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HYPOTHETICAL POSSIBILITIES OF DEVELOPMENT OF NON-HODGKINS LYMPHOMA DURING LYME BORRELIOSIS

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ABSTRACT

The hypothetical possibilities of the infectious genesis of malignant diseases in the past could not be confirmed, in proportion to the degree of development of science and technology development level. Contemporaneous medicine has numerous proves of these possibilities. Among them, the evident coherency of borrelia burgdorfferi (bb) with different kinds of primary skin lymphoma (1), but it is certainly necessary to do a lot of additional scientific research to get the exact answers to many unknown immunogenic mechanisms, which bring to that condition.

Modern studies show great difference and prevalence rates, which could be consequence of geographical heterogeneity and variability of bb, and common participation of numerous other zoonotic and non-zoonotic agents in co-transmission, co-infections and their contribution to chronic Lyme borreliosis (LB), as well as many other factors, which usually proceed to a development of malignant diseases (2).

Our investigation during the period from 2015 - 2018, even in modest possibilities and confined diagnostic conditions, were directed onto four cases, in which epidemiological, clinical data, laboratory and pathohystological diagnostic allowed assumptions about possibilities that bb is the causer and/or actuator of malignant course of the disease. In diagnosis, besides serological Elisa test onto specific bb antibodies, and confirmative (WB) test was used too. Prevalence bb DNA was researching with the help of three different Polymerase Chain Reaction (PCR) protocols.

Bb DNA was detected in all four cases. Direct sequencing of cleansed PCR products confirmed specificity amplified fragments. The specificity is evaluated using the tools for research the basic local alignments and with a use of adequate software for research the basic local alignments and with use of adequate software for inquiring heterogeneity (Multalin software) of the aimed sequences of the genes in different bioptic samples.

***KEYWORLDS**: Borrelia Burgdorferi, Cause, Actuator, the Malignant Diseases*

Article History

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INTRODUCTION

Lymphomas are malignant diseases of lymphocytes of the immune system. They may develop anywhere in the body, but mostly they develop in lymph nodes. According to historical reasons, they have divided into two categories: Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL), which refers to all others lymphoma with over 30 different types. The most common are B cell lymphoma in 85% of cases and T cell lymphoma in 15%. Histological visage of the lymphoma and their clinical behavior are determined by the following factors (3): kinds of cells, the level of cell

differentiation, localization of the cell origin (humoral factors, growth factors). Recent years, a great number of infective agents is etiologically connecting with the development of NHL among humans, including Chlamydia psittaci, Hepatitis C virus, Campylobacter jejuni, Helicobacter pylori (4-7) and many other infectious agents, which in different proportion take part in the total number of diagnosed NHL cases. The observed associations are significant from the aspect of clinical and therapeutic amplifications'' and, as in another side, they indicate the possibilities of the influence and participation of the infections onto mechanisms of malignant diseases occurrence. (8). According to the mechanisms of action, infectious agents associated with NHL are dividing into 3 groups:

First Group: This group contains viruses that can directly transform the lymphocytes, is called the viruses of lymphocytic- transformation (VLT). In this group is also Epstein Barr Virus (EBV), connected to Burkit s lymphoma (9), NHL at immunosuprimated cases, extranodal cellsnatural killers /T-cell NHL/, humanherpes virus 8 (HSV-8), /primary cause of lymphoma/. Human T lymph tropic virus /which can cause T-cell leukemia / lymphoma in adults/. In this group, many other infectious agents can be associated with NHL. (9 – 11)

In the **Second group** is a virus of the human immunodeficiency (HIV), unique for its causing of the hard immunodeficiency because of its exhausting of CD-4+ T lymphocytes, which could lead to acquired immunodeficiency (AIDS) with the risk of occurred of some NHL subtypes (12, 13). In this group can be many other infectious agents including bartonellae, brucellae, borrelia burgdorferi (bb), according to evidence that show they cause chronic infections and bring to a specific and non-specific immunodeficiency (14).

The third group includes infectious agents, which based on evidence increase the risk of NHL development through the chronic immune stimulation. This group includes hepatitis C virus (5) as well as many other viral and bacterial agents, which cause chronic "site-specific" inflammations possible, connected by lymph tissue (15 - 17). The hypothesis under which infectious agents may cause NHL in many cases has confirmed by their presence in tumor tissue at diseased humans as well as the results of experimental laboratory research of immune-genetic mechanisms of the malignant diseases (18-23).

Contemporary epidemiological studies also provide evidence of the fact that infections are a significant risk factor for malignant development, demonstrating that infection is more frequent in NHL cases than in controlled group's cases. According to numerous mechanisms, which help the infection to be the reason on NHL, appear with increscent molecular understanding of the malignancy process, this research field deserves constant scientific, multidisciplinary attention (16, 17, 23)

Lyme borreliosis (LB) is a zoonosis, multi-systemic infectious disease, with an acute, sub-acute and chronic course, from the group of vector-borne transmissible diseases (VBD). Etiologic agent, spirochete borrelia burgdorferi (bb) has been isolated in 1982. In addition, later is defined borrelia sensu lato complex. Nowadays there are 20 types of bb and is confirmed that at least 8 types are pathogenic for humans. Infection is related to different clinical manifestations: skin, neurological, rheumatological, and cardiovascular. European investigation on a sample of over 3000 serums of the patients with chronic LB showed that in over 50% there was about co-infections, of which 30% of cases were evidenced as multi etiological participation of numerous infectious agents in the co-infectious basis of disease (21 - 23). Compound mechanisms of humoral and cellular immune response classify the LB into a group of dangerous infections that destroy the immune system (15).

Primary vectors, reservoirs, and sources of infection for bb are Ixodes ticks, and possibly other hematophagous vectors. Ticks are the most frequent transmitters of bb in the world and they transmit other infectious agents of the *vector-borne diseases* complex. (VBD) (14,17,18)

The involvement of different infectious agents in polyvalent transmission throughout the ticks allows co-infectious involvement of these ageneses in common immunogenic base of chronic LB, and possibly malignant diseases (19-21, 23).

Besides the numerous and known spectrum of infectious agents, which take part in LB, significant is the potential transmission of "new" infectious agents, among which special attention is directed to numerous oncogenes and retroviruses that, at least for now in enzootic cycle, as causes immunodeficiency and malignant diseases of the animals, and in zoonotic cycles, possibly the humans, too. (24)

Hypothesis and popular theory that human HIV has the origin in monkey's SIV, asks the justified question - can other lentiviruses of the animals also cause human diseases? Data of numerous new studies warn about the fast natural transformation of infectious agents, and about their transmissions from enzootic into zoonotic cycles, without need to evolve in enzootic cycles for a long time. It is known that some cat and cattle lentiviruses cause the immunodeficiency. For Cattle Enzootic Leucosis (ELG) has been proved to cause human diseases too. It is chronic proliferative disease which cause RNA virus of enzootic leucosis of cattle (VELG) which has been classified as Deltaretrovirus genus, Retroviridae family, whose basic characteristic is having an enzyme of Reverse Transcriptase which allows transcription of nucleic acid in reverse way, into complementary DNA and which Integraze enzyme imbeds into the host's genetic code.(24, 25).

VELG instigates lymphocytosis with a characteristic increase of the number of lymphocytes in peripheral blood and in advanced clinical results with leucosis changes causes permanent lymphocytosis and development of lymphoma. At most the infected animals (about 70%), the disease does not develop, and if it does it is clinically manifested between 4 - 8 weeks after the infection. When they are present, clinical signs of disease refer to infected organically systems. Diseases that viruses of this family cause are leukemia, lymphoma, and sarcoma among different kinds of animals (among humans they are less researched). Our knowledge is wider concerning human immunodeficiency (AIDS) and animals (FAIDS-feline AIDS and SAIDS –simian AIDS).

The virus shows expressive tropism towards lymph tissue and leukocytes. It can multiply and survive only in lymphocytes (13, 26, 27).

According to data from the literature numerous members of lentiviruses subfamilies cause the immunodeficiency among animals, and potentially possible among humans. They have shown in Cart 1.

Table 1

Major Members of the Lentivirus Sub Family		
Source	**Virus**	**Diseases**
Humans	HIV-1 HIV-2	HIV / AIDS
Primates Makaki monkeys: Rhesus African green monkeys Cercopitecus Mandrili Simpanze monkeys	SIV	Monkey AIDS
Other animals Cat Cow sheep horse cocoon	Virus of cat imunodeficijency Virus of bef imunodeficijencije V. visna / maedii Equine infectious anemia Equine arthritis / encephalitis	Cat AIDS Pulmo, CNS, anaemia,artritis,encephalitis

Epidemiological conditions important for the development of NHL are numerous, firstly because of evidenced hospitability of the ticks for the whole spectrum of different microorganisms. The knowledge's about possibilities of co-transmission, an exchange of genetic material through the plasmids in natural hosts and vectors, participation in co-infections of two or more infective agents from of the vider Vector-borne disease complex (VBD). It is evidential that, in natural hosts and vectors, infectious agents can exchange their DNA and certain proteins with each other. Which, from infectivity aspect, can significantly amplify or change the pathogenetic, i.e. immunological potency and widening possibilities of complex immune and autoimmune mechanisms, direct tissue damages and even malignant diseases occurrence (28-30)

Ticks are obligate hematophagous ectoparasites that parasitize on mammals to maintain their own development and reproduction. Humans are incidental victims if they accidentally get involved in the tick's food chain. These predators have a characteristic that their sting may stay hidden for a long time, without any clinical manifestations that could indicate infection, so the World Health Organization (WHO) has accepted the presence of most frequent skin change on a tick's sting point, Erythema migrans (EM) as pathognomonic sign and important diagnostic criteria of an early stage of LB. In addition, in dr. Andric Doctoral dissertation it is confirmed that EM is an indicator of bb involvement into co-infections after the tick's sting (26). (Figure 1)

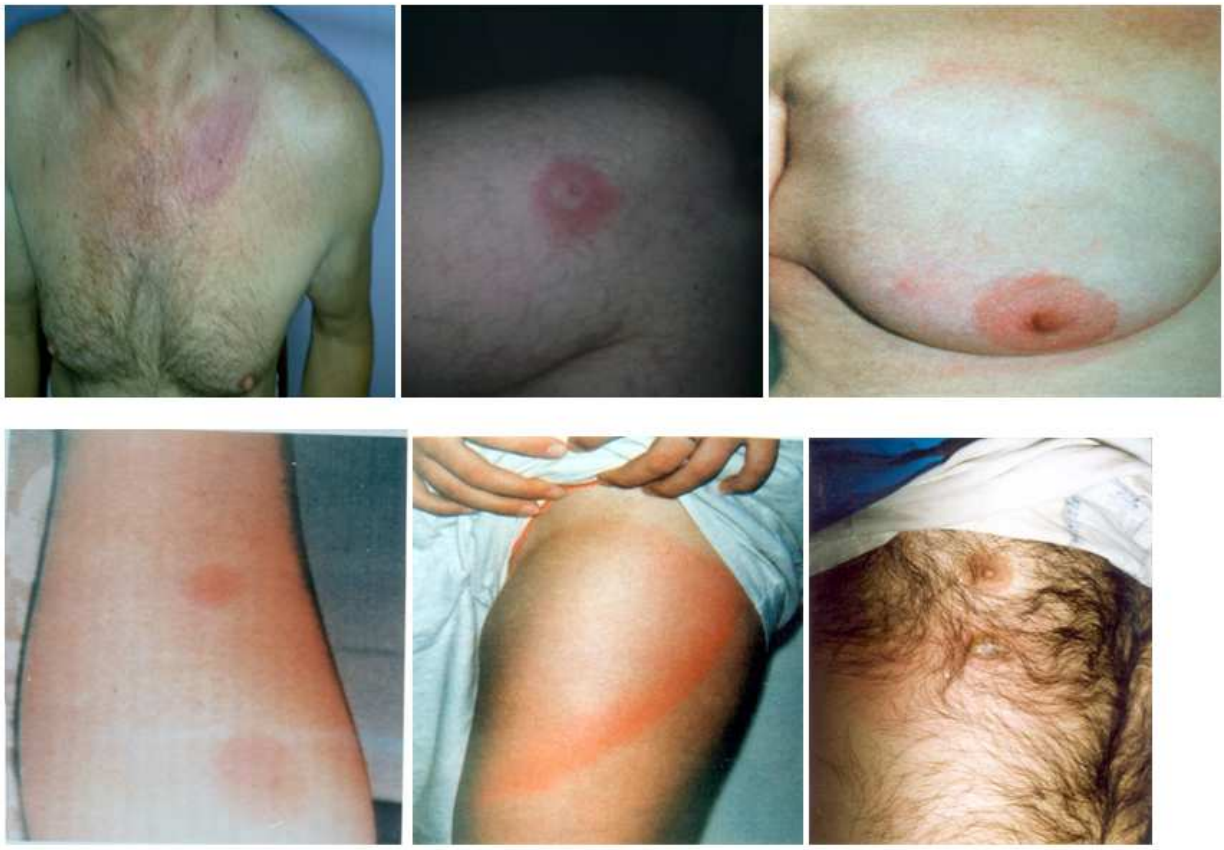


Figure 1: Erythema Migrans is a Characteristic Dermal Lesion of an Early Stage of Lyme Borreliosis at 35-75% of Infected Patients. being Present, it Represents the Specific Sign and Important Diagnostic Criteria of an Early Stage of the Disease. (Photo Documentation of Prof Bogdanka Andric)

The most dangerous thing about ticks is their role of biological vectors for numerous infectious agents. There is a part of the life cycle of these microorganisms inside of them, with the possible exchange of parts of genetic and extra-genetic structures, proteins, with consequent creating of new characteristics of infectious agents, which imply an increase of immunogenic potentials. There are evidence that bb in co-infections with HIV brings to hard immunological irreversible changes of the Central Nervous System (CNS). It is also proven that in human infections it establishes long latency in macrophages, with the possibility of reactivation of EBV, which also lives in macrophages for a long time and whose oncogene potentials are known nowadays (27-30).

The start of autoimmune mechanisms, which is preventing in chronic LB, can also be the first step towards induction and the beginning of malignant development (31, 32). Identification of the numerous, very significant competitive behavior (i.e. E-valuation < 1 x 10-9) for RNA viruses in genomes of mammals and arthropod vectors, EVE which refer to seven families were identified, including viruses with double connection (ds-RNA) (Reoviridae) and positive RNA (RNA+ve) viruses (Flaviviridae), as well as segmented (Ortomyxoviridae, Buniaviridae) and non-segmented (Borna-, Filo-, Rhabdo- viridae) families by negative RNA (RNA-ve) viruses. In accordance with integration process which doesn't t include genomic RNA, and includes viral m-RNA, all EVE derived from RNA virus contained genetic structures that included one virus transcript (or fragments derived from individual transcripts). EVE derived from different genes have never showen up as contiguous sequences and therefore it was not possible to establish if EVE-s from different genes of a certain family of viruses come from the same or different virus lines/infections (30 – 34).

Since it has discovered in 1975, LB has been on a top of the focus of the world health and scientific public's interest (16, 18, 32). Current problem and modern significance of LB could be summarized through several segments:

The registered fact is evident: increase of LB diseased in a world, widening of infectious agents spectrum, that besides bb can be transmitted by ticks, more and more frequent is the registration of the late, chronic forms of diseases, co-infections and is more frequent connected with malignant development. During LB, bb leads to chronic progressive immune and autoimmune disorders, resistant to therapy with the development of chronic, progressive forms of the disease with possible disability and death.

The particular problem related to LB is a discovery of co-infections. Patients with co/infections are classified into 2 groups (35)

In the first group in common endemic areas, the co-infections of bb with different agents of the tick-borne complex were detected and they are limited with their geographical distribution and kinds of natural hosts (33, 34). In our environment, the research showed that rickettsial agents are the most frequent participants in co-infection through ticks and the most frequent participants in co-transmission through ticks and the most frequent participants in co-infection with bb (35). In the second group co-infections are consequences of endogenous immunological reactivation of different intracellular agents that were initiated by bb. Among first proven is reactivation of Epstein Baar Virus (EBV) under the influence of bb from macrophages in which both infective ageneses establish latency for a long period of time (35). The evidence that show bb can be included in dermal B-cell lymphoma development is significant, but there are opened questions if associations can also refer to non-cutaneous lymphoma. According to the fact that LB is a systemic disease, it is clear that infection with bb isn't limited only on the skin. A wide spectrum of clinical manifestations includes many other lymphoid tissues.

Many world's research is directed towards exploring the evidence that infection with bb is connected with a higher risk of the total development of NHL or specific subtypes of NHL among humans (33, 34).

During 2015 to 2017, in Clinic for Infectious Disease in Podgorica, four cases suspect of NHL were analyzed, in pursuit for evidence that infection with bb could be etiologic cause/ initiator of malignant diseases development.

METHODOLOGY

When suspecting NHL, the biopsy of the enlarged lymph nodes is of special importance. To analyze available lymph nodes we can use the usual excision procedure of taking the tissue sample. When the lymph nodes or other suspicious changes are not available, the biopsy procedure is preceded by scanning and photographing, so the needle aspiration biopsy and pathohistological verification can be applied. However, an aspiration needle biopsy cannot give us a good sample as an excision biopsy for initial NHL diagnosis. The biopsy of bone marrow can also be using, but it is not a usual part of routine evaluation for early NHL diagnostics, but it can be significant for differential diagnosis. Blood tests, i.e. total blood analysis and differential blood analysis include inspecting different kinds of blood cells so it can be examined whether their number reduces in normal or pathological range. These results are not specific for the NHL. Sometimes other blood markers can be used to expand the information that is in accordance with prognosis.

The screening for certain virus infections is also a part of starting laboratory evaluation. PET scan and CT scan have two different kinds of rays that are often used together, so they obtained the result of anatomy can be shown by CT, and PET scanner is used to detect the inflammation areas. The mass in the chest area, i.e.mediastinum is very frequent

result in classic NHL on PET/ CT examination (36, 37, 38). PET scan has lately become primarily significant for initial setting up the diagnosis. This method can also be helpful to determine the difference between active tumors and fibrotic areas that stay after treatment of NHL

In our investigations diagnosis, besides serological Elisa test onto specific bb antibodies, and confirmative Western blood (WB) test was used, too. Prevalence bb DNA was reseacher shed with the help of three different Polymerase Chain Reaction (PCR) protocols. Bb DNA was detected in all four cases. Direct sequencing of cleansed PCR products confirmed specificity amplified fragments. The specificity is evaluated using the tools for research the basic local alignments and with use of adequate software for inquiring heterogeneity (*Multalin software*) of the aimed sequences of the genes in different bioptate samples.

RESULTS

Our four patients, all females, aged 32, 65, 66 and 69 years old, from different Montenegrin areas. The first patient we have been observed, 65 years old, about two years being a patient at hematology Clinic in Podgorica, with a diagnosis of systemic NHL. Physical decay and hematologic results made her look for medical help. When she first came to the Clinic in 2015 an isolated tumor change 5 x 4, 5 cm has not been detected on her back, which did not hurt and did not itch nor did it show any signs of dermal inflammation. Retrospective epidemiological data showed that she lives in a rural areanear Podgorica. She has contacts with many domestic animals. She can t remembers of any neither tick sting nor Erythema migrans (EM), and she noticed the occurrence of dermal tumor change on her back skin 3-4 years ago. The dermal tumor has been developing slowly and gradually sizing up. As she has thought it was atheroma and it has not actually bothered her with its localization, she repeatedly refused surgical intervention.

By serological analyses with Elisa method, specific IgG antibodies to bb have been evidenced, followed by positive confirmative Western blood (WB) test in IgG classis of antibodies, and with a help of Polymerase Chain Reaction (PCR) method, DNA of LB was detected. Except for bb, serological and PCR search for potential co-infect agents have not been done. It is decided to do the surgical removal of tumor lesion and pH verification. Before the surgical intervention, ultrasound and CT skin examination were done (Figure 2).

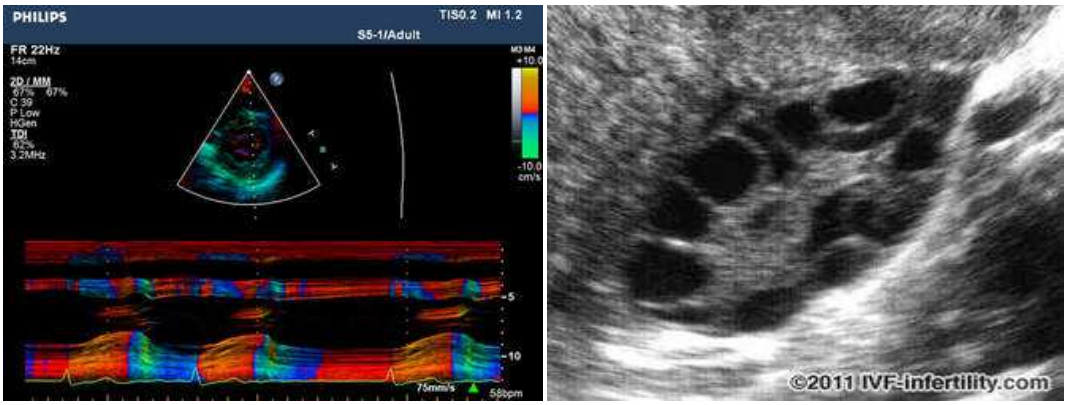


Figure 2: As usual, before the Biopsy, Diagnostic Methods Included Ultrasound and CT Dermal Examination. (https.//www.gougle.com./ultrasonographia+on+non+hodkins+cutis+lymphoma a&rtz=1C1AVSA_enMES72MES677)

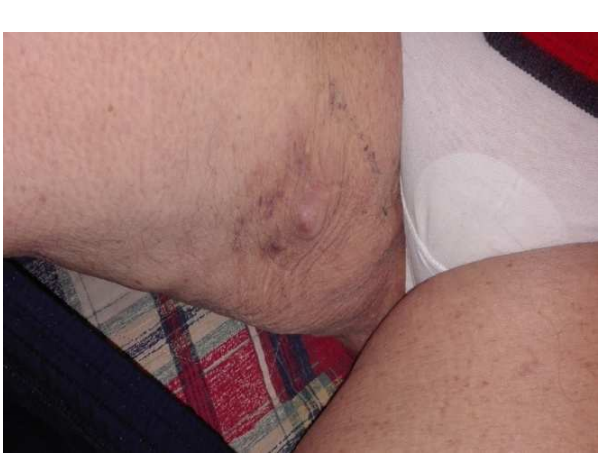
After primary cut, from tumorous formation, (A) the big tick (B) was isolated, it sized 4, 0 x 4, 5 cm, full, pale green colored (because of biliverdin presence). The tick was alive, hardly movable, identified as Ixodes ricinus according to Pomerncev key (Figure 3 A, B)

A **B**

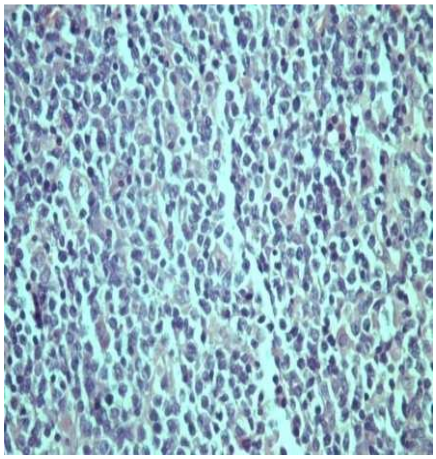
Figure 3: Pathohistological Analysis of Tumor Change had the Following Characteristics: Relation between B.burgdorferi Primary Dermal Lymphoma of B-cells (PCBCL) has been Confirming and for the First Time Published in December 2017, after the Demonstration of Microorganisms into Dermal Infiltrations of B-cells Present in Places in which PCBCL was Developing Afterward. Thanks to Claudia Scholikop et al. (In "Blood2008"ICEN 15 111 (12)5524-5529.)

Follicular lymphoma is the most frequent type of indolent non-Hodgkins lymphoma. It is developed from B lymphocyte and grows very slowly. It influences an increase of lymph nodes and can be transmitted to bone marrow or spleen. Most of the patients with follicular lymphoma are about 50 years old or older. Follicular lymphoma may retreat and disappear without therapy treatment. Patients have to follow carefully the signs or symptoms in case the disease progresses or comes back. Rarely, but in some cases, follicular lymphoma may become aggressive like diffusive lymphoma of big B-cells.

Second case, patient aged 69 living in Montenegrin coast (Tivat). During her life, she had several tick strings. In her right inguinal area, about 15 years ago, she had multiple stings of a tick and EM. Because of multiple stings, she has been testing and had positive serology IgM and IgG-specific antibodies to bb with Elisa method, which is also confirming by WB test. She had been treating for 4 weeks with dovicin capsules 2x2. Since then she hasn't had any troubles. During 2018, she asked for medical help because she noticed a small tumor in the right inguinal area (A), she felt general weakness and exhaustion. Local treatment of tumor change understood as purulent inflammation, brought to the local appearance of redness and pain (secondary infection). According to lab results, what is registered was a temporary fall of all lineages of blood elements. Microbiological Elisa bb testing is IgG positive, WB test positive, as well as PCR –DNA. Pathohistology the lymphoma of B-cells is diagnosed (Figure 4 A, B).



A



B

Figure 4: Primary Dermal Anaplastic Lymphoma of Large Cells Appears only on the Skin as a Benign Node or May be Expanded around When it Requires Therapy. Original Photo Documentation by B. Andric

Two other cases from Nord Montenegro: daughter, 32 years old and mother, 67 years old, both with diagnosed systemic NHL. For both of them after a one-year period the disease ended with death outcome. Both of them had positive serological tests Elisa and WB on bb, as well as positive DNA –PCR. According to epidemiological data, they had a dog pet, which they have been in close contact with for years. Four or five years before their disease the dog got asthmatic attacks and diffusive subcutaneous nodes, many of them on the dermal petiole, of which some have exuded. The dog was not diagnostically processed. Veterinarian diagnostic of tumor changes was missed out since the dog was old and the owners refuse to expose it to stress and surgery. The dog was occasionally treated with antibiotics in cases when it came to some regressive changes, but even after that, the disease rapidly progressed and death.

The mother and the daughter were constantly beside the sick dog, nursing it until it died. The death of the dog was proceeding by blindness, developed ataxia and progressive loss of weight. First, the daughter got diseased, who previously has never been sick. The disease started in July 2015. What dominated in clinical simptomatology was high body fever, hard breathing and to that matter RTG, pulmonum was done. The result showed significant packages of mediastinal lymph nodes and left-sided pleuritis, which shadowed the completely left lung with drastic propulsion of mediastinum into the right. In the right hemithorax, a basal, a small amount of fluid was found. The patient had been examined to sarcoidosis, prospective specific etiology of pulmonary changes, psittacosis, Q fever, Brucellosis, Ehrilihiosis, Leishmaniasis. The results were negative. She was treated at the Clinic for infectious diseases in Podgorica with antibiotics during the period of four weeks and after that her clinical state was improved-there was retreatment of exudates, improvement of general state and blood parameters which showed significant depression in segments of white and red lineage. Mediastinallymphocytes showed certain regression, but not any significant regression. After cessation of antibiotic treatment, her state got worse. With aimed hematological examination, systemic NHL was diagnosed and hematological

therapy treatment was enclosed. She passes through 3-chemotherapy treatment, the transplantation of the stem cells, but without any improvement of her state. Mediastinal lymphatic maintains and grow, once again there are exudates detected, first in left, then in the right lung, and at the end pancarditis and death. Among other serological analysis, Elisa testing of bb was also done and elevation of specific IgG antibodies. There was bb DNA found by PCR method. The examinations of animal onco and retroviruses weren't done because there weren't conditions for that. According to pathohistological, it was about primary mediastinal lymphoma of large B cells, which grows fast in lymph nodes and often

in spleen, liver, marrowbone or other contaminated organs. The signs and symptoms of the disease can be heterogeneous and may include fever, overnight sweating, weight loss (B symptoms). Lymphoma of large B cells primary mediastinal is a subtype of this lymphoma, it is characteristic for creating exceeding fibrotic tissue (similar to scar) in lymphatic tissue. A tumor is usually forming behind the sternum, which affects respiratory passability, causes coughing and wheezing. Taking a cue from pleural effusion, it represents a large risk factor of pulmonum overloading. Most patients are women aged 30-40, as in our case.

Mother aged 67, had chronic gastritis for many years and she had a positive serological result to helicobacter pylori, despite specific helicocin therapy she was using. At the end of 2017, the disease started with an inflammatory cough, feeling of choking, infirmity, and sometimes moderately high temperature, followed by progressive weight loss. She hasn't reported any inconveniences until February 2018, when her medical state drastically got worse. Because of dominant symptom- choking, RTG pulmonum was done and we detected distinctively enlarged Hilary lymph nodes and small and densely positioned diffusive changes in parenchyma of both lungs, suspicious to secondary deposits. Similar changes were found in kidney tissue, adrenal glands, gizzard, and disks. As for CNS, the changes turned up later, expanding on nervus opticus, and were manifested by a vision disorder. Somnolence and disorientation, hemiplegia and incontinence showed up before death outcome, in July 2018. She refused all medical examinations, interventions, and therapy. Besides RTG pulmonum and CT of a head and lungs, basic laboratory search and microbiological examinations were also done. It is proven, with the help of PCR method, that there was bb DNA in blood, and Elisa and WB test proved specific IgG antibodies to bb in diagnostic titers. Microbiological examination of blood showed a positive result on helicobacter pylori and biopsy of gastric mucosal lining showed suspicious subtype of gastric MALT lymphoma, composed of post germinant center of B cells.

DISCUSSIONS

According to the fact that infection with bb isn't localized dermal disease, but systemic infection, which under the definition may seize all the organs and organic systems and has been reflected with wide range of clinical manifestations, disseminated and distant regions, including lymphoid tissue. Numerous results confirm this statement. (Figure 5). Research proves that there is a presence of bb DNA in malignant lesions with nodal B cellular lymphoma, and after that the research started in Sweden and Denmark and hypothesis that bb infection is associated with high risk of NHL development with specific subtypes of NHL, which is primarily reflected on high risk of development of dermal, i.e. non-dermal lymphoma, which is proven and accepted by WHO. (37 -- 40).

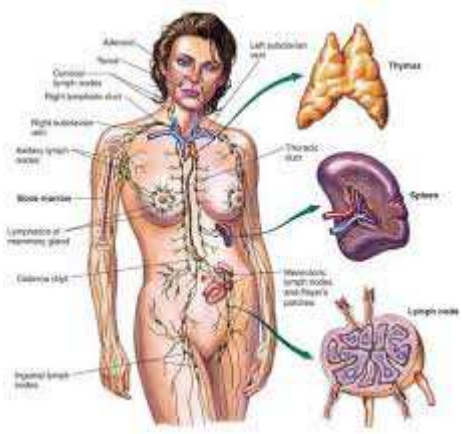


Figure 5: It is Believed that there isn't any Tissue or an Organ bb can't Get in and Cause Pathological Changes. Nowadays, it is Categorized as a Dangerous Disease because it, using Different Mechanisms, Destroys the Immune System. (htps:// admin@budwigcenter.comdmin@budwigcenter.com)

The suspicious mechanism by which bb may induce lymphoma occurrence represents the start of chronic immune-stimulation supported by continuous lymphoid proliferation,(41, 42) with analogously oligoclonal and ultimate monoclonal B cell selection, analogical as in helicobacter pylori of associated gastric MALT lymphoma. With those mechanisms bb tends to favor subtype of lymphoma as MALT lymphoma is like and which consists of germinative center or post germinative center of B cells. (41). Extra gastric MALT lymphoma (41, 42, 44) ensues in the same cells as gastric does, but it may also appear in any other organ and there are chances for this disease to come back after many years. (43).

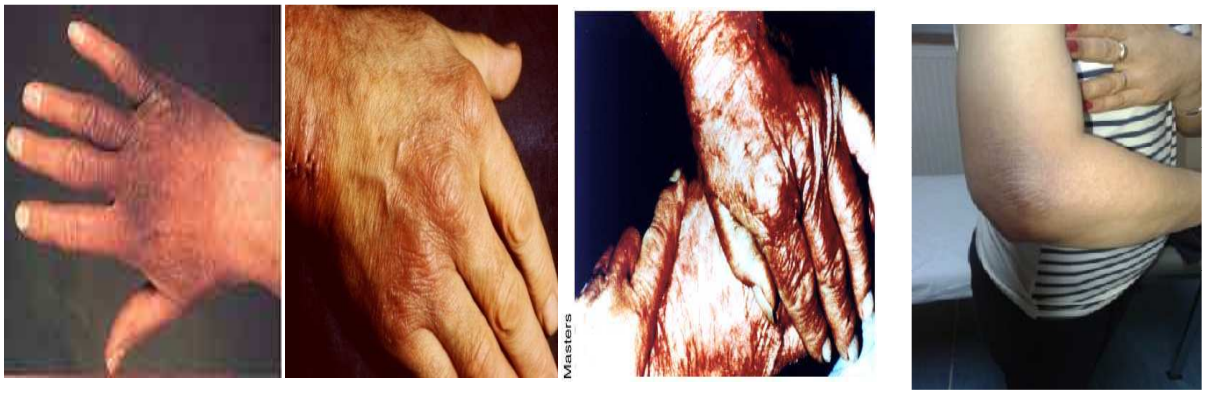
Numerous examinations in the world showed surprising results, that specificities of borrelia associates with malignoma of mantle cell lymphomas (MCL) and are malignomas of B-cells, predominantly made of pre-germinal basis. (44)

Borrelia DNA is also evidenced in cases of nodal MCL, but in some other reports, hepatitis C virus RNA in MCL were described, with complete regression after antiretroviral therapy, which indicates that some additional research is needed. *Jares* (45) published a study in which 15-40% of MCL-s occurs because of somatic hyperactive mutation, suggesting that these tumors vary depending on germinative centers, which means that high risk for MCL is biologically possible, but this needs to be proven.

Association between MCL risk and bb seropositivity can also be founded among the patients who have not had clinical manifested borrelia infection. Asymptomatic cases that had positive borrelia serology have been observed primarily based on immune response and it could be seen that immune response between patients with and without clinic simptomatology of LB was different. According to *Still's* (46), observation and also on observations of other Swedish researchers (44) there isn't a differentiation of dominant Th-1 immune response, which is considered as primarily important for the eradication of borrelia spirochete among bb positive asymptomatic persons and patients with clinical manifested borrelia infection.

Until today there aren't entirely known numerous and different strategies which bb can damage the host's immune system with during the infection. Strategies include antigen variations of the surface membrane of bb, protein control, including or not including complement system into immunogenic mechanisms of infection, intracellular persistence, according to *Singh* and *Girschick* (47). In one study, patient with acute LB and manifested EM initially didn't have a high

level of Th-1 cytokine interferon gamma (IFN-gama) followed by a high level of Th-2 cytokine interleukin-4 (Il-4).(48) Contrary to patients with chronic borrelia infection who manifested acrodermatitis chronica atrophicans (ACA) and who persistently had a high level of Il-4 and vary small or non-IFN-gama expressions. This chronic dermal inflammation with dense lymphocyte infiltration and dermal atrophy named ACA seems like chronic helicobacter pylori infection, which indicates mucosa-associated lymphoid tissue (MALT) and abdominal lymphoma (7). An interesting fact is that hard cases of dermal B-cellular limphocytoma are evident and they started into the context of ACA (6,47,48). According to numerous studies, it is established that the expression of IFN-gamma has particular significance for control and resolution of bb infection. These conclusions are based on associations with borrelia lymphomas, because dominant Th-1 immune response is related to increased risk for other chronic inflammatory diseases (38) which can be related to increased the risk to develop NHL (49,50). In addition, Th-1 is the dominant immune response registered at helicobacter pylori positive gastric MALT lymphoma (41). Acrodermatitis chronica atrophicans (ACA) is the late dermal form of LB. The early inflammatory stage is manifested with blue-red discoloration and diffusive skin swelling. The atrophic stage represents the manifestation of late-stage with red discoloration and thin wrinkled appearance of the skin (Figure 6- A, B, C, D).



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

Figure 6: (A, B, C, D) ACA Diagnosis is very Difficult. It can be Wrongly Diagnosed. During Inflammation, Stage as Vascular Disorders, Erysipelas, or Bursitis / Arthritis and in the Atrophic Stage as Lichen Scleroses Atrophicus, Morphea or other Dermal us Diseases. It is Evidenced that Difficult Cases of skin B-Cellular Lymphocytes Have been Initiated within ACA Context. (Photo Documentation by B.Andric)

Besides ACA, in new literature, for the first time, another form of late LB was described, (LCLB) with feet tumor. The examined woman, aged 64, had a big tumor change from the back on the right hemithorax, with conspicuous deformities on both feet, which created because of wearing tight shoes, back 2-3 years ago. With histological dermal examination, the lymphocytic granulomatosis infiltrates with plasma cells was founding. Serology of bioptic skin material to bb was significantly positive and PCR analysis bioptic material of the skin discovered bb sensu lato, genospecies B. afzelii. After antibiotic treatment, the tumor totally disappeared, the skin was atrophic and dry.(53, 54, 55). The diagnosis of ACA is often missed and may can be wrongly diagnosed while in inflammation stage, as vascular disorders, erysipelas or bursitis / arthritis, and in atrophic stage as lichen sclerosis atrophicus, morphea or other skin disorders. Cutaneous lymphoid infiltrates (CLI) are frequent in routine dermal pathology. However, differentiation of the reactive CLI from malignant lymphocyte often represents a challenge, because many inflammatory dermatoses may clinically and/ or histopathologycaly imitate cutaneous lymphoma. (44, 45).

Reading the literature from 1966 up to July 1st at PubMeD.gov. the diagnostic approach to CLI and most common differential imitators of the lymphoma are here differentiated according to six dominant morphologic and immune-phenotypes, histopathological patterns: (1) surface dermal T-cell infiltrates, (2) surface and deep skin perivascular and/or nodular natural killers T-cell infiltrates, (3) pan-dermal diffusive T-cell infiltrate, (4) paniculit T-cell infiltrates, (5) infiltrates of B-cells of dominant cells, and (6) dominant infiltrates of B cells. (42) Since none of the histopathological characteristics is enough to clarify the difference between benign and malign CLI, it is necessary to carefully consider the total balance of clinical, histopathology, immune-phenotypicall and molecular characteristics so the diagnosis can be established. In spite of the progress of auxiliary studies, like immunehystochemy and molecular clonality, these studies often shoe specificity and limited sensitivity. Therefore, detection and proper understanding clinical-pathological correlation still stay the golden standard for precise diagnosis of CLI-s. (40, 45, 46).

CONCLUSIONS

According to our cases, the suspicious clinical association between bb and NHL has been complementing with positive epidemiological data, serological and PCR confirmation of bb infection of our four patients. The evidence bb can initiate the development of dermal B-cellular lymphoma, were intriguing even as an assumption- can it also be associated with systemic, non-dermal lymphoma.

We are aware of the insufficiency of the applied diagnostic methods that we used in our research to diagnose the potential infectious genesis of some malignant diseases, as well as the small sample of patients we examined.(28, 37) However, we believe that research is useful for practice. We know that we need to talk seriously about these possibilities. Lyme borreliosis has been comparing to the "iceberg summit" for a long time. To date, much research has been done in the scientific research of this strange disease, but the fact that it is still missing is putting under control. The fact that LB is among us is a compulsory factor for further multidisciplinary scientific work and exchange of experiences.

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