is transferred by tick stitch, the ways of transferring hp infection aren’t entirely known. It can be transferred by direct contact, oro-oral or fecal-oral way. Also with contaminated instruments (endoscopes, dental and other) or drinking water. What is also proved is vertical transfer from mother to a baby, which all indicates direct infection transfer. Transfer of hp from an

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

animal to a human hasn't been proved. Hp was found in cats' gizzard, but its frequency on cats' owners in regard to general population hasn't been confirmed (45). Hp has also been found in pigs' gizzard and in flies' intestine, which can theoretically present potential way of infection.

Pathogenetic mechanisms of human hp infection are not entirely known. They are best examined in digestive tract (35, 46, 47). Most of colonized population remains asymptomatic for the whole life. Hp is highly adapted to gastric mucosa, where it colonizes mucosa and sub-mucosa. It is attached to gastric mucosa by numerous adhesive molecules: BabA, which is attached to Lewis antigen on the surface of mucous gizzard cells, and the resulting damages of gizzard mucosa develop by apoptosis induction, through bonding with MHC II molecules. The production of urease allows conversion of urea into ammoniac and chloride, which are directly cytotoxic. Ulcers usually appear in CagA (cytotoxic protein) and VacA (vacuolated toxin) gene expression. The expression of these genes is connected to increased induction IL-8 production, potential mediator of gastric inflammation. Genetic variations of a host also have a significant role in these processes, as polymorphism, which leads to high level of IL-1beta related to atrophic gastritis and gastric cancer. Except in gastrointestinal tract, hp is also a causer outside the gastrointestinal tract (48, 49).

Diagnostic of bb and hp infection is difficult. Bb is facultative intracellular agens which can be protected , not only from immune system but also from antibiotics influence. Borrelia can change its surface antigens as they become unrecognizable to immune system; it is more often registered in multietiological co-infections than as independent entity. During the bb infection, cellular immune response (CIR) precedes to measurable humoral immune response (HIR). For diagnosing a disease, there is a significant number of serological tests available, but the problem is late advent of specific antibodies and their specificity and sensibility. False positive results (on patients with different diseases) and false negative results (premature testing, anticipatory antibiotics treatment) are frequent. ELISA and Indirect Immune fluorescence (IIF) are mostly used. Western blot (WB) based on detection of antibodies of 39kDa protein is considered as most sensitive. PCR method is used for borreli DNA detection (50).

For diagnosing hp, the most common are the tests which detect specific antibodies; tests of urea stench, using special per oral dilution, decomposes hp. Products of this decomposing can be detected in patient's breath. Hp proteins can also be detected in excrement (51).

Numerous studies document the fail of standard antibiotic therapy on patients with LB. Previous uncontrolled examinations, as well as controlled placebo examinations, put attention on a fact that wanted results can't be achieved with long lasting antibiotics therapy. The extended antibiotics therapy can be useful and justified on patients with long-lasting symptoms of LB and in case of co-infection with other TAZ complex agenses which are transferred by ticks (52).

Speaking about Hp infections, antimicrobial therapy is also a problem and is rarely optimal. The efficiency of numerous often recommended treatment regimes is more and more endangered with development of antimicrobial resistance. By non-invasive molecular methods (in excrement isolates), it is possible to estimate sensibility of hp on claritromycine. General rule is that we should prescribe therapeutic regimes that have an effect from >90% to >95%. If we can't achieve the >90% rate of eradication, then we should use the most effective local regimes. After the treatment, you should always confirm the efficiency of the applied therapy. In most world's regions, the regime of treatment obtains four medications, including PPI + three antimicrobial items (claritromycine, metronidazole/ tinidazole and amoxicillin), or PPI + bismuth + tetracycline +metronidazole, which give best results. Previously used therapy (PPI, amoxicillin and claritromycine) should be avoided, as for drastic resistance increase (53).

II. MATERIAL AND METHODOLOGY

In 2012-2017 period of time, on Infectious diseases Clinic in Podgorica, 406 patients with confirmed diagnosis LB were treated, and on 268 patients helicobacter pylori was simultaneously detected. For Etiologic confirmation of diagnosis the following serological methods were used: Elisa, Western blood, IIF. For detection DNA of bb, Hp and other co-infectious agenses, we used PCR method, too. According to the need, additional methods were used: bone marrow biopsy, peripheral blood smear, X-ray diagnostics, hematological diagnostics, Ultrasound, CT, NMR, EMG, EKG, Echocardiography, and other methods.

Co-infectious forms were divided into 2 groups. In first group, multietiological co-infections bb+ Hp with

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

infective agenses from TAZ complex were registered on 212 patients. In second group, bb+Hp with immunologically conditioned co-infections a were registered on 194 patients.

III. RESULTS

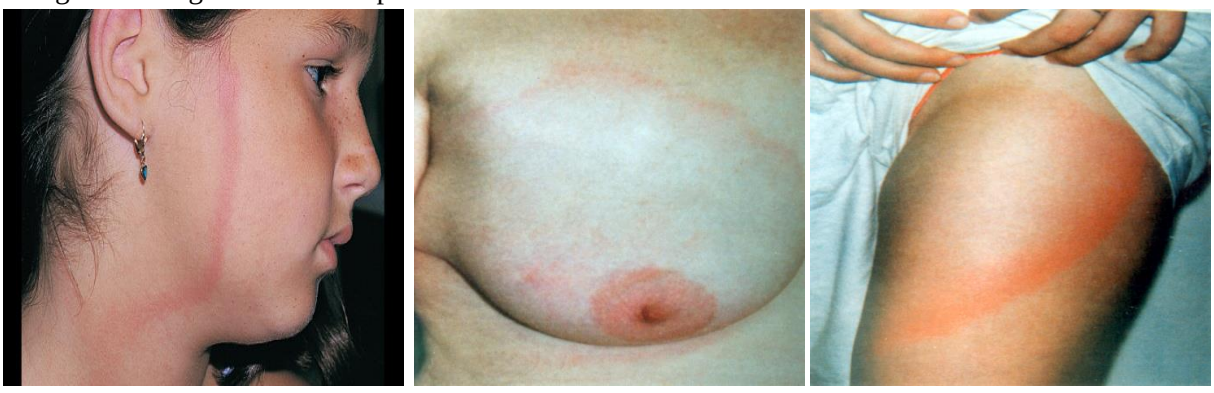
Clinically, LB is defined as multi-systematic disease with small number of specific and wide range of nonspecific clinical manifestations. Clinical course of the disease over short acute phases takes characteristic slow, chronic, cyclic infections very early, with numerous remissions and exacerbations. Clinical characteristics of dermal manifestations in examined patients with co-infective participation of bb and Hp in LB are shown in Table 1

Table 1 Frequency and characteristics of skin manifestations in examined patients with coinfective participation of bb and Hp in Lyme borreliosis No 268

SYMPTOMS	CASES	%
Skin manifestations		
Primo affect – Erythema migrans tipical in city (EM)	112	
Metastatic	8	
Multilocular	6	
Chronic, recurrent EM	24	
Generalized maculopapular exanthema / vasculitis / urticaria	103	
Localized dermal exanthema	2	
Acrodermatitis Chronica Atrophicans (ACA)	3	
Lymphocytoma cutis benigna solitaria (LCB)	4	
Sclerotic dermal changes	3	
Morphea	1	
Malignant lymphoma of the skin	2	

Because of difficult clinical diagnostics, WHO accepted Erythema Migrans (EM), the most frequent dermal manifestation of LB, on the spot of tick stitch for significant diagnostic criteria of early phase of the disease. It appears on 30-75% of diseased. In our series, EM of typical or untypical appearance has been registered on 150 patients. Other dermal changes were found on 118 patients, or , in total examined sample, skin changes were registered on 268 patients.

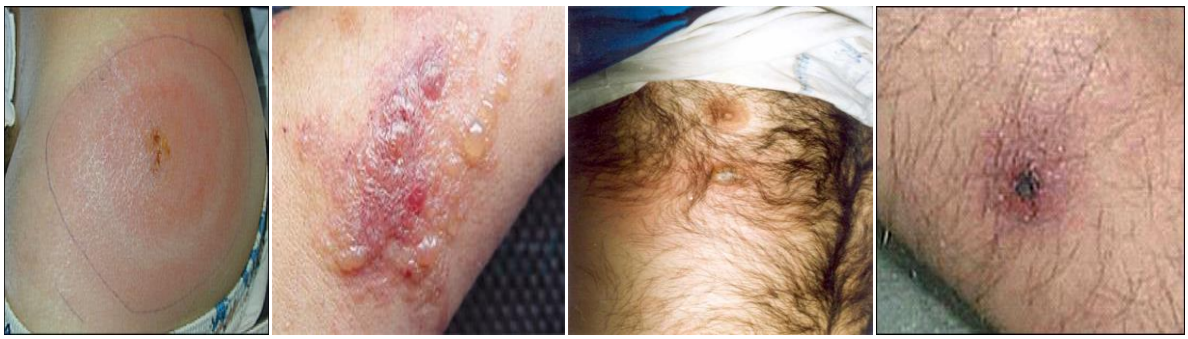
Erythema migrans (EM) is repercussion of local immunological reaction on bb presence in skin, which isn't able to stop dissemination of infective agens. The classical EM is different size, with central papule on the spot of tick stitch, around which pale zone is formed and it ends with bright red demarcation edge (Photo 1). Inside the pale zone, one or more demarcation lines, bullae or necrotic changes can appear (Photo 2). It can be hemorrhagic or metastatic (Photo 3).



A ***B*** ***C***

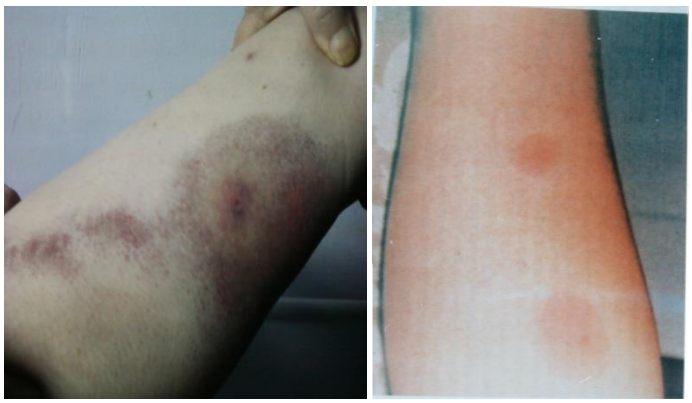
Photo 1: Erythema migrans with classic typical appearance (A, B, C).

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718



A B C D

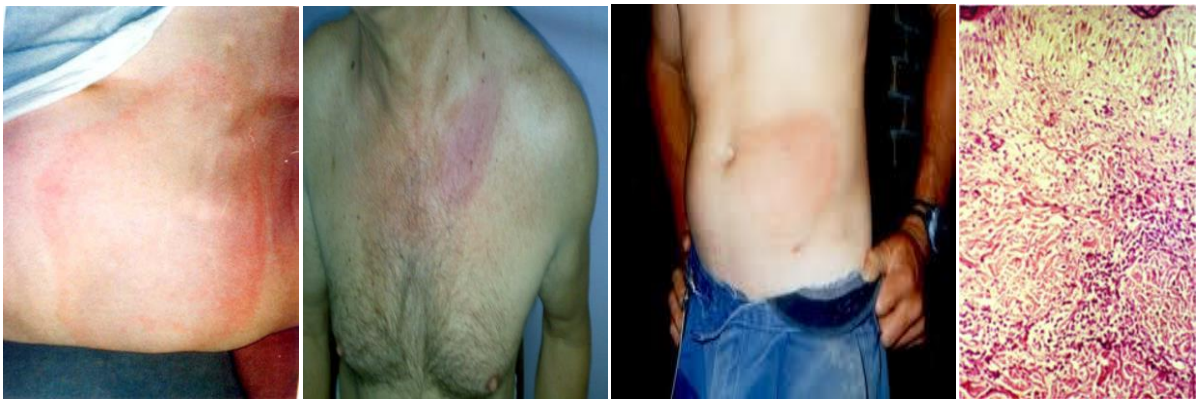
Photo 2: Erythema migrans: A-EM with more demarcation lines, B and C – bullous EM from the liquid of bullous change on patient. C- we isolated spirochete, D- necrotic changes of EM.



A B

Photo 3: EM can be hemorrhagic- A, or metastatic-B

In acute stage, EM is often followed by systemic disorders: high temperature, headache, muscle and joint spain, exhaustion, flu-like syndrome. EM belongs to an early stage of LB, but it can last for weeks or months and can relapse (Photo 4)



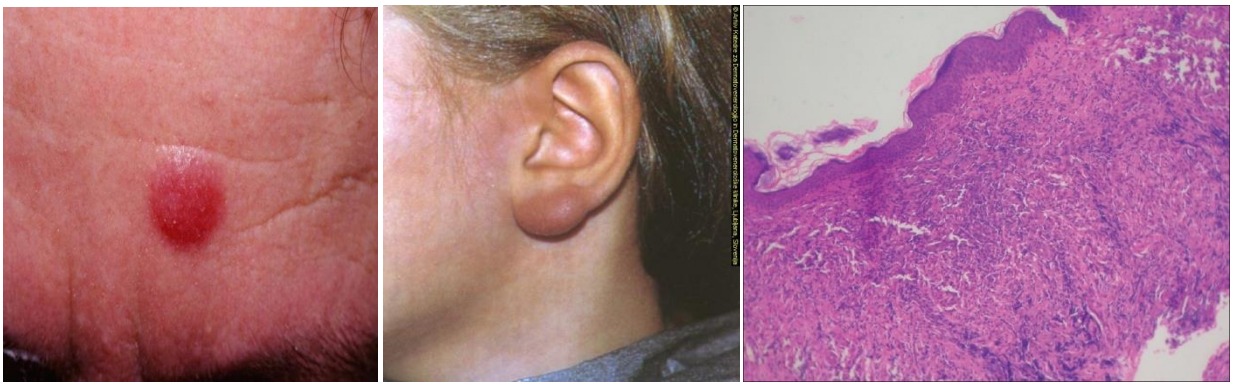
A B C D

Photo 4: Chronic and recurrent EM, registered on 24 examinees. During the diagnostic procedure, co-infections with rickets anaplasmas, coxiella burnetti, bartonella henselae had been proven. With serological methods, we diagnosed presence of hp on 25% of patients with chronic EM. Dense lymphocytic infiltrates were found in dermis. (D)

Lymphocytoma cutis benigna solitaria (LCB) in 4 of our patients, is a manifestation of an early disseminated stage

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

of LB. It belongs to very frequent common types of cute B-cell pseudolymphoma. It is usually registered on patients with co-infections in endemic areas of LB in Europe and in our country (Mediterranean area). In USA it isn't registered. It is considered to be a cause of b.afzelli. It represents big differentially –diagnostic problem from the aspect of clinical and histological recognition (Photo 5).



A B C

Photo 5: A and B – Typical appearance of LCB in LB, C- histological result: dense lymphocytic infiltrates in dermis with a presence of histiocytes. Lymphocytes don't show any atypia. Immunehistochemical analysis shows diffusive presence of CD 20 and variable presence of CD 3 of small lymphocytes, and CD 30 cells are negative.

In differential diagnosis it is sometimes difficult to distinguish it from persistent stitch reaction of arthropod, nodular scabies, sarcoidosis, borderline tuberculosis, Hansen's disease, granuloma annulare, primary cutaneous B-cell lymphoma, especially in non-endemic areas of LB. Among dermatological manifestations which are related to hp infections we can also find chronic idiopathic urticaria (HIU), or chronic urticaria (HU) in 5 ptients. It represents dermal inflammation which is defined by appearance of urtricas on skin, almost daily, during 6 weeks at least. It appears with releasing histamine and resulting increase of permeability of small blood vessels walls and "leakage" of the content, which leads to the rash (urtica) on skin and dermal swelling (angioedema). Infections are on the first place in responsibility for HU occurrence. Among them, hp infection takes significant place, especially on predisposed persons (Photo 6).

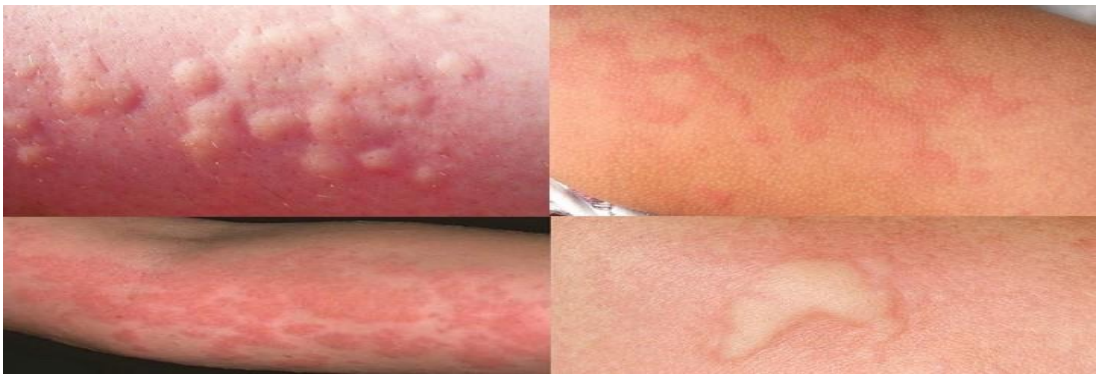


Photo 6: Chronic urticaria, as a result of hp infection and with different presentations. If it's a case of co-infection where, besides hp and bb, r.conorii also takes part, differential diagnosis can be extremely difficult, because of similarity of the rash, and more frequent progress of harder changes (angioedema).

Vasculitis – blood vessels walls inflammation. It's a consequence of unrecognizing own endothelial cells of blood vessels by immune system, and damage of these cells looking like it's about bacteria or viruses. Many viruses, rickets – groups of freckle fevers, typhus groups and other numerous infective agenses may cause these changes. Besides infections, some forms of malignancy,

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

certain immune system disorders and allergic reactions can also be a trigger to vasculitis appearance. Affinity of rickets agenses towards endothelial of blood vessels, has qualified vasculitis as their basic clinical manifestation, whether it's about self-contained or co-infectious participation with bb and/or other agenses. In our country (Mediterranean area), ricketsial agenses (freckle fevers groups- r.conorii and typhus groups- r.typhi) are the most frequent participants in co-infectious forms of LB, and vasculitis changes are categorized as most frequent manifestations, registered on 103 of our patients. (Photo 7). Involvement of hp into co-infections in our series can develop additional differentially-diagnostic problems on 32%.

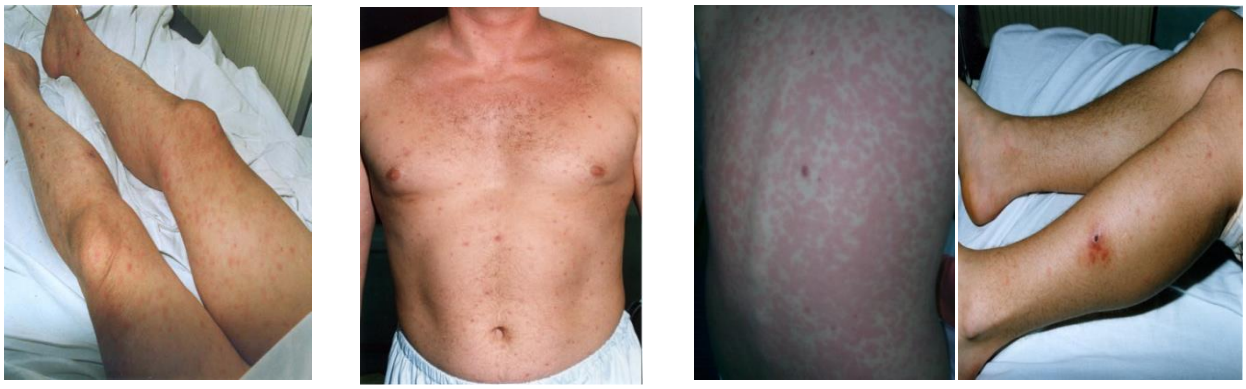
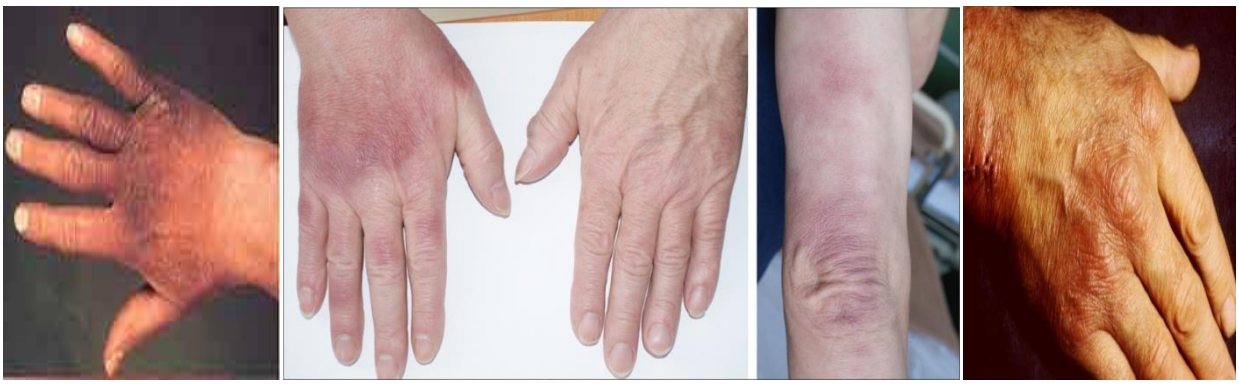


Photo 7: Vasculitis modifications as a consequence of affinity of rickety agenses towards blood vessels endothelial, whether it is about self-contained diseases or co-infections. Participation of Hp in co-infections can create differential diagnostic problems in clinical recognition of exanthema.

Acrodermatitis Chronica Atrophicans (ACA) in 3 our cases, present dermal manifestation of LB's late stage. It occurs 2 months - 2 years after the beginning of the disease. On 77% of the patients it envelops to acral parts of extremities, but also can occur on other places – on abdominal skin, knees, in case of scleroderma. It can also span the muscles. It represents the influence factor onto diabetes mellitus development. In these cases, bb has been proven as concomitant infection (Photo 9)



A ***B*** ***C*** ***D***

Photo 9: Acrodermatitis Chronica Atrophicans usually precedes chronic atrophic modifications occurrence. It signifies persistent inflammatory reaction of the skin, in late stage of LB (A,B,C,D).

Diagnostic difficulties of ACA refer to two stage. In inflammatory stage, this change is sometimes hard to differentiate from vascular diseases (erysipelas or bursitis / arthritis). In atrophic stage there are difficulties to differ it from lichen sclerosus et atropicus (LSA), morphea, or other chronic dermatosis. The evident thing is the initiation of difficult patients with dermal B-cell lymphoma with ACA. Sclerotic dermal lesions belong to late stage of LB. They usually have heterogeneous origin. High affinity of bb towards collagen and connective tissue could be a reason for pathological modifications in

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

sclerotic dermal lesions. (Photo 10)

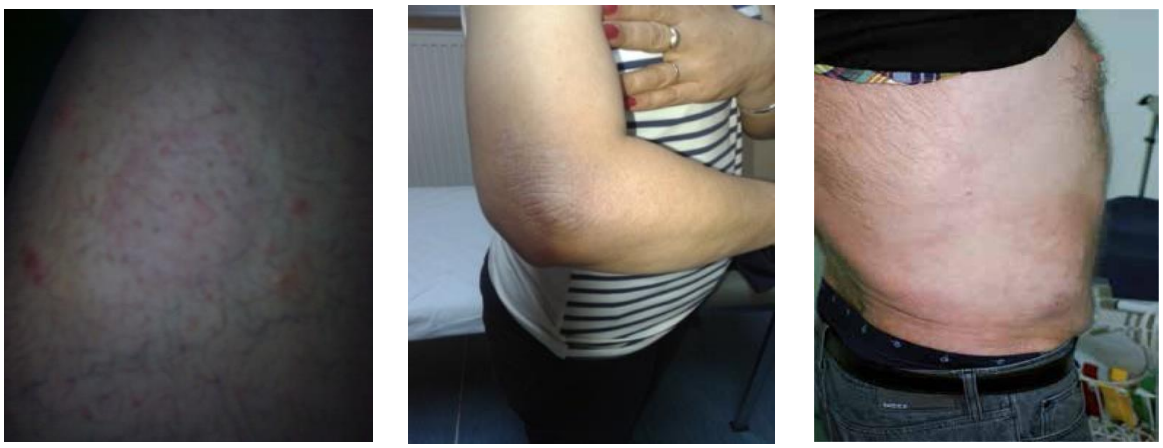
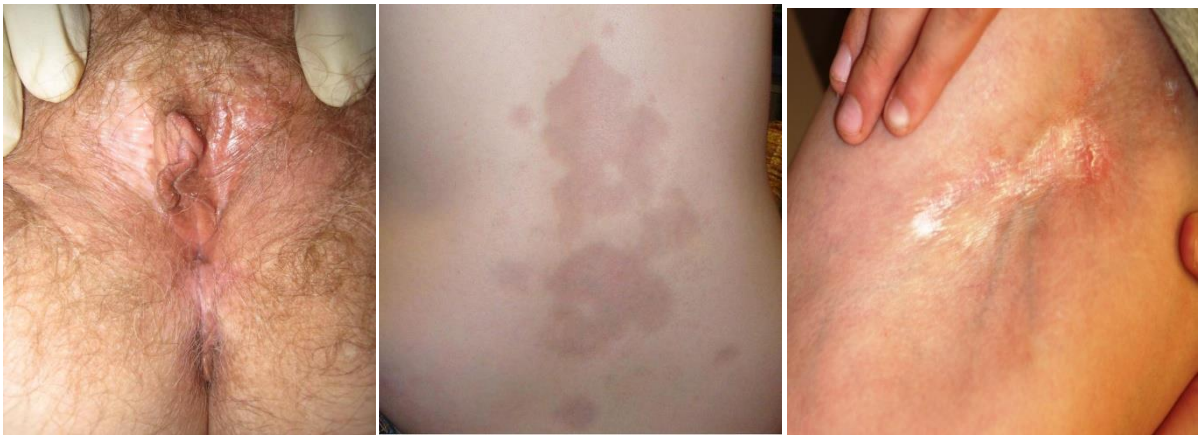


Photo 10: Sclerotic modifications on skin during LB are the result of primary affinity bb towards collagen and connective tissue in general. They occur in late stage of LB, mostly in those areas where b.afzelii is endemic, among untreated patients and patients with co-infection. In chronic dermal lesion Hp was positive on 57% of patients.

Sclerotic modifications, like Morphea, Lichen Sclerosus et Atrophicus (LSA), represent chronic inflammatory mucocutaneous disorders, with sclerotic and atrophic lesions of the skin and subcutaneous tissue. The reason for their appearance is collagen subsidence. The manifestations are different, from small dermal plaque to widespread diseases that cause functional and cosmetic deformations.

Association of morphea with LB is initially described in Europe. In Japan and USA, b.afzelii had been identified in ACA at first, and then in morphea too. It is also noticed that sclerotic modifications, which look like morphea, lichen sclerosus et atrophicans (LSA), often appear among older women in anogenital region – 85-98% of cases, and they also appear in extragenital regions. They coexist with ACA and show clinical histological similarity (Photo 12).



A B C

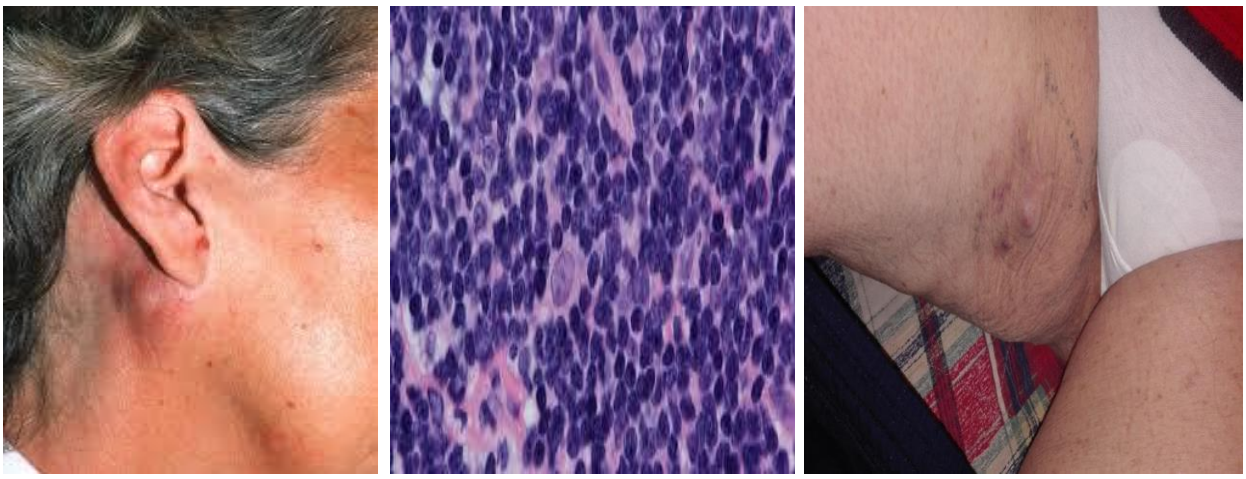
Photo 12: Sclerotic modifications on skin and subcutaneous tissue are the results of collagen over-subsidence. They were found in ACA, morphae, LSA. They often appear among elder women, in anogenital region – A , but also in other areas – B and C. They often coexist with ACA and show clinical and histological similarities.

Serological results of bb in these dermal manifestations are contradictory. In some reports, antibodies on bb are detected among 50% of patients with LSA, while bb DNA was detected in 33% of cases with morphae, and on 50% of patients with LSA. Borrelia is

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

visualized histological with polyclonal antibodies on around 69% morphae cases, and 63% cases on patients with LSA. Doubts about connection of bb infection with non-Hodkins lymphoma development (NHL), apropos primary dermal lymphoma of B-cells (PCBCL), had appeared prior to spirochete identification in 1982. But what was missing, were the evidences about connection of bb with PCBCL in studies outside Europe. When variations of clinical manifestations of LB with different forms of borrelia were considered, and whose geographic distributions differ, it became clear that infections with b.afzelii (which often aims skin) and b.garinii as causer of NHL development, dominate in Europe. The results are corroborated with serological evidences and demonstration of bb DNA in proportion of lymphatic dermal damages. Regression of the lymphoma after treating LB was recorded.

Regarding hp, there are uncontested evidences about its involvement in MAALT lymphoma appearance, within chronic antigen simulation in digestive tract. Later was discovered that numerous infective agenses are also connected to MAALT lymphoma development outside digestive tract, including bb in the skin (6), Chlamydia psitacci in respiratory system, and is also proven their co-infectious influence with hp (Photo 11).



A B C

Photo 11: Primary dermal non-Hodkins lymphoma of large cells appears only on the skin as benign node or may be expanded around when it requires therapy.

IV. DISCUSSION

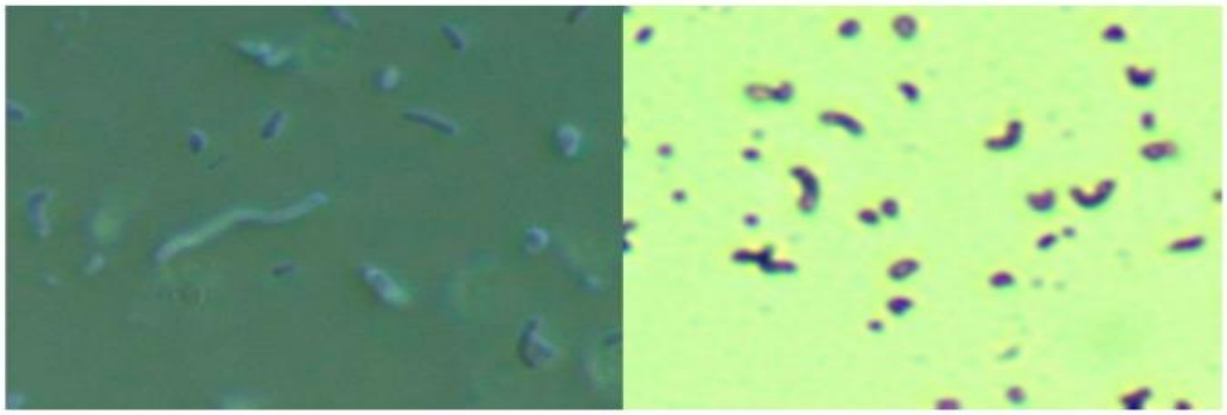
Since its discovery, LB represented big diagnostic and therapeutic and prognostic problem (1-3, 54). According to wide spectrum of clinical manifestations that were ascribed to it, with unclosed list of possible new clinical manifestations, it was suspected that those manifestations could be caused by one infectious agens (14). Differences in results within certain manifestations of LB in Europe, USA, Asia had also aroused certain doubts, until epidemiological researches drew attention to the fact that differences in geographic distribution of different kinds of bb could be one of the reasons for differences in clinical presentation, course and prognosis of disease (14, 55). In newer history of LB, many cognitions have expanded by intensive scientific research work, but they are still insufficient to explain numerous unknowns related to this disease. Since 2000 Burrascano has asserted the possibility of co-infectious involvement of numerous infective agenses of zoonotic and non-zoonotic series into co-infections with bb (56), which could explain extremely complex immunopathogenetical mechanisms of infections, wide spectrum of clinical manifestations, chronic course of LB, ability to cause immunological, autoimmune, malignant diseases (56-59). It is categorized as dangerous infectious agenses that destroy immune system (57, 60).

Although it isn't entirely known, bb has different strategies to avoid immunological system of the host and to maintain the infections (61- 65). These strategies may include antigenic variations of superficial spirochete membrane, attaching to complementary proteins, intracellular persistence, etc. At the beginning of examination of the patients with acute LB and present EM, high level of Th1 cytokine , interferon-gama (IFN-gama) were present, and after that levels of Th2 cytokine

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

interleukin-4 (IL-4) have also increased. Vice versa, patients with chronic LB infection, manifested as EM, ACA, had permanent high levels of IFN-gama (62), but without IL-4 level increase (36). The examination of patients with ACA confirmed presence of cytokine in EM, but what has also been detected are high levels of IL-4 (62) and very small or non expression IFN-gama among these patients. According to these results, the conclusion was that expression of IFN-gama is significantly important for control and solving bb infection. This result is relevant to Borrelia-lymphoma occurrence, as it is immunological response which dominates Th1 at the beginning of an infection, related to increased risk from other chronic inflammatory diseases which are connected to the risk of NHL occurrence. Also a dominant Th1 is spotted in immunological response on Hp-positive gastric MALT lymphoma (62).

Interest in Helicobacter pylori initiated studies that tried to connect this infective agens also with extragastric diseases and malignant tumors. Knowing immunopathogenetic potentials of hp and its evolutional adaptability first of all indicates its immunological potency. WHO has already categorized it in category of first order cancerous causers, primarily in digestive tract, but according to the results of certain serious examinations, numerous parameters also indicate the possibility of its action outside digestive tract. Every beginning is difficult, so is demystification of hp, but possibilities of modern medical science, based on practical experiences, represent precious help. In regard to co-infectious participation of bb and hp, it was proven that these two agenses have the ability to establish biofilm in vivo (63, 64) on which bases is set up the hypothesis that, by forming of biofilm they can be included in progress of numerous disorders and dermal modifications. Evidences showing that bb and hp have tendency for co-localization in focuses of epithelium tissues, so mixed (co-infections) bb and hp biofilms that contain beta amyloid and phosphorylated tau, may have a significant role in evolution of dermal manifestations.(Photo 12)



A ***B***

Photo 12: bb and Hp culture from the skin sample of Morgellons (MD) in modified Hp medium: A- Darkfield microscopyshows larger Borrelia spirochete, surrounded by smaller microorganisms. B- By crystal purple coloring, gram-negative microorganisms in comma shape, consistent with hp, are shown

Additional examinations with confocal microscopy discovered that distribution of bb and hp within common biofilm structures is specific for species. Hp cells are localized on the surface of bb cells, and bb cells are mainly on the inside. Specific distribution suggests that these bacteria may have specific synergistic functional roles. There were mixed biofilms and synergetic operation were also reported for other mixed bacterial infections. It was shown that they have high level of antibiotics resistance (64).

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

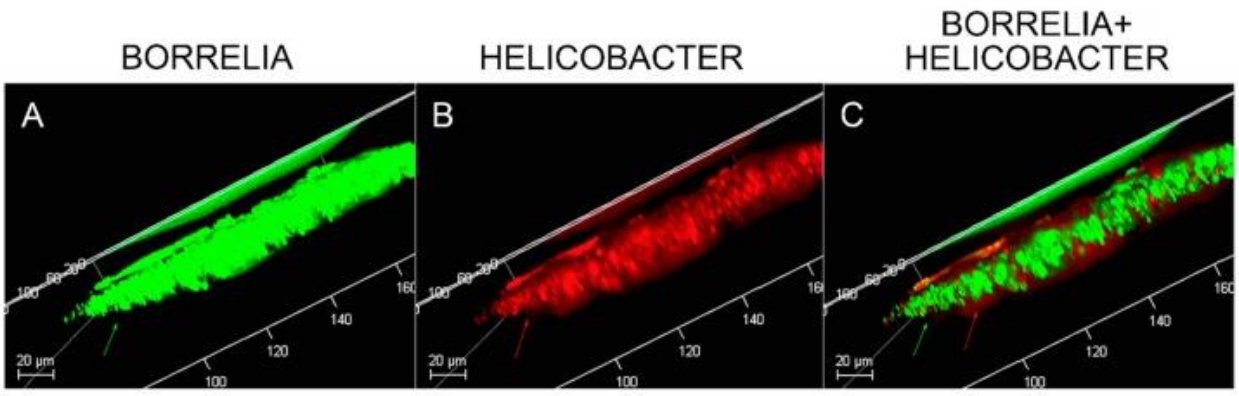


Photo 13: Confocal microscopy of MD skin parts. A- coloring only for bb (green); B- coloring only for Hp (red); C- coloring both for bb and Hp. Hp localization is registered mostly external, and bb localizationmostly on the inside. This photo was taken on 200x enlargement.

Mechanisms for establishment and maintain of co-infections still haven't been known enough. Regardless it is about epidemiologically conditioned (with numerous agenses from TAZ complex) or immunologically conditioned co-infections (mikoplasmas, EBV, etc) inside the infected organism. Results of co-infectious participation of bb and trichinellae spiralis, as well as bb with leishmania parasite, represented a puzzle for a long time, even besides laboratory results that confirmed their participation in diagnostic titre on heavy diseased from LB (14).

In study which we implemented during 1999-2002 in Clinic for Infectious diseases in Podgorica, we considered these results as rare "unusual co-infections", which we couldn't explain. We considered and accepted the possibility they occurred by reactivation of these agenses inside the organism under the influence of bb. It was later proven that many infective agenses of zoonotic order are able to exchange their functions with bb in natural hosts, vectors, during the infection (14) (rickettsial agents, bartonella henselae, and many others). It is further proven that bb may carry trihinele spiralis, and establishment of co-infective relations is already possible in common natural hosts for both of these agenses (wild boars, rodents). It has recently been proven that bb may carry leishmania parasite and in that manner it can provide co-infectious participation of this agens in infected organism. However, we have to consider possibilities of leishmaniae to establish latency in organism, after the infection. After bb arrives in infected organism, the reactivation and cooperation between these microorganisms can be accomplished inside the organism itself. According to our experiences, these co-infections usually affect CNS, which isn't able to defend against "modified immune response of the host". Co-infections that are based on immunological bases are the consequence of immunopathogenetic reactivation of numerous infective agenses, among which special places are occupied for herpes viruses and other infective agenses, contributing to a wide spectrum of clinical manifestations, co-infective forms of LB. In Czechoslovakia, via electronic microscope it was possible to visualize co-infectious participation of EBV and bb (14), which was known for a long time based on serological results; and the results were interpreted as false positive, according to immunological crossing. It is later affirmed that the first cells of immunological defense- phagocyte cells, are the spots where certain matters of immunogenic line between bb and EBV are met and exchanged. It is known that EBV stays in macrophages for a long time. By phagocytosis of bb, the possibility to establish co-infection with EBV is a reality and the problem for clinical manifestation of co-infections through chronic course, malignant diseases, and regarding therapy, increasing resistance. It is known for a long time that cutaneous pseudolymphoma is one of LB manifestations. It occurs due to motion of the spirochetes from skin surface into dermis. However, newer researches connect marginal-zone lymphoma (MZL) with Lyme disease. Spirochetes are evidenced inside malignant cells in MZL. Pathogenesis of this phenomenon doesn't have certain explanations for now. However, with analysis of some cutaneous modifications that are frequent manifestations of LB, some interesting results are reached. It was found out that EM contained increased concentration Il-4 in its structure, which indicated activation Th-2, the response which is proved to

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

be necessary for the disease not to transfer into chronicity. Unlike it, moderately high values of IFN-gama are evidenced with analysis of ACA, which indicates persistence of Th-1 response (62). Deficit of cellular immune response which conditions lower concentration of IFN-gama, doesn't lead to activation of humoral immune response and that brings to long-lasting maintain of lower levels of IFN-gama, namely to chronic immunological stimulation, which leads to lymphoproliferation and appearance of MZL.

V. CONCLUSION

Interest in Hp and its participation in extragastrointestinal diseases, started when its immunogenic potentials and possibility of participation in coinfections with other infective agenses were perceived. Attempts to incriminate it as a cause of numerous and unexplainable extragastric disorders, can't be unreservedly accepted, but no beginning is easy. We categorized our discoveries about co-infections bb with parasites Trichinella spiralis and Leishmania infantum, in lack of evidences, into "strange co-infections" according to hypothesis they can reactivate in infected organism under the influence of bb. The exact evidences about their co-infectious influence have been introduced later. In this study, we proved presence of bb and hp among some patients, but without possibility of exact evidences that they are in co-infectious cooperation. Among examined patients, we didn't have any evidences new manifestations of dermal modifications, or more difficult than usual clinical results. Chronic evolution of dermal changes was registered among small number of our patients who haven't been treated. What could be perceived, theoretically, partly and practically, was the fact that these two different microorganisms have numerous immunological and other very similar characteristics, so we can't repel their potentially co-infectious action in and out of digestive tract. There are exact evidences that they were found in dermal changes of so-called Morgellon's disease, and with a help of confocal microscopy their togetherness has also been proved.

VI. REFERENCES

1. Ivanko Boic (2000): European Borreliosis and other diseases that are transmitted through tick bite Monographic publication, Medical faculty Belgrade.
2. Stanek G.,Strle F (2003) : Lyme borreliosis, Lancet. ;362:1639 1647. [PubMed]
3. Steere AC.(2001): Lyme disease. N Engl J Med.;345:115–125. [PubMed]
4. Joppe W.R.Hovius, Alje P.van Dam, Erol Fikring (2017) : Tick –host –pathogen interaction in Lyme borreliosis. Trend in Parasitology, Vol.23, Issue 9, September 20017, pp 434 – 438.
5. Mayne P,J.: Clinical determinations of Lyme borreliosis, Babesiosis, Bartonellosis, Anaplasmosis and Ehrlichiosis in Australian cohort. Int.J.Gen.Med., 2014 : 8 : 15 – 26, doi:10.2147/IJ6M.S 75825. (Pub Med)
6. Raileanu C et al.:(2017) : Borrelia diversity and Co-infection with other Tick Borne Pathogens in Tick. Front.Cell.Infect.Microbiol.
7. Gray J.(1990):Tick borne disease interaction, Abstract, proceeding of the I-th Congress of the European Society for Emerging infect. Dis., Budapest.
8. Weis J, (2010): Pathogenesis of Borrelia burgdorferi infection and disease. Universiti of Utah Scool of Medicine.
9. Mayne P.,English J.S., Kilbane E.J., Burke J.L.Middelveen MJ, Stricker RB (2013) : Morgelons : A novel Dermathological perspective as the multisystem infective disease borreliosis. F1000 Research :2 :118, doi: 10.12688/f1000 research. 2-118 vl. (Pub Med)
10. Margos G.,Gatewood AG.,Aenensen DM,.Haninkova K.,Terekhova D.,Vollmer SA.,Cornet M.,Piesman J.,Donaghy M.,Bormane A, et al. (2008):MLST of housecreting genes captures geographic population structure and suggest a European origin of Borrelia burgdorferi. Proc.Natl.Acad.Sci.USA : 105:8730-8735, doi: 10.1073/pnas.0800323105 (Pub Med.)
11. WagnerB.(2015) : BB 0744 Afect Tissue Tropism and spetial Distribution of borrelia burgdorferi. Infection and Immunity Journal.
12. Smedby KE.,Hjalgrim H.,Askling J.,et al: (2006) : Autoimmune and chronic inflammatory discorders and risk of non-Hodgkins lymphoma by subtype. J. Natl. Cancer Inst.: 98 : 51 – 60. Pub Med.
13. Munksgaard L.,Obitz ER.,Goodlad JR. et al.(2004) :Demonstration of B. burgdorferi-DNA in two cases of nodal lymphoma. Leuk.Lymphoma, 45 : 1712 – 1723 (Pub Med)

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

14. B. Andric (2002) : Clinical Characteristics and Diagnosis of Associated Transmisible tick born zoonoses. Doctoral Disertation, Medical Faculty Universty in Novi Sad, Serbia
15. Bandoski C.,(2014) : Evidence for te presence of human pathogens Borrelia and Helicobacter in Morgelon sDisease. Austin, TX, USA, 29-30 March (accessed on November 2018). Available online: http://www.theceht.org./julie-levis.html
16. Hall T.(2011): Identification of DNA of Borrelia burgdorferi in homo Thyrolicus. A multidisciplinary community creating experiences that connect people to place (SEGD) WCBI images for Hall 2012.
17. BerglundJ.,Eitrem R.,Ornstein K. et al.:(1994) : An epidemiologic study of Lyme disease in southern Sweden. N.Engl.J.Med.: 333 : 1319 – 1327 (Pub Med).
18. Munksagard L.,Frish M.,Melbye M.,Hjalgrim H.(2000):Incidence patterns on lyme disease and cutaneous B-cell non-Hodgkins lymphoma in the United States. Dermathology :201:351-352 (Pub Med).
19. Li C.,Inagaki, Kuo TT.,et al.(2003):Primary cutaneous marginal zone B-cell lymphoma : a molecular and clinicopathologic study of 24 asian cases. Am.J.Surg.Pathol. : 27 : 1061 -1069 (Pub Med)
20. Andres, C., Ziai, M., Bruckbauer, H., Ring, J., and Hofmann, H.(2009) Acrodermatitis chronica atrophicans in two children. Int J Dermatol.; 49: 180–183
21. Pinto DS., (2002) : Cardiac manifestation of Lyme disease. Medical Clinic of North America, Volume 86, Issue 2, Page 285 -296.
22. Hulinska D. et al. (2007) : Persistence of Borrelia garinii and Bottsia speciesrrelia afzelii in patients with lyme arthritis. Zeintra fur Bacyeriologiaeblatt
23. Hildenbrand P. et al.(2009) : Lyme neuroborreliosis : Manifestations of a Rapidly Emerging Zoonosis. American Journal of Neuroradiology. 1079 – 1087.
24. Tijsse – Klasen E., Hein Spong, Nenad Spandak (2013) : Co-infection of borrelia burgdorferi sensu lato and Ricketsia species in tick and in an erythema migrans patients,Parasites and Vectors 6, Article Number 347, (2013)
25. Raileany C.,et al. (2017) : Borrelia diversity and Co-infection with Other Tick Borne Pathogens in Tick.Front. Cell. Infect. Microbiol., 14 February 2017 | https://doi.org/10.3389/fcimb.2017.00036
26. Lewis J.,Lloyd VK.,Robichaud G.A.,(2017) : Evidence of bacterial co-infection in Morgellons and Lyme patients. Procedings of the 11-th Annual Medical – Scientific Conference on Morgellons Disease : Austin, TX, USA, 14 – 15 April (acceseed on 18 Avgust 2018) Aviable online : http://www.thecehf.org/julie-lewis.html.
27. Wang IN.,Dykhuizen DE.,Qui W.,Dunn JJ.,Bosler EM., Luft BJ.(1999),: Genetic Diversity of OspC ai a local population of Borrelia burgdorferi sensu strict. Genetics : 151: 15 – 30, ((PMC free article), (Pub Med)
28. Margos G.,Gatewood AG.,Aanensen DM., Haninkova K.,Terekhova D.,Vollmer SA.,Cornet M.,Piesman J.,Donaghy M., Bormane A., et al. (2008): MLST of housekeeping genes captures geographic population structure and suggest a European origin of Borrelia burgdorferi. Proc. Natl.Acad. Sci USA : 105 : 8730 – 8735, doi:10.1073/pnas.0800323105. (PMC free article), (Pub Med).
29. Zuckert, W. Kerentseva, T.Lawson, C.Barbour A., (2001) : Structural Conservation of Neurotropism-associated VspA within the Wariable Borrelia Vsp-OspC Lipoprotein Famyli. Molecular microbiology 59 (5), 1473-1484
30. Abhishek Chandra, Norman Latov, Gery P. Wormser, Adriana R. Margues, Armiih Aladim (2011) : Epitope Mapping of Antibodies to VlsE protein of borrelia burgdorferi in post Lyme Disease Syndrome Clin. Immunology, Okt. 141 (1): 103-121, doi: 10.1016/j.clin 2011.06.005.
31. Cutler SJ.,Ruzic-Sabljic E.,Podkonjak A.(2017) : Emerging borrelia expanding beyond Lyme borreliosis. Mol.Cell Probes : 31 : 22 – 27, doi:10.1016/j.mep.2016.08.003 (Pub Med)
32. CI Wiliams, B.Strabino, A.Weinstein, P.Spierling, F.Medici (1995) : Maternal Lyme disease and congenital malformation : a cord blood serosurvey in endemic and control areas. Pediatr. Perinat. Epidemiol, 9 (1995), pp 320 – 330. Cross Ref, Viev Record in Scopus, Gougle Scholar.
33. Bogdanka Andric (2008): Vectors Transsmisible

Zoonoses. Monographic publication, p. 1 – 249, Library of Biomedical Sciences, Edition Medicine, University of Montenegro, ISBN978-86-7664-056-0, CIP-616.993 (075.8)
34. Paul S.Mead, MD,MPH: (2016) : Epidemiology of Lyme disease Infections Disease. Clinics, Vol.29, Issue 2,p:187-210.
35. Tobias Straub,Marma A, Freudenberg Ulrice Schleicher, Cristian Bogdan, Georg Gasteriger and Hanspoter Pircher (2018): Bacterial coinfection restrains antiviral CD8 T-cell response via LPS induced inhibitory NK cells. Nature Communications 9, Article number 4117 (2008).
36. Konturek JW (2003) : Discovery by Jaworski of Helicobacter pylori and it s pathogenic role in peptic ulcer, gastritis and gastric cancer. J.Physici Pharmacol, 2003, Dec 54, Suppl.3, p 23 – 41.
37. In 1983 Australian doctors J.Robin Warren and Barry Marchall Isolated Helicobacter pylori. (Delayed Grafication: WHO it took Everibody so long to acknowledge that bacteria cause ulcers..
38. Johannes G. Kusters, Arnoud HM van Vliet and Ernest J.Kuipers (2006) : Pathogenesis of Helicobacter pylori infection. Clin. Microbiol, Rev 2006, Jul, 19 (3), 449-390, doi:10.1128/CMR 00054 – 05.
39. Frank Wong, Enn Rajner-Hartley, Michael F. Bume(2014) : Extraintestinal Manifestations of Helicobacter pylori : A concise Review. World J.of Gastroenterol. (WJG), 2014, Sept 14, doi: 10.3748/wjg.v.20 I 34.1195.
40. Kira J.I.,Isobe N. (2019) : Helicobacter pylori infection and demyelinating disease of the Central Nervous System. J. Neuroimmunol., April 15, 329 : 14 – 19, doi: 10.1016.j.neuroim.
41. Williams MP.,Pounder RE. : (1999) : Helicobacter pylori: from the benign to the malignant. Am. J. Gastroenterol., 1999, Nov 94 (11 Suppl) : p 11 – 16, doi: 10.1016.s0002-927 (99)00657-7.
42. Godwin CS.,Amstrong JA.: (1990) : Microbiological aspects of Helicobacter pylori (Campylobacter pylori). European Journal of Clinical Microbiol., 1990 : 1 – 13.
43. Johannes, G.Kusters, Amoud HM van Vliet and Ernest J.Kuipers (2006) : Pathogenesis of Helicobactter pylori infection. Clin.Microbiol. Rev.: 19 (3) : 449 – 490. Doi:10.1128/CMR00054-05. (Pub Med)
44. Allaker RP.,KA.Yung.,JM.Hardie, P.Domizio, N.J. Meadows (2002) : Prevalence of Helicobacter pylori at oral and gastrointestinal sites in children evidence for possible oral to oral transmission. J.Med. Microbiol.. 51 : 312 – 317 (Pub Med)
45. Bazile,D.S., Wangler, T.Weitzenegger, W/Steinbber, J.Volz, M/ Przyhkerski, KP. Schafer, B Sach (2002) , Biology and Helicobacter pylori and a felis. J. Bacteriol: 180 : 317 -329 (Pub Med)
46. Ando TRM Pek, D.Pride, Smilevine T. Takata, JC Lee, K.Kusugami, A.Vander Ende, EJ Kuipers, MJ Blaser (2000) : Polimorphism of Helicobacter pylori HPO 638 reglect geographic origin and correlate vith cagA status. J.Clin.Microbiol. 40 : 239 – 246 (Pub Med)
47. Blaser MJ., and LC Atherton (2004): Helicobacter pylori persistence and disease. J.Clin.Investig.: 113 : 321 – 335. (Pub Med)
48. Gasberrini A., Carlani, G. Gasbanini, SA Chiholm (2004): Extrn,MD, Veronicaagastric diseases – other Helicobacters. Helicobacter 9 (Suppl 1), 57 -66 (Pub Med)
49. Leontiades G.I, VK Sharma, CW Holdew (1999): Non gastrointestinal assotiation of Helicobacter pylori infection with is the evidence? Arch. Intern, Med, : 189 : 925-940 (Pub Med).
50. Lohr B.,Fingerle V.,Norris DE.,Hunfeld KP.:(2018) : Laboratory diagnosis of Lyme borreliosis : Current state of the art and future perspectives. Crit. Rev.Clin.Lab.Sci : 55 (4) : 219 - 214
51. Skrebinska S.,Megcaud F.,Bessede E. (2018) : Diagnosis of Helicobacter pylori infection. Helicobacter2018, Sept 23, (Suppl.1 : e 12515, PMID 30203584, doi: 10.1111/hel125.5 (Indexed for MEDLINE).
52. Pavlovic M.,Dulovic O. (2002): A therapeutic approach to various forms of Lyme disease. Acta Infectol Yugoslav : 7: 55-59.
53. Dino Vaira,MD,Angelo Zullo MD, Nimish Vakil, MD, Luigi Gatta MD, Chiara Ricci MD, Federico Perna, PhD, Cesare Hassan MD, Veronica Bernabucci MD, Andrea Tampieri MD, Sergio Morini MD. (2007) - Sequential Therapy versus

March - April 2020
ISSN: 0193-4120 Page No. 5703 - 5718

Standard Triple – Drug Therapy for Helicobacter pylori eradication - A Randomised Trial.Ann.Internal.Med., April 2007, 27: 146 (8) : 556 – 563, doi: 10.7326/0003-4819-146-8-200704170-00006.

54. Kalish RA.,Kaplan RE. et al (2001) : Evaluation of Study, patients with Lyme disease 10 – 20 – year follow-up. J.Infect.Dis. : 183 : 453 (Pub Med).
55. Burrascano J. R. (2000) : Advance Topics in Lyme Disease : Diagnostic Hints and treatment guidelines for Lyme and other tick borne illnesses, Lyme Net, Sixtent Edition, copiring October 2008.Board Member, International Lyme and Associated Diseases Society. http://www.cliktoconvert.com
56. Schwan TG.,Piesman J. (2000) : Temporal changes in outer surface proteins A and C of the Lyme disease – associated spirochete Borrelia burgdorferi durin the chain of infection in ticks and mice. J.Clin.Microbiol. 38 : 382 (Pub Med).
57. Grimm D.,Tilly K., Byram R. et al.(2004) : Outer – surface protein C of the Lyme disease spirochete a protein induced in tick for infection of mammals. Proct.Natl.Acad.Sci. USA. 101 : 3142.
58. Angiuta J.,Ramamorthi N., Hoviks JW. Et al. (2002): Salp 15 an Ixodes scapularis salivary protein inhibit (CD4+) T cell activation. Immunity. 16 : 849 (Pub Med)
59. Sarkar A.,Hayes BM.,Dulebohn DP.,Rosa PA (2011) : Regulation of the virulence determinant OspC by bbd 18 on linear plasmid Ip 17 of borrelia burgdorferi.Journal of bacteriology, 139 : 5365. doi: 10.1128/JB.00383-11
60. Gubler DJ. (1998) : Resurgent vector –borne diseases as a global health problem. Emerg. Infect. Dis. : 4 : 424 – 450. (Pub Med)
61. Tilly K.,Bestor A.,Rosa PA.(2013) : Lipoprotein succession in borrelia burgdorferi similar but distinct roles for OspCand VisE at different strateges stages of mammalian infection.: Mol. Microbiol. : 89.216. (Pub Med).
62. Keane – Myers A.,Nickell SP. (1995) : Role of Il-4 and INF-gama in modulation of immunity to borrelia burgdorferi in mices. J. Immunol.: 155.2020. (Pub Med).
63. Wormser GP, Brisson D, Liveris D et al. (2008) Borrelia burgdorferi genotype predicts the capacity for hematogenous dissemination during early Lyme disease. J Infect Dis, 198(9), 1358-1364. (Pub Med)
64. Anguita J.,Samanta S.,Revilla B.,Suk K.,Doss, Bartold SW.,Fikrine E. (2000) : Borrelia burgdorferi gene expression in vivo and spirochete pathogenicity. Infect Immun. 68 : 1222 – 1230 (Pub Med).
65. Barthold SW.,de Souza MS.,Janotka JL.,Smith AL.,Persing DH. (1993) : Chronic Lyme borreliosis in the laboratory mouse. Am. J.Pathol. 143 : 959 – 971 (Pub Med).
66. Bugli F.,Pelmieri V.,Torreli R.,Papi M., De Sjoirito M.,Cacaci M. Galganos S.,Macucci L.,Paroni S. F., Vella A et al. (2016) : In vivo effect of elarithromycin and alginate lyase against hp biofilm. Biotehnol. Prof. : 32 : 1584 – 1591, doi:10.1002.btpr.2339 (Pub Med).
67. Sepi E.,Bastrian SL.,Mfooj CM.,Scott S.,Rattelle A.,Pabati N.,Poruri A.,Barugu D.,Theophilus PA., Pham TV. Et al. (2012) : Caracterisation of biofilm formation by borrelia burgdorferi in vitro. PloS ONE. : 7 : e 48277. doi: 10.1371/journal.pone.0048277 (Pub Med)
68. Souhila, Tabak, Doukani Koula, and Bensoltane Ahmed. "Effect of Bifidobacterium Bifidum cuetm 89/29 on helicobacter pylori 158 san responsible for gastroduodenal diseases." International Journal of Applied and Natural Sciences (IJANS) 4.1 (2015): 25-32
69. Mehde, Atheer a., and Takwa A. Chechan. "Study the effect of oxidative stress and several biochemical features in patients with helicobacter pylori (h pylori) bacteria." International Journal of Applied and Natural Sciences (IJANS) 2.4 (2013):, 109-116
70. Mulchandani, Rupa, et al. "Helicobacter Pullorum model on Balb/ca mice." International Journal of Research in Applied, Natural and Social Sciences (IJRANSS) 1.2 (2013):85-92
71. Mulchandani, Rupa, et al. "Helicobacter spp. Detected in liver, pancreas in human patients with tumours." International Journal of Research in Applied, Natural and Social Sciences (IJRANSS) 1.2 (2013):81-84
72. Sawadogo, Amidou, Brigitte Nana, and Mansourou M. Lawani. "Organization and

hazards of occupational diseases among women in textile crafts in the city of Ouagadougou." International Journal of Medicine and Pharmaceutical Science (IJMPS) 9.6 (2019):83–94

73. Abdulwahab, Hamid, and Abdulameer Abdalbari. "Coronary artery diseases in Iraqi women angiographic study and risk factor correlation." International Journal of Medicine and Pharmaceutical Sciences (IJMPS) 5.4 (2015):27-40
74. Gill, Mukti. "Evade communicable diseases through Handwashing." International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS) 2.12 (2014):1-12
75. Al Maghrabi, Katibah. "The pattern of skin diseases among patients attending primary health care centers in Jeddah city, Saudi Arabia, 2012." International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS) 2.2 (2014):93-100.