The Chronic Manifestation Knot (CMK) Model: A holistic and unified approach to facilitate graphical data visualization and statistical predictive modelling for diseases at the microorganism level

Kunal Garg
Master's thesis
University of Jyväskylä
Department of Biological and Environmental Science
Cell and Molecular Biology
19.05.2015

Preface

This thesis would not have been possible without the unwavering support from my mother and late grandmother. I owe an enormous thanks to Dr. Leona Gilbert (a.k.a Lee) for believing in me and for providing me with multi-faceted opportunities to learn science in all shapes and sizes. Learning statistics and applying it for developing a novel model was a nerve cracking yet exciting process. I would like to thank Dr. Stephen Croucher and Dr. Leena Lindstrom for providing me with their time and expertise in statistics. Kanoktip Puttaraksa (a.k.a Kai) and Louise Thuesen Hermansen (Denmark collaborator), thank you very much for all the fruitful discussions with regards to statistics, Tick-borne diseases, and arthritis. I have realized that science can be a little less daunting for me if I am surrounded by awesome people. I would like to thank all the awesome Lee's research group members for establishing a stress-free and warm dynamics within the group (by the way guys, I would not mind little more silence in the joint office). I also owe an enormous thanks to the Nanoscience establishment and their master's scholarship provision. Thank you, Dr. Janne Ihalainen and Dr. Riitta-Liisa Kuiittinen for your incredible support.

University of Jyväskylä **Abstract of Master's Thesis**
Faculty of Mathematics and Science

Author: Kunal Garg
Title of thesis: The Chronic Manifestation Knot (CMK) Model: A holistic and unified approach to facilitate graphical data visualization and statistical predictive modelling for diseases at the microorganism level.
Date: 19.05.2015 **Pages:** 51+7

Department: Department of Biological and Environmental Sciences
Chair: Cell and Molecular Biology
Supervisor: Leona Gilbert

Abstract

Borrelia species are predominantly responsible for the most common form of Tick-borne diseases (TBD) which is Lyme borreliosis (LB). Episodes of Lyme arthritis (LA) is a hallmark symptom of LB patients. LA is an atypical form of reactive arthritis (ReA) that is a sub-type of Spondyloarthritis (SpA). Coinfections such as *Ehrlichia chaffeensis* accompany primary *Borrelia* species during the later stages of LB infection to induce severe symptoms. Currently, there is no holistic approach that would unify different TBD pathogens into independent and multiple TBD infection (MTBDI) combination categories to facilitate graphical and statistical analysis between any two patients groups. The chronic manifestation knot (CMK) model with its eight categories is a holistic and unified approach that facilitates data visualization and predictive statistical modelling (binary logistic regression) between two patients groups (LB and SpA patient groups). The CMK model helps to discover and clinically understand different developmental aspects of a disease at the microorganism(s) level. In order to validate the newly proposed CMK model, immune response frequencies of LB and SpA patient groups were statistically moulded to clinically understand relevant MTBDI combination(s) that may play an imperative in inducing chronic conditions. The immune response frequencies by LB and SpA patient groups (n = 54) to *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis* were obtained from two independent studies that emphasized on recording the immune responses on the enzyme-linked Immunosorbent assay (ELISA) platform. The raw optical density (OD) values from both the studies were converted into the optical density index (ODI), and finally transformed into binary codes for establishing the eight CMK categories. Graphically, immune responses by both patient groups to the CMK categories revealed that the outer surface membrane proteins across both *Borrelia* morphologies are different. Statistically, the results revealed that *Borrelia*'s pleomorphic ability may play a crucial role for *Borrelia* spirochete to progress through the early to late stages of LB, to induce chronic conditions like arthritis together with coinfections. From statistical and experimental standpoints, the inclusion of *Borrelia* RB for diagnostic purposes should strongly be considered to improve the detection efficiency of the ongoing diagnostic tools.

Keywords: Chronic manifestation knot model, *Borrelia* Round Body, and Lyme Borreliosis.

Table of content

Abbreviations

ATP	Adenosine Triphosphate
AFM	Atomic Force Microscopy
BSK	Barbour-Stoenner Kelly
BSA	Bovine Serum Albumin
CMK	Chronic Manifestation Knot
ELISA	Enzyme Linked Immunosorbent Assay
EM	Electron Microscope
EXPC	Expected Counts
HRP	Horse Radish Peroxidase
HLA	Human Leukocyte Antigen
HGA / HGE	Human Granulocyte Anaplasmosis / Human Granulocyte Ehrlichiosis
IFN-γ	Interferon gamma
IL	Interleukin
IgM	Immunoglobulin M
IgG	Immunoglobulin G
LB	Lyme Borreliosis
LD	Lyme Disease
LA	Lyme Arthritis
MTBDI	Multiple Tick-borne Disease Infection
NA	Not Applicable
OD	Optical Density
ODI	Optical Density Index
OBC	Observed Counts
PCR	Polymerase Chain Reaction
RB	Round Body
ReA	Reactive Arthritis
SpA	Spondyloarthritis
TBD	Tick-borne Disease
TNF-α	Tumour Necrosis Factor-α
TMB	3,3’,5,5’ Tetramethylbenzidine

1. Introduction

Ticks are ectoparasites (external parasites) that belong to the Arachnid class. They feed on Homo sapiens, mammals, birds, reptiles or amphibian's blood (hematophagy) for survival (Barker and Murrell, 2004). Ticks are responsible for one of the most common vector-borne diseases called Lyme Disease (LD) or Lyme Borreliosis (LB) (Aucott *et al*., 2009). *Borrelia* species that are spirochete in morphology are primarily responsible for LB (Burgdorfer *et al*., 1982; Radolf *et al*., 2012). Currently, *Borrelia* strains that have been recorded as the most prominent causative agents for multisystem disorder in LB patients (Steere, 2001) are *Borrelia burgdorferi* (Kalish *et al*., 2001; Mayne, 2014), *Borrelia afzelii*, *Borrelia garinii* (Strle *et al*., 2006), *Borrelia miyamotoi* (Krause *et al*., 2013), and *Borrelia valaisiana* (Diza *et al*., 2004). Onset post tick bite, symptoms start developing as early as a week and may prolong for years progressing towards chronic conditions due to the increase in the severity of the symptoms (Steere, 2001). Importantly, the Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibody responses to *Borrelia* strains (Figure 1) are segregated into two stages, early and late stage (Kalish *et al*., 2001). Subsequent to a tick bite, in the first stage of a *Borrelia* infection, the IgM response prevails for several initial weeks (Figure 1) and sustains unto early dissemination of the *Borrelia* infection (Kalish *et al*., 2001). In the Second stage of a *Borrelia* infection, certain additional weeks are required to build up an IgG response against the infection (Kalish *et al*., 2001). An IgG response to a *Borrelia* infection (Figure 1) sustains for months and years (Kalish *et al*., 2001) in individuals experiencing chronic conditions such as Lyme Arthritis (LA). Traditional indications for LB are an Erythema Migrans rash, disruption in cardiac muscle's electrical conduction, neurological abnormalities, and episodes of arthritis (Steere, 2001; Kalish *et al*., 2001).

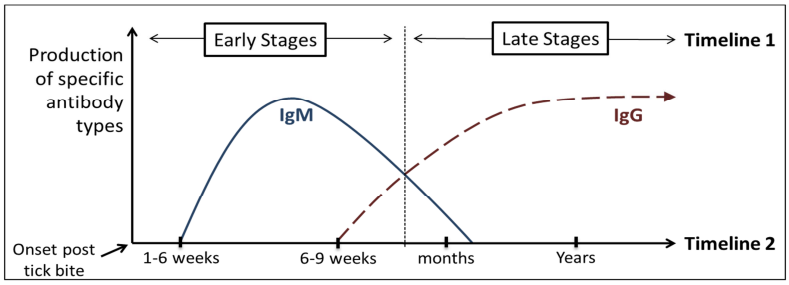

Figure 1: Graphical representation of typical IgM and IgG response to *Borrelia* strains in an individual onset post tick bite. An IgM response prevails for several initial weeks, but an IgG requires certain additional weeks to build up a response. The latter immunoglobulin sustains for years (Steere, 2001; Kalish *et al*., 2001).

In 1976, the clinical feature that helped bring LB in patients to light was arthritis (Steere, 2001; Kalish *et al*., 2001). Therefore, arthritis has been at the grassroots of LB. It is a disorder that results in inflamed connective tissues (such as cartilage, tendons, and synovial tissue) and swollen joints (Fries *et al*., 1980a). Tabulated in Table 1 are a few examples for different arthritis types and subtypes with their characterization / symptoms. Spondyloarthritis (SpA) effects pelvic joints, spine and joints in the arms and legs (Kim *et al*., 2005). Among different types of arthritis, SpA is a distinct kind as it affects ligaments and tendons that support the spine (Kim *et al*., 2005). SpA symptoms such as pain and stiffness explain the inflammation in the spine (Rudwaleit *et al*., 2004). Further, for chronic / late form of SpA, i.e., ankylosing SpA, symptoms are presented in the form of deformities in spine structure due to bone destruction (Kim *et al*., 2005). Patients also experience some degree of spinal fusion in the latter (Kim *et al*., 2005). SpA is a hereditary disease; 30 genes that make an individual susceptible to SpA have been reported (Benjamin and Parham, 1990; Fries *et al*., 1980b; Kim *et al*., 2005). Among the 30 genes, individuals with Human Leucocyte Antigen (HLA)- B27 genetic predisposition have been recorded as the most susceptible group to develop SpA (Benjamin and Parham, 1990). Further, it has also been reported that genetic susceptibility for SpA extends beyond HLA-B27 with genes such as HLA-B60, HLA-DR1 (Brown *et al*., 1998), Tumour Necrosis Factor-α (TNF-α) (Milicic *et al*., 2000), and a few more. SpA is of particular interest in this study due to three fold. Firstly, Reactive Arthritis (ReA) is a SpA subtype (Colmegna *et al*., 2004a). Secondly, Lyme Arthritis (LA) is the most frequent and significant differential diagnosis for ReA (Berghoff, 2012). Third and most importantly, Hermansen, L and colleagues (2015) demonstrated Tick-Borne Disease (TBD) pathogen's diagnostic value for SpA patients when compared with ongoing techniques. Their study validates that SpA patients immunological response against *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis* was distinctly elevated when compared with healthy controls.

ReA is an asymptomatic form of arthritis that develops in reaction to a bacterial infection (Colmegna *et al*., 2004a; Panush *et al*., 2007). Onset post infection, within one to two weeks duration patients start expressing asymmetric oligoarthritis. Further, several days or at maximum several weeks are required by the bacterial infection to induce ReA (Braun *et al*., 2000; Carter and Hudson, 2009). Conventionally, ReA becomes chronic if an

infection persists for more than six months (Carter and Hudson, 2009). Patients present symptoms either in the form of pain and swelling in joints (such as ankle or knee), heels, toes, fingers or as persistent lower back pain (Carter and Hudson, 2009). Different bacterial infections are responsible for triggering reactive arthritis manifestations at four different sites of entry. Four examples of microbial involvement and their clinical consequences with regards to their different entry sites are; firstly, through gastrointestinal tract, clinical manifestations are presented as diarrhoea for *Yersinia enterocolitica* infection (Granfors *et al*., 1998), gastroenteritis for *Salmonella typhimurium* infection (Falkenhorst *et al*., 2013) or asymptomatic signs for *Campylobacter jejuni / fetus* infection (Braun *et al*., 1997). Secondly, through urogenital tract, clinical manifestations are presented as urethritis for *Chlamydia trachomatis* infection (Braun *et al*., 1997; Butrimiene *et al*., 2006), cervicitis for *Ureaplasma urealyticum* infection (Butrimiene *et al*., 2006) or endometritis for *Neisseria gonorrhoeae* infection (Liebling *et al*., 1994). Thirdly, through bronchopulmonal tract, clinical manifestations are presented as bronchitis / pneumonia for *Chlamydia pneumonia* infection (Braun *et al*., 1997). Lastly, through skin / mucosa clinical manifestations are presented as an EM rash for *Borrelia burgdorferi* infection (Jaulhac *et al*., 1996; Braun *et al*., 1997), cat-scratch disease for *Bartonella* infection (Arnold and McKenna, 1994) or brucellosis for *Brucella abortus* infection (Mohamed Zahidi *et al*., 2015).

Table 1. Different types of arthritis and a brief indication with regards to their character / symptom (Fries *et al*., 1980c; Kim *et al*., 2005; Berghoff, 2012).

Arthritis Type	Characterization / Symptoms
Spondyloarthritis	Affects spine and inflames tendons in hips, knees, and shoulders
Fibromyalgia	Affects tendons and muscles that support joints
Gout	Uric acid crystals in joints
Juvenile idiopathic arthritis	Results in swelling and loss of joint function
Lupus	Autoimmune form of arthritis
Osteoarthritis	Results in bone and cartilage destruction
Polymyalgia rheumatic	Damages tendons, muscles, ligaments and joint tissue
Psoriatic arthritis	Affects joints of toes and fingers
Rheumatoid arthritis	Affects synovial tissues
Sclerodoma	Overproduction of fibrous material to support skin structure
Reactive arthritis	Resultant of a bacterial or viral infection

As discussed earlier, LA is the most common and imperative differential diagnosis for ReA patients. It is an atypical type of ReA because of its association with Major Histocompatibility Complex class II (MHC class II) molecules (i.e, HLA-DR2, and HLA-DR4) instead of HLA-B27 (Hill *et al*., 1997; Kim *et al*., 2005; Kovalchuka *et al*., 2012). In the past, like Jaulhac, B and colleagues (1996), many (Braun *et al*., 1997) have confirmed detection of chromosomal *Borrelia burgdorferi* DNA in the synovial tissue and fluid of LB patients, but have not emphasized with regards to the inflammatory events that progress towards development of arthritis. Though animal models do not imitate pathological, immunological or clinical manifestations of LB, they do help comprehend pathogenic mechanisms, possible targets for therapeutic approaches, and most importantly the inflammatory events that progress towards development of arthritis (Barthold *et al*., 1993; Barthhold, 1996; Burchill *et al*., 2003; Christopherson *et al*., 2002; Nardelli *et al*., 2005; Peterson *et al*., 2007). Two animal models have been reported to correlate reasonably with LA conditions in humans. The first model emphasizes initial inflammatory events onset post infection (Barthold *et al*., 1993; Barthhold, 1996). Briefly, when a mouse was intradermally injected with *Borrelia burgdorferi*, within five days, the bacterium was found approaching the connective tissues around the joints, thus, inducing mild arthritis. This mild inflammation events in mice's joint capsules were characterized by the inflow of leukocytes (mostly neutrophils) and lymphocytes. Several weeks later, substantial damage to the synovial linings, ligaments, and tendons was observed due to a massive inflow of neutrophils, synovial hyperplasia, and fibrin deposition (Barthhold, 1996). Recurring arthritic episodes were brought to light when the mice were examined for a long-term duration (Barthold *et al*., 1993). The second model emphasizes sustenance of arthritis for several months (Christopherson *et al*., 2002; Burchill *et al*., 2003; Nardelli *et al*., 2005; Peterson *et al*., 2007). Briefly, two mice were independently treated, i.e. vaccinated against and infected with *Borrelia burgdorferi*, respectively. Several weeks later, on examining the tibiotarsal joints from both mice, it was revealed that histopathology of the mice vaccinated against *Borrelia burgdorferi* was similar to the mice that was infected with *Borrelia burgdorferi*. Therefore, the vaccinated mice developed arthritis with the same severity as that of the *Borrelia burgdorferi* infected mice. Identical histopathology lasted for more than a year, and the mice were found to have developed severe destructive arthritis (Christopherson *et al*., 2002). Both models not only validate the relationship between

Borrelia burgdorferi infection and arthritis, but also indicate the need for examining a broad range of inflammation and severities that could result in chronic arthritis manifestations in humans.

LB patients may also experience chronic manifestation in the form of coinfections (Eskow *et al*., 2001; Swanson *et al*., 2006). Coinfection is a late stage conditon in LB patients wherein *Borrelia* infection is accompanied by one or more than one bacteria that belongs to a different genus (Hunfeld, 2002). Multiple bacterial infections hamper the therapeutic success, and as a result, lead to worsening / intensifying of a patient's symptoms (Carter *et al*., 2010). Coinfections that are associated with LB are *Bartonella* species (Eskow *et al*., 2001), *Yersinia enterocolitica* (Saebø and Lassen, 1994), *Chlamydia pneumonia* / *trachomatis* (Braun *et al*., 1997; Butrimiene *et al*., 2006; Carter *et al*., 2010), *Mycoplasma pneumonia / fermentans* (Berghoff, 2012), *Babesia microti* (Mitchell *et al*., 1996), *Rickettsia* species (Hunfeld, 2002), *Franscisella tularensis* (Berghoff, 2012), *Coxiella burnetii (Berghoff, 2012), Campylobacter jejuni* (Braun *et al*., 1997; Berghoff, 2012), Human Granulocytic Anaplasmosis / Ehrlichiosis [HGA / HGE (including *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*)] (Mitchell *et al*., 1996; Thomas *et al*., 2001), and a few others. Effects of a coinfection (especially HGE) in LA patients can be devastating as demonstrated by Thomas, V and colleagues (2001) in a mouse model experiment. Briefly, three mice were intradermally inoculated with *Borrelia burgdorferi*, HGE and a mix of both *Borrelia burgdorferi* and HGE, respectively. The latter group was of particular concern as Thomas, V and colleagues had hypothesized that the mice infected with more than one bacteria would experience intensified symptoms when compared to the mice infected with a single bacteria type (*Borrelia burgdorferi* or HGE) . It was found that, the mice that was infected with only *Borrelia burgdorferi* developed arthritis within two weeks, but the mice that was infected with only HGE did not develop arthritis as the bacteria was cleared from the bloodstream (Thomas *et al*., 2001). Interestingly, the host immune response of the mice infected with both bacteria expressed a bacterial burden in two distinct ways (Thomas *et al*., 2001): 1) reduced Interferon gamma (IFN-γ) receptors on macrophages indicated decrease in phagocytosis activity, and 2) elevated levels of Interleukin- 6 (IL-6) noted increase in Th_2 activity. Not only did the authors find results supporting their hypothesis but also established that coinfection in LA conditions can progress to develop into a chronic condition with severe manifestations.

Mice models have indeed enabled our understanding regarding possible clinical outcomes in patients experiencing chronic manifestations such as LA and coinfections. Additionally, *Borrelia burgdorferi* has been reported to persist through antibiotic treatment (Embers *et al*., 2012). In a study carried out by Embers, E and colleagues (2012) 24 rhesus macaques (non-human primates) were infected with *Borrelia burgdorferi* to evaluate the efficiency of an antibiotic therapy (28 days routine of oral doxycycline) that is traditionally prescribed to humans (Wormser *et al*., 2006). Among 24 rhesus macaques that were infected with *Borrelia burgdorferi*, 12 rhesus macaques received an aggressive antibiotic treatment (28 days routine of oral doxycycline) after 4-6 months. Subsequently, techniques such as Polymerase Chain Reaction (PCR), immunofluorescence, culturing, and others were utilized to examine any residual *Borrelia burgdorferi* post-treatment. It was established that the *Borrelia burgdorferi* withstood the antibiotic therapy and this findings led to questioning the pathogenicity of antibiotic resistant / tolerant persisting *Borrelia* forms. In addition, *Borrelia burgdorferi* has also been reported to alter its morphology when exposed to unfavourable conditions (Miklossy *et al*., 2008; Meriläinen *et al*., 2015). Induction and isolation of a persistent *Borrelia* form was reported by Milkossy, J and colleagues (2008). The group observed that on exposing *Borrelia burgdorferi* culture to unfavorable conditions such as osmotic and heat shock the bacterium expressed itself in an atypical cyst like morphology. Similar *Borrelia* phenotypes were also isolated from the brain of three patients experiencing Lyme neuroborreliosis (LNB). Succeedingly, detail characterization of *Borrelia burgdoferi*'s atypical / pleomorphic form was recently provided by Meriläinen, L and colleagues (2015). Briefly, with regards to the *Borrelia burgdorferi'*s pleomorphic form, the study established four fold, firstly, *Borrelia burgdorferi'*s pleomorphic form was not cell deficient as it had a flexible yet intact cell wall, (therefore Meriläinen, L and colleagues termed it as Round Bodies (RB) instead of cyst). Secondly, adenosine triphosphate (ATP) studies revealed that the pleomorphic form displayed negligible metabolic activity when compared to its native spirochete form. Thirdly, the bacterium was able to transform its morphology (into RB) when exposed to stressful environmental conditions. Last and most importantly, *Borrelia burgdorferi* was able to change its morphology (into RB form) when exposed to human serum. In fact, the Atomic Force Microscopy (AFM) micrographs from the Milkossy J and colleagues (2008) study and the Electron Microscope (EM) micrographs from the Meriläinen, L and co-

workers (2015) study reported the same atypical / pleomorphic morphology of *Borrelia burgdorferi*. In general, bacterial pleomorphic forms have been reported to evade and challenge host's immune system by either changing their pathogenic mechanism or by experiencing reduced susceptibility towards antibiotic treatments (Domingue and Woody, 1997; Justice *et al*., 2008). In addition, Thammasri, K and colleagues studied the diagnostic value of *Borrelia burgdorferi* RB, (Thammasri *et al*., 2015) on an advance enzyme-linked immunosorbent assay (ELISA) multiplex array platform. The group demonstrated a high number of LB patients responding to the RB cell lysates when compared to the spirochete cell lysates. Also, it was reported that there were LB patients that did not respond to spirochete cell lysates, but were found to respond to the pleomorphic (RB) form cell lysates (Thammasri *et al*., 2015).

Essentially, chronic conditions indicate multiple overlapping etiologies that are manifested in a LB patient due to multiple TBD infections (Fukuda *et al*., 1994). In a LB patient, multiple tick borne disease infections (MTBDI) represents a combination of more than one bacteria that belongs to a different bacterial genus (Hunfeld, 2002) co-existing (i.e. coinfections) to intensify symptoms. As discussed earlier, under the TBD infections umbrella, there are diverse types of *Borrelia* spirochete strains (Rudenko *et al*., 2011; Radolf *et al*., 2012; Horowitz, 2013) and coinfections (Berghoff, 2012) that indicate a plethora of combinations through which chronic conditions with severe manifestations may be induced in a LB patient. Statistical tests such as binary logistic regression (Field, 2013) can help in modelling the immunological response frequencies and discovering / predicting key MTBDI combination(s) that may play an imperative role in inducing multiple overlapping chronic etiologies. The binary logistic regression modelling can be successfully implemented between two patient group's immunological response frequencies, but not between two individual LB patient's (Field, 2013) immunological response frequencies. However, implementing the test between two patient groups with supernumerary MTBDI combinations or individual TBD infections will be daunting, impractical and most importantly, difficult to infer results from a clinical perspective due to lack of clinical indications (early / late stage). Therefore, in the interest of discovering / understanding key MTBDI combination(s) that may play an important role in inducing chronic conditions, a holistic approach that unifies different types of *Borrelia* spirochete strains and co-infections is required. In addition to *Borrelia* spirochete strains and co-

infections, the approach is also required to include pleomorphic forms of *Borrelia* such as *Borrelia* RB. In accordance with recent findings, accounting *Borrelia* RB under the TBD infections umbrella is of paramount importance (Miklossy *et al*., 2008; Embers *et al*., 2012; Meriläinen *et al*., 2015). Currently, there is no holistic model that would facilitate implementation of predictive modelling such as the binary logistic regression.

In this study, a "Chronic Manifestation Knot (CMK)" model has been proposed for the first time to address the need for a holistic and unified approach that conviniently facilitates data vizualization (graphical analysis) and predictive modelling (statistical analysis) between two patient group's immunological response frequencies (Figure 2). Inspiration for constructing the model was obtained from "Triquetra" an ancient symbol that is also known as a "Trinity Knot" (Blindheim, 1985). As illustrated in Figure 2, three categories that establish the CMK model are, C1) Primary infection (i.e. *Borrelia* spirochete infections), C2) Persistent infection [i.e. *Borrelia* Round Body (RB) infections] and C3) Coinfections [Example: HGE (*Ehrlichia*)]. Practically, *Borrelia* spirochete genospecies such as *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia miyamotoi*, *Borrelia valaisiana*, and many others can be unified as "only *Borrelia* spirochete" category (C1) because they belong to the same family i.e. *Borrelia burgdorferi* sensu lato (Busch *et al*., 1996; Wang *et al*., 1999; Girschick *et al*., 2009; Rudenko *et al*., 2011). The same applies to persistent infections, wherein different *Borrelia* RB genospecies can be unified as "only *Borrelia* RB" category (C2). Persistent infection is an independent CMK category (C2) because, although *Borrelia* RB is an extension of its natives spirochete form (C1), the pleomorphic (RB) phenotype exhibits aberrant characteristics such as its antigenic properties (Thammasri *et al*., 2015), reduced susceptibility towards antibiotic treatments, and many others that result in changing its pathogenicity against the host (Domingue and Woody, 1997; Justice *et al*., 2008; Miklossy *et al*., 2008; Meriläinen *et al*., 2015). On those grounds, primary (C1) and persistent (C2) infections are two independent but overlapping CMK categories (Figure 2, right side). On the contrary, all coinfections belong to different genus (Hunfeld, 2002), but under the TBD umbrella they share the same pathogenesis mechanism, that is to accompany primary infection and induce chronic conditions with severe manifestations (Fukuda *et al*., 1994; Eskow *et al*., 2001; Swanson *et al*., 2006; Carter and Hudson, 2009; Berghoff, 2012). As discussed earlier, *Borrelia burgdorferi* accompanied by *Ehrlichia chaffeensis* results in

manifesting destructive arthritis conditions (Thomas *et al*., 2001), therefore *Ehrlichia chaffeensis* [only *Ehrlichia* (C3)] is of special interest in this study. For implemeting CMK model, at least 3 infections that correspond to categories C1, C2 and C3, respectively, are required.

The triangle in Figure 2 represents three primary ends (C1, C2 and C3) of a knot. MTBDI categories (MTBDI C4, MTBDI C5, MTBDI C6 and MTBDI C7) between the primary ends signify alternative ways in which a knot can be tangled / interlaced. Basically, a particular knot (MTBDI categories) qualitatively and quantitatively specifies the primary ends that interlace / combine to represent a type of MTBDI. Thus, MTBDI C4 (i.e. only *Borrelia* spirochete and Round Body) is the result of an interlace between C1 and C2, MTBDI C5 (i.e. only *Borrelia* spirochete and *Ehrlichia*) is the result of an interlace between C1 and C3, MTBDI C6 (i.e. only *Borrelia* Round Body and *Ehrlichia*) is the result of an interlace between C2 and C3, and MTBDI C7 (i.e. *Borrelia* spirochete, *Borrelia* Round Body and *Ehrlichia*) is the result of an interlace between C1, C2 and, C3. The conception behind the word “only” from C1 to MTBDI C7 is discribed below in section 3.8. The circle around the triangle in Figure 2 is called “Severity circle”. It denotes relative tightness / tension between all five knots (MTBDI C4, MTBDI C5, MTBDI C6 or MTBDI C7) that directly relates to severity in symptoms. Essentially, the maximum possible tension is located at the centre of the severity circle and the minimum possible tension is situated on the circumference of the severity circle. Thus, the area inside the circle signifies a tension gradient that originates on the circumference and strengthens radially up till the centre. Accordingly, MTBDI C4, MTBDI C5 and, MTBDI C6 share the same severity in symtomps, but MTBDI C7 is the most severe category. As illustrated in Figure 2, C8 represents the Nil response group, wherein presumably an individual is healthy.

Clinical indications (early and late stage) will facilitate interpretation of statistical results from a clinical perspective. Hence, all CMK categories (except C8) signify a clinical indication. In Figure 2 (right side), CMK categories and their clinical indications are evident in accordance with timeline 1 from Figure 1. Take note that unlike timeline 1 in Figure 1, early and late stage clinical indications presented in Figure 2 (right side) are independent of timeline 2 from Figure 1. Therefore, in Figure 2, IgM and IgG antibodies

represent early and late stages, respectively, but do not relate to seroconversion timeline (timeline 2) as shown in Figure 1. As discussed earlier, a *Borrelia* spirochete infection [primary infection (C1)] can persist from early (IgM) stage to late (IgG) stage (Steere, 2001; Kalish *et al*., 2001; Embers *et al*., 2012), but coinfections such as HGE [*Ehrlichia chaffeensis* (only *Ehrlichia* (C3)] accompany a primary infection only in the late (IgG) stage of the disease to manifest chronic conditions in a patient (Jaulhac *et al*., 1996; Berghoff, 2012). Also, in accordance with the one month antibiotic treatment timeframe that is prescribed to humans (Wormser *et al*., 2006), it is presumed that residual spirochete *Borrelia* would induce into RBs [persistent infection (C2)] sometime during the early stage (IgM) and then persist towards the late chronic stage (IgG). On those grounds, MTBDI C4 indicates progression from early stage into the late stage whereas MTBDI C5 indicates progressed into late stage, and MTBDI C6 indicates late stage infection. MTBDI C7 represents deep late stage infection.

Accordingly, all categories except C8 apply to the late stage / IgG response, but only categories C1, C2 and, MTBDI C4 apply to the early stage / IgM response. Ideally, a patient with IgM response should not be responding to any MTBDI categories except MTBDI C4. On the basis of a serological test (that results with an immunological response frequency), each category is intended to help in indicating the clinical standpoint either of an individual patient or a group of patients. For example, with respect to IgM response, if patient / patient group A and B respond to category C1 and MTBDI C4, respectively, then the latter is more inclined towards progressing into the late stage. Similarly, with respect to IgG response, if patient / patient group A and B respond to category MTBDI C5 and MTBDI C7, respectively, then the latter is presumably experiencing the effects of chronic conditions to a higher degree in comparison with the former. Among the 8 CMK categories, MTBDI C7 is the most severe category. Seemingly, a patient / patient group responding with IgG will always be more inclined towards developing / experiencing chronic conditions when compared to a patient / patient group responding with IgM. Therefore, CMK is a timeline gradient model that not only helps in clinically indicating a patient's infection / symptom stage but also helps in comparing two patient groups based upon their immunological response frequencies towards the 8 CMK categories. This study has compared two patient groups on the CMK platform that are characterized by LB (Thammasri *et al*., 2015) and SpA (Hermansen *et al*., 2015), respectively.

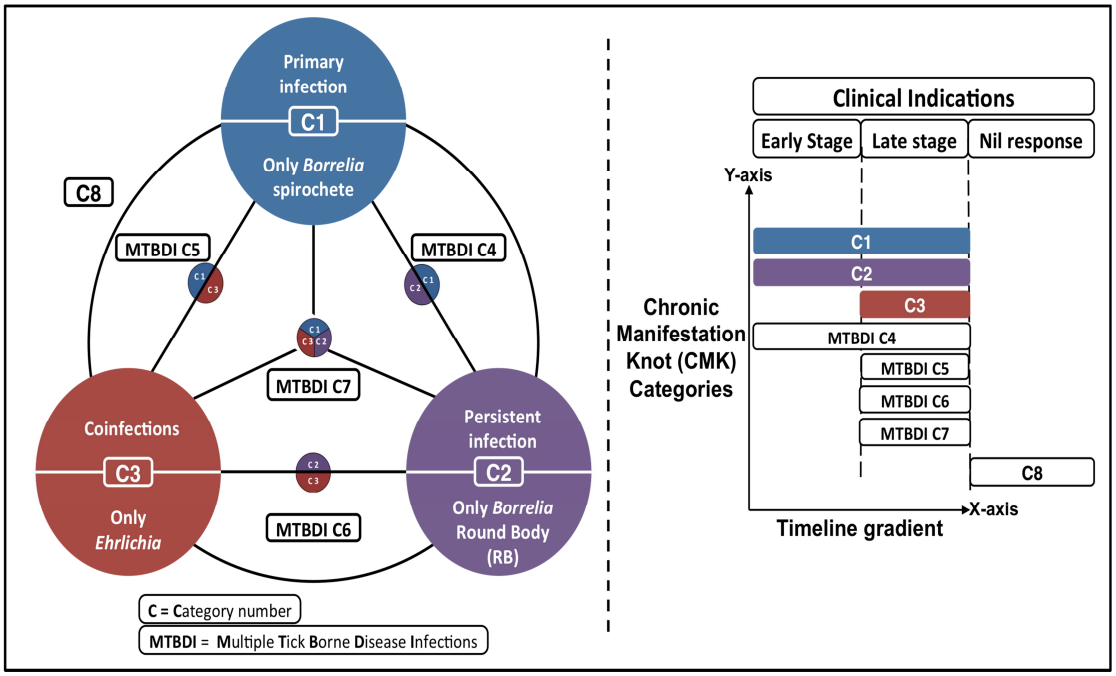

Figure 2: Chronic Manifestation Knot (CMK) model is a holistic and unified approach that facilitates graphical analysis and statistical analysis between two patient group's immunological response frequencies to help discover / predict key Multiple Tick Borne Disease Infection (MTBDI) combination(s) that may play an imperative role in inducing multiple overlapping chronic etiologies. Primary infection / C1 (i.e. *Borrelia* spirochete infections), Persistent infection / C2 (i.e. *Borrelia* Round Body (RB) infections), and Coinfections / C3 are the 3 primary categories upon which the CMK model has been established. The model is comparable with the widely known method of fastening a linear material (for example, a rope) by tying / interweaving / interlacing the respective ends of the material. All 3 primary categories (C1, C2, and C3) on the triangle embody ends to a particular knot, wherein the knots are represented by MTBDI categories. The MTBDI categories (MTBDI C4, MTBDI C5, MTBDI C6, and MTBDI C7) signify alternative ways in which a knot can be interlaced between the 3 primary categories. MTBDI C4 is the result of an interlace between C1 and C2, MTBDI C5 is the result of an interlace between C1 and C3, MTBDI C6 is the result of an interlace between C2 and C3, and MTBDI C7 is the result of an interlace between C1, C2, and C3. Further, the circle around the triangle is called "Severity circle". It denotes relative tightness / tension between all five knots (MTBDI C4, MTBDI C5, MTBDI C6 or MTBDI C7) that directly relates to severity in symptoms. Essentially, the maximum possible tension is located at the centre of the severity circle and the minimum possible tension is situated on the circumference of the severity circle. The C8 category represents healthy individuals that do not experience any severe conditions and are therefore outside the severity circle. Clinical indications for all 8 CMK categories on the right side aids in analyzing the statistical results from a clinical standpoint. All CMK categories (except C8) have been mapped on an time-gradient scale that ranges from early (IgM) to late (IgG) disease stage (see text below) in accordance with previous reports (Jaulhac *et al*., 1996; Domingue and Woody, 1997; Steere, 2001; Kalish *et al*., 2001; Wormser *et al*., 2006; Embers *et al*., 2012; Berghoff, 2012; Meriläinen *et al*., 2015). The model proposed is intended towards not only clinically indicating patient(s) infection / symptom stage but also comparing two patient groups based upon their immunological response frequencies towards the 8 CMK categories.

2. Aims of the study

It is known that LB patients may experience chronic manifestations in the form of LA (Berghoff, 2012), which is an atypical form of ReA (Hill *et al*., 1997; Kim *et al*., 2005; Kovalchuka *et al*., 2012). Also, ReA is a subtype of SpA (Colmegna *et al*., 2004b). Therefore, ReA in the form of LA could be a link between LB and SpA, wherein the latter is chronic by character (Kim *et al*., 2005). In addition, Hermansen, L and co-workers (2015) have demonstrated TBD pathogen's diagnostic value for SpA patient group. Their study (Hermansen, L *et al*., 2015) validates that SpA patient groups immune response to *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis* was distinctly elevated when compared with the healthy controls. Similarly, Thammasri, K and colleagues (2015) validated LB patient groups immune response to the same 4 antigens as Hermansen, L, and co-workers (2015). Thammasri, K *et al*., (2015) study concluded that the antigens utilized were found to be highly specific and sensitive when compared with the antigens being used in commercial serology based diagnostics for LB.

Aims of this study is to graphically and statistically compare the immune response frequencies between LB patients from Thammasri, K and co-workers study (2015) and SpA patients from Hermansen, L and colleagues (2015) study with the help of CMK model and it's 8 categories. In this study, it is hypothesized that;

1. Graphically, since SpA is chronic by character (Kim *et al*., 2005), response by SpA patients to CMK categories will appear more inclined towards developing / experiencing chronic conditions when compared to LB CMK categories across both antibody types.
2. Patients with an IgM response in both LB and SpA groups will not respond to any CMK categories except 1, 2, and 4.
3. Statistically (binary logistic regression), at least one CMK category among 2, 4, 6, and 7 between LB and SpA groups will result as a potential predictor variable. The *Borrelia burgdorferi* RB is a common factor among categories 2, 4, 6, and 7. This result will indicate that *Borrelia* RBs may serve as a bridge between a primary infection and a coinfection in LB patients that leads to the development of chronic conditions such as LA / SpA.

3. Materials and methods

Though this study emphasizes graphical and statistical analysis of LB (Thammasri *et al*., 2015) and SpA (Hermansen *et al*., 2015) patient groups, experimental validation only for the latter was performed as a part of this study. In addition, to match the number of antigens between both the studies and also to abide to the CMK model's pre-requisite for at least 3 infection types that correspond to C1, C2, and C3 categories (Figure 2), SpA patients were also tested for *Borrelia burgdorferi* RB. Experimentally, both studies differ with regards to their serum sample collection process, and apart from this all other materials as well as the methods were the same.

3.1. Serum sample collection

As reported by Thammasri, K and colleagues (2015), 54 well-characterized serum samples for Lyme disease (LD) were received from the Borreliose Centrum Augsburg (BCA) clinic, which was approved by the Federal Institute for Drugs and Medical Devices, Germany (Ethical approval number: 95.10-5661-7066). Under the same ethical approval, 15 healthy serum samples were obtained to serve as negative controls. The author kindly provided raw experimental results (Optical density (OD) values) for this thesis.

Similarly, for this study as reported by Hermansen, L and colleagues (2015), 85 well-characterized serum samples for Spondyloarthritis (SpA) were received from the King Christian 10th Hospital for Rheumatic Diseases, Denmark, which was approved by the Danish data protection agency and the regional ethics committee of Southern Denmark (Ethical approval number: S-20110029). Under the same ethical approval, 40 healthy serum samples were obtained to serve as negative controls. In order to match the sample sizes of SpA and LD, 54 SpA serum samples were randomly chosen for graphical and statistical analysis performed in this study. Therefore, the sample size (n) in this study from both patient groups is 54 serum samples (n = 54).

3.2. Preparation of antigens for Enzyme Linked Immunosorbent Assay (ELISA)

Five antigens were utilized in this study: *Borrelia burgdorferi sensu stricto B31* (ATCC35210), *Borrelia afzelii P12* (ATCC 51567), *Borrelia garinii Fuji P1* (ATCC 51991), *Borrelia burgdorferi sensu stricto B31* Round Bodies, and *Ehrlichia chaffeensis*.

Cultures were obtained from the American Type Culture Collection (ATCC) for the former three infectious pathogens. For *Ehrlichia chaffeensis*, a peptide with the following amino acid sequence, NH_2 – SAVSNRKLPLGGVLMALVAAVAPIHSALLA - COOH (Dumler and Bakken, 1995; Brown *et al*., 2001) was used (GeneCust, Luxembourg). The peptide was > 95% pure and weighed 1 mg.

3.3. Culturing and isolation of *Borrelia* in spirochete and pleomorphic form

Barbour-Stoenner-Kelly (BSK) medium was utilized for growing all three *Borrelia* cultures. The BSK medium was prepared in accordance with previously reported instructions (Barbour and Hayes, 1986). In order to culture and isolate *Borrelia* species in their native spirochete form, each *Borrelia* strain was independently grown in BSK medium at 37 ˚C for 5-7 days (Meriläinen *et al*., 2015). After 5-7 days of incubation at 37 ˚C, the cells were isolated by centrifuging the cell culture tubes at 5000 × g for 10 min (Meriläinen *et al*., 2015). The supernatant was discarded, and the pellet was stored at -80˚C until further use. For culturing and isolating *Borrelia burgdorferi sensu stricto B31* Round Bodies, a similar methodology was used. Further, instead of storing the spirochete cells at -80˚C, the cells were resuspended in 2 ml of distilled water (Meriläinen *et al*., 2015). Post 2 h incubation of spirochete *Borrelia* cells in distill water, the solution was centrifuged at 5000 × g for 10 min (Meriläinen *et al*., 2015). Again, the supernatant was discarded, and the *Borrelia burgdorferi sensu stricto B31* Round Body pellet was stored at -80˚C until further use.

3.4. Culturing and Isolation of *Escherichia Coli* (*E. coli*)

Laboratory grade *E.coli* DH5α cells were cultured in luria broth (LB) medium. The cells were incubated for 2h and 30 min at 37 ˚C on a shaker [250 rpm, New Brunswick Scientific Classic Series (C24 incubator shaker)]. Once the Optical Density (OD) value at 600 nm was measured as 1.0 (i.e., 1×10^9 cells/ml), aliquots of 200×10^6 cells were made into 2 ml eppendorf tubes. The tubes were centrifuged at 13,000 × g for 5min after which the supernatant was discarded, and the pellet was stored at -80 ˚C until further use.

3.5. Processing of isolated bacterial pellets for utilization in ELISA

All pellets were thawed on ice and were carefully resuspended in 100 µl of phosphate buffered saline (PBS, pH 7.4). To dissociate the cell wall in the lysates and homogenously dissolve the contents in PBS, all solutions in tandem were sonicated for 15 min (Bransoni C220), heated at 99.9 ˚C for 15 min and sonicated again for 15 min. Finally, 1 mg/ml stock concentration for all antigens was stored at +4 ˚C.

3.6. ELISA procedure

The 1 mg/ml antigen stocks were diluted at 1:100 dilutions in 0.1 M carbonate buffer (0.1 M Na_2CO_3 + 0.1 M $NaHCO_3$, pH 9.5). In addition for the antigens, similar dilution was used for two positive controls, human IgG (Sigma #I251T) and human IgM (Sigma #I8260). As a negative control, *E. coli* DH5α was diluted to 1:400. In order to realize consistency with regards to resulting Optical Density (OD) value at 450 nm, these positive and negative controls were used.

A 100 µl of antigens and controls were coated in triplicates and duplicates, respectively, on a flat bottom 96-well polystyrene ELISA plates (Nunc) and were incubated at +4 ˚C overnight. Post incubation, the plates were washed three times with 300 µl of PBS-Tween (PBS + 0.05% Tween 20) and were then coated with 100 µl of 2% BSA (Sigma #A7030) in PBS. After an overnight incubation at +4 ˚C, the 2% BSA in PBS was discarded, and 100 µl of patient serum diluted to 1:200 in 1% BSA/PBS was added. The plates were then allowed to incubate for 2h at room temperature, and washed five times with 300 µl of PBS-Tween. An amount of 100 µl of Horse Radish Peroxidase (HRP) conjugated to mouse anti-human IgG (Abcam #ab99765) or IgM (Novus #NBP1_42415) was introduced to the plates at 1:10000 or 1:1000 dilutions, respectively. After 1.5 h incubation at room temperature, plates were washed five times with 300 µl of PBS-Tween and were then supplemented with 100 µl of 3,3',5,5' Tetramethylbenzidine substrate (TMB, 1-Step ultra TMB-ELISA substrate, Thermo-Piercenet #34028). Plates that were previously supplemented with HRP conjugated mouse anti-human IgG or IgM were incubated at room temperature for 5 min or 1 h, respectively. The reaction between the secondary antibodies and TMB substrate was stopped by adding 100 µl of 2 M H_2SO_4.

Further, Victor^{TM} X^{4} multi-label plate reader (Perkin Elmer 2030 manger) was utilized to measure the OD values at 450 nm at 0.1 sec.

3.7. Data processing for graphical and statistical analysis

In the interest of establishing a cut-off value for all antigens in both the studies and for SpA patients response to *Borrelia burgdorferi sensu stricto B31* Round Bodies, average absorbance (OD at 450 nm) from respective healthy sera samples for individual antigens was added to three times standard deviation of the average absorbance (Thammasri *et al*., 2015; Hermansen *et al*., 2015). Finally, raw data set was obtained in the form of IgM and IgG OD values for all 5 antigens. To maintain uniformity with regards to the differing OD intensities between the patient groups, all 54 original IgM and IgG OD values for every antigen were divided by their respective cut-off values. Thus, resulting in normalizing original values to create an Optical Density Index (ODI) data set for each antigen's IgM and IgG responses in both patient groups. Finally, the ODI values were converted into a binary data set that contained 1 or 0 to denote positives or negative, respectively. To establish a binary data set post normalization, all values below the borderline threshold (i.e., 0.9) were denoted as 0 and values at or above the borderline threshold were denoted as 1. In the end, there were four binary data sets, i.e., LB IgM, SpA IgM, LB IgG, and SpA IgG.

Further, the same binary data set from individual antigens with IgM and IgG responses were independently utilized to organize the 8 CMK categories for both patient groups. Firstly, *Borrelia burgdorferi*, *Borrelia afzelii* and *Borrelia garinii* were unified as "Only *Borrelia* spirochete" category (discussed earlier). Secondly, as it is already evident, the persistent infection and the coinfection in this study are *Borrelia burgdorferi* RB and *Ehrlichia chaffeensis*, respectively. To obtain immunological response frequencies out of any one particular binary data set of four, a simple scoring approach was adopted for all patients independently. For example, if a patient had only responded to any one of the *Borrelia* spirochete species, then only CMK C1 was scored as 1 and all other 7 categories were scored as 0. Similarly, is the case for C2 and C3. With regards to MTBDI CMK categories, if a patient had responded to any one of the *Borrelia* spirochete species and *Borrelia burgdorferi* RB, then only MTBDI C4 was scored as 1 and all others as 0. Similarly, based on respective MTBDI knots / combination 5 and 6 were scored.

Seemingly, if a patient responsed to any one *Borrelia* spirochete species, *Borrelia burgdorferi* RB and *Ehrlichia chaffeensis*, then only MTBDI C7 was scored as 1 and all others as 0. Lastly, if a patient had not responded to any of the five antigens in a particular binary data set (or for an antibody type), then category 8 was scored as 1 and all others 0. Therefore, in all four binary data sets, each patient could be scored as 1 only for one of the CMK categories based on its response to five antigens. In the end, there were four CMK binary data sets, i.e., LB IgM, SpA IgM, LB IgG, and SpA IgG. In all cases, binary data sets reflect the immunological response frequencies / proportions for graphical and statistical analysis.

3.8. Software and instruments used

ND 1000 spectrophotometer (finnzymes) was used to measure protein concentration of cell lysates at 280 nm. Victor^{TM} X^4 multi-label plate reader (Perkin Elmer 2030 manger) was utilized to measure the OD values at 450 nm at 0.1 sec. Microplate washer DNX-9620G (Nanjing Perlove Medical Equipment Co., Ltd) was used for washing ELISA microplates.

Microsoft Excel 2010 was utilized to convert OD values and establish all 8 binary data sets. It was also used in plotting pie charts and pie of pie charts that have been used for graphical representation of the results. IBM SPSS version 23 was utilized to compare the immunological response frequencies proportions between LB and SpA patient groups with the help of Fisher 's exact test. It is a test for independence / association between two proportions (Field, 2013). Therefore, the Fisher 's exact test statistically compares two proportions of binary data sets from LB and SpA groups with the intent to examine whether they are significantly dependent or independent. Also, binary logistic regression was performed to discover possible predictor variables between both the patient groups (Field, 2013). Predictor variables will help in understanding statistically relevant proportions from a clinical perspective.

4. Results

4.1. Response by SpA patient group to *Borrelia burgdorferi* Round Body (RB)

Figure 3 presents a scatter plot for IgM (Figure 3A) and IgG (Figure 3B) responses by SpA patient's sera samples to *Borrelia burgdorferi* RB. The cut-off mark for IgM and IgG antibody types was computed as 0.341 and 1.037 (cut-off line), respectively. Figure 3A and 3B demonstrate 4 data point categories, SpA sera samples that were found to be positive, borderline (in very close proximity with the cut-off line) or negative to *Borrelia burgdorferi* RB and the healthy / control sera samples. In Figure 3A, 19, 8, and 27 SpA patients were recorded as positive, boderline, and negative, respectively to *Borrelia burgdorferi* RB. In Figure 3B, 29, 4, and 21 SpA patients were recorded positive, boderline, and negative, respectively to *Borrelia burgdorferi* RB. It was inferred that SpA patient's positive immunological response against *Borrelia burgdorferi* RB is distinctly elevated when compared with healthy controls.

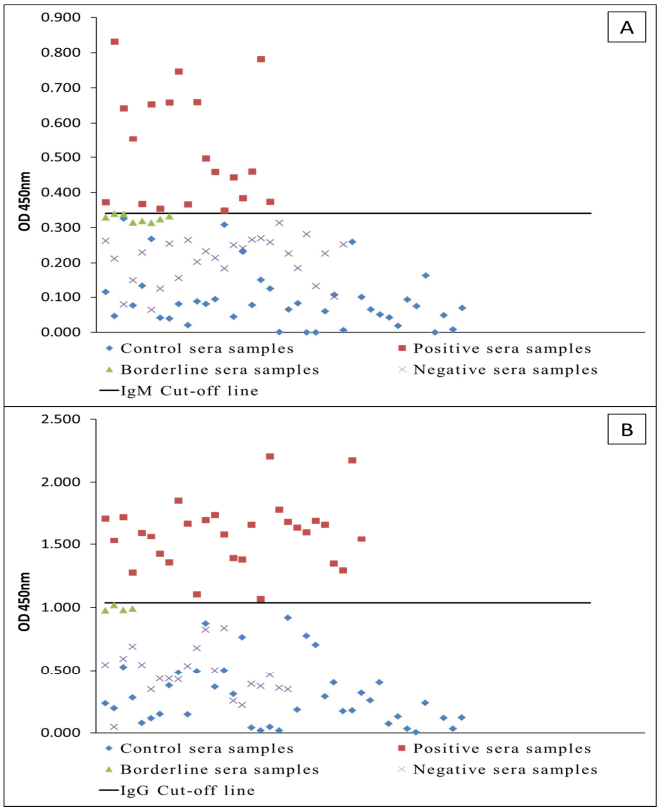

Figure 3: Immune response (IgM and IgG) by SpA patients to *Borrelia burgdorferi* RB. A) IgM response by SpA patient sera samples to *Borrelia burgdorferi* RB, and B) IgG response by SpA patient sera samples to *Borrelia burgdorferi* RB. Positive IgM and IgG immune responses by SpA patients to *Borrelia burgdorferi* RB was recorded to be noticeably elevated when compared with the 40 control sera samples.

4.2. Graphical analysis between LB and SpA non-CMK binary data sets

As mentioned earlier, Hermansen, L and colleagues (2015) experimental validation was performed as a part of this study. Though detailed analysis and presentation of the experimental results from both the studies (Hermansen *et al*., 2015; Thammasri *et al*., 2015) are beyond the scope of this study, presented below in Figure 4 are pie charts with percentages of positive IgM and IgG responses by LB and SpA patients to *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii* and *Ehrlichia chaffeensis* independently. In total, 55, 120, 134, and 184 positive responses to all five antigens were recorded in LB IgM, IgG, SpA IgM, and IgG groups, respectively. Specifically in LB IgM (Figure 4A), 4 (7%), 10 (18%), 4 (7%), 26 (48%), and 11 (20%) positive responses were recorded for *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis*, respectively. In SpA IgM group (Figure 4B), 42 (32%), 27 (20%), 23 (17%), 27 (20%), and 15 (11%) positive responses were recorded for *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis*, respectively. In LB IgG group (Figure 4C), 27 (23%), 29 (24%), 17 (14%), 16 (13%), and 31 (26%) positive responses were recorded for *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis*, respectively. Lastly, in SpA IgG group (Figure 4D), 40 (22%), 33 (18%), 34 (18%), 38 (21%), and 39 (21%) positive responses were recorded for *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis*, respectively.

In Figure 4, pie charts are easy to visualize and compare when viewed in percentages instead of individual counts. Percentages offer a more relative and holistic outlook making it easy to compare and contrast between the IgM and IgG responses in both patient groups. In general, with IgM, LB and SpA patients were found dominantly responding to *Borrelia* spirochete species i.e. *Borrelia garinii*, and *Borrelia burgdorferi*, respectively. In contrast, with IgG, LB and SpA patients were found responding to all five antigens quite equally. On comparing IgM and IgG responses of patients within LB group, it was observed that all four antigens except *Borrelia garinii* witnessed an increase in their respective IgG response proportions when compared to IgM. Evidently, LB IgG proportions were quite uniformly segregated among the five antigens presumably indicating that most patients have

responded to multiple antigens when compared with their IgM responses. Also, the total number of positive frequencies observed in LB IgG group was 120, which is a little more than two fold when compared to the total number of IgM (55) positive responses, meaning, all five antigens have experienced at least two fold number of LB patients responding to them, respectively. On comparing IgM and IgG responses of patients within SpA group, unlike the LB group, there was no particular pattern observed explaining the increase or decrease in proportions among antigens between the two antibody types. Seemingly, SpA IgG proportions were also quite uniformly segregated among the 5 antigens presumably indicating that most patients have responded to multiple antigens when compared with their IgM counterparts. Also, the total number of positive frequencies observed in SpA IgG group was 184, which is a little more than one fold when compared to the total number of IgM (134) positive responses, meaning, all five antigens have experienced at least one fold number of SpA patients responding to them, respectively.

On comparing LB IgM and SpA IgM positive immune response proportions, it was noticed that only *Borrelia burgdorferi* RB had maintained a relatively consistent positive response proportion between both patient groups. Clearly, SpA IgM positive immune respone proportions were quite uniformly segregated among the 5 antigens indicating that most patients have responded to multiple antigens when compared with the LB IgM response group. Also, the total number of positive frequencies that were observed in SpA IgM group was 134, which is a little more than two fold when compared to the total number of LB IgM (55) positive responses, meaning, all 5 antigens have experienced at least two fold or number of SpA patients responding to them, respectively. Similarly, on comparing LB IgG and SpA IgG positive immune response proportions, it was observed that almost all proportions had maintained relatively consistent positive response proportions between the two patient groups, but SpA IgG was slightly more uniformly segregated among the 5 antigens when compared with LB IgG, indicating that SpA IgG patients have responded to multiple antigens at a higher degree of uniformity when compared with LB IgG response group. Therefore, in descending order, patient groups that have relatively responded to all 5 antigens in a uniform manner are SpA IgG, LB IgG, SpA IgM and LB IgM. Similar is the order for total number of positive frequencies in each group in descending order.

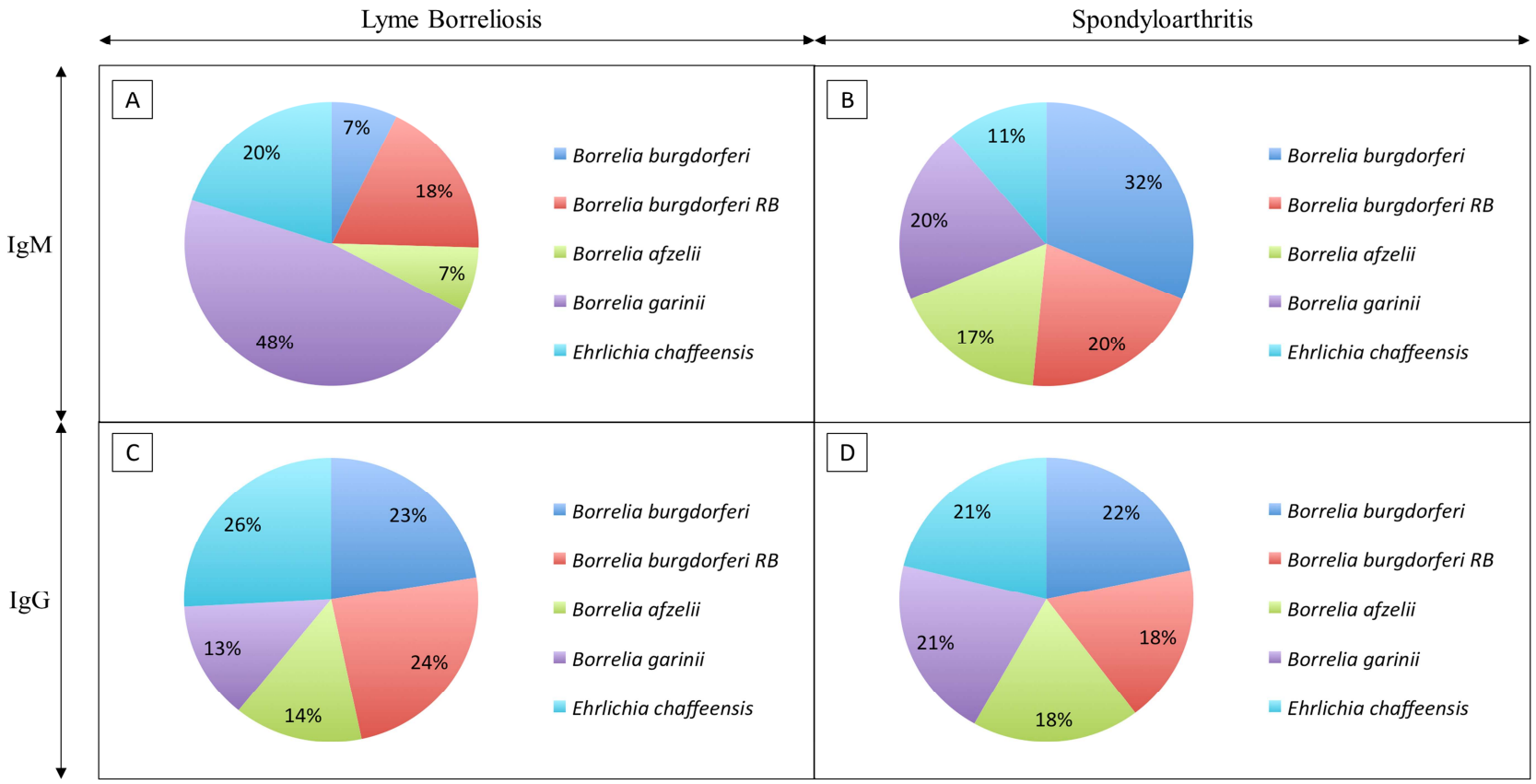

Figure 4: Graphical representation of positive IgM and IgG responses by LB and SpA patients to *Borrelia burgdorferi, Borrelia burgdorferi* RB, *Borrelia afzelii, Borrelia garinii,* and *Ehrlichia chaffeensis* independently. A) Collectively, 55 positive IgM immune responses by LB patients to all 5 antigens were recorded. B) Collectively, 134 positive IgM immune responses by SpA patients to all 5 antigens were recorded. C) Collectively, 120 positive IgG immune response by LB patients to all 5 antigens were recorded. D) Collectively, 184 positive IgG immune response by SpA patients to all 5 antigens were recorded. The difference in the total number of positive responses by both patient groups against both antibody types was due to patients responding to multiple antigens.

4.3.Statistical analysis between LB and SpA non-CMK binary data sets

Graphical analysis between LB and SpA non-CMK binary data sets gave an outlook with regards to only the positive immunological response frequencies. Further, in order to statistically examine whether immunological response frequencies / proportions of any two antigens between LB and SpA groups are significantly dependent or not, both negative and positive frequencies are imperative for analysis. Also, the negative response by a patient when tested for an antigen, is of paramount importance because in real-time diagnosis this would indeed save the patient from enduring a possible antibiotic treatment. As discussed earlier, Fisher 's exact test was used to statistically compare all five antigens between both patient groups against each other for significant or non-significant association. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG as shown in Appendix A, Table A.

Briefly, for all 50 Fisher's exact test proportions (IgM and IgG included), observed counts (OBC), expected counts (EXPC) and Cramer's V (φ_C) values have been tabulated. Pair of OBC and EXPC is listed for all negative and positive immunological response frequencies from LB and SpA. Simply, the number of negatives and positives counts observed in the binary data set corresponding to a particular Fisher's exact test proportion has been tabulated in the OBC columns. EXPC differs from OBC in the way that, rather than physical negative and positive counts, it is a statistically computed value that assumes no-hypothesis condition. At the outset, test for independence between two proportions initiates with a no-hypothesis condition where it is presumed that a particular Fisher's exact test proportion is non-significantly dependent / associated solely based on the EXPC values. Thus, EXPC is the number of counts that we would expect to observe if there was no significant dependence /association between the two proportions that are being tested, meaning, OBC should be different from EXPC. Fisher's exact test informs us if the difference between OBC and EXPC counts is diverse enough for the proportions to be statistically and significantly dependent or not. In addition, Fisher's exact test sheds light only on the association between two proportions, but not the strength of association, which is also called an "effect size." φ_C value, indicates effect size. It ranges from 0 to 1 where a particular Fisher's exact test proportion is eminently associated / dependent at 1 and vice

versa at 0. In appendices Table 1, φ_C value has been tabulated in the last column for each Fisher's exact test proportion. Associated with the φ_C values is an asterisk that signifies the level of dependence. i.e. * implies that a Fisher's exact test proportion is significantly dependent at $p \leq 0.05$.

Practically, when testing *Borrelia burgdorferi* antigens between LB and SpA patients with IgM antibody type (Appendix A, Table A), if there was no association between the negatives and positives from both the patient groups then the OBC would have either exactly matched or had been closely comparable with their respective EXPC. From the φ_C value and an asterisk (*) it is clear that the difference between the OBC and EXPC is diverse enough for this particular Fisher's exact test proportion to be significantly dependent on $p \leq 0.05$ with 0.712 φ_C value implying eminent dependence. Another way of looking at this is that, the difference between the positive and negative OBC between LB and SpA is not diverse enough for this particular Fisher's exact test proportion to be significantly independent. Not all proportions are dependent, as seen in a few proportions such as the Fisher's exact test between *Borrelia burgdorferi* and *Borrelia burgdorferi* RB. Here, the difference between the OBC and EXPC was not diverse enough for both proportions to be statistically dependent. Mainly, the proportions did not qualify because they were non significantly associated below or at least at $p \leq 0.05$ that resulted in a very low φ_C value. Another way of looking at this is that, the difference between the positive and negative OBC between LB and SpA was diverse enough for this particular Fisher's exact test proportion to be significantly independent. Similar dependence or independence approach on the basis of OBC and EXPC can be noticed in all other Fisher's exact test proportions in Appendix A, Table A.

Table 2 holistically represents all the details provided in Appendix A, Table A. All SpA antigen types accommodate two columns that represent the Fisher's exact test results for two particular antigen types, each from LB and SpA. The left column represents Fisher's exact test results for IgM antibody type, and the right column represents Fisher's exact test results for IgG antibody type. As presented in Table 3 footnotes, each test result is colour coded to indicate significant dependence between two particular antigen types, each from LB and SpA. Dark Blue and Dark Red colours represent significantly dependent Fisher's exact test proportions for IgM and IgG, respectively. White / blank represents

non-significant / independent tests results for both antibody types. In addition, φ_C value range associated with dependent ($p \leq 0.05$) and independent Fisher's exact test proportions have been tabulated in the footnotes of Table 2. In total, 50 Fisher's exact test proportions were tested, of which 40 proportions were found significantly dependent i.e. 21 proportions were significantly dependent for IgM, and 19 proportions were significantly dependent for IgG. It is difficult to place statistically dependent proportions tabulated in Table 2 into any clinical perspective because individual antigens do not correspond to any particular scale bar like the time gradient scale for CMK categories. Therefore, Binary logistic regression was not performed on the 40 significantly dependent Fisher's exact test proportions.

Table 2: Holistic representation of Appendix A Table A wherein Fisher's exact test was used to statistically compare immunological response frequencies (binary data set) of *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis* between both patient groups against each other for significant or non-significant association. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG. All SpA antigen types accommodate two columns that represent the Fisher's exact test results for two particular antigen types, each from LB and SpA. The left column represents Fisher's exact test results for IgM antibody type, and the right column represents Fisher's exact test results for IgG antibody type. Dark Blue and Dark Red colours represent significantly dependent Fisher's exact test proportions for IgM and IgG respectively. White / Blank represents non significant / independent tests results for both antibody types. In addition, φ_C value range associated with dependent ($p \leq 0.05$) and independent Fisher's exact test proportions have been tabulated in the footnotes.

Patient Type	Spondyloarthritis									
Lyme Borreliose	**Antigen Types**	*Borrelia burgdorferi*		*Borrelia Round Body*		*Borrelia afzelii*		*Borrelia garinii*		*Ehrlichia chaffeensis*	
	Borrelia burgdorferi										
	Borrelia Round Body										
	Borrelia afzelii										
	Borrelia garinii										
	Ehrlichia chaffeensis										
	Antibody Types*	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**	**IgG**	**IgM**	**IgG**

* Color code for IgM and IgG associated with P and Cramer's V value are as follows

Parameter / *P* Value	IgM	IgG	Cramer's V Value (φ_C) range
$P \leq 0.05$			0.172 – 0.712
Independent proportions			0.019 – 0.134

From Table 2 / Appendix A, Table A, we know that more than ¾ of the Fisher's exact proportions were found to be significantly dependent, signifying that the immunological response frequencies of antigens across both antibody types between LB and SpA groups statistically correlate at 80%. This implies significant dependence between the LB and SpA patient groups immunological response frequencies. Further, out of 18 Fisher's exact

proportions (IgM and IgG included) between *Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia garinii* from both patient groups, 15 proportions were found to be significantly dependent i.e. more than 80% correlation, indicating high dependence between both patient groups immunological response frequencies towards *Borrelia* spirochete species. This observation also supports unification of all *Borrelia* spirochete species as "only *Borrelia* spirochete / C1" in CMK model (Figure 2). On that account, CMK categories qualify for graphical and statistical analysis.

4.4. Graphical analysis between LB and SpA CMK categories binary data sets

On the basis of the previously discussed approach behind the CMK and its 8 categories, presented below in Figure 3 are pie charts and respective pie of pie charts with percentages of positive IgM and IgG responses by LB and SpA patients towards all 8 CMK categories. Unlike Figure 4, scoring methodology for CMK categories resulted in a uniform total number of positive responses that is equal to the sample size (n = 54) in LB IgM, IgG, SpA IgM, and IgG. As discussed earlier (Figure 2), 8 CMK category names that have been used in this study are "only *Borrelia* spirochete / C1", "only *Borrelia* Round Body RB / C2", "only *Ehrlichia* / C3", "only *Borrelia* spirochete and *Borrelia* RB / MTBDI C4", "only *Borrelia* spirochete and *Ehrlichia* / MTBDI C5", "only *Borrelia* RB and *Ehrlichia* / MTBDI C6", "*Borrelia* spirochete, *Borrelia* RB and *Ehrlichia* / MTBDI C7", and "Nil response / C8". These categories will be interchangeably referred by their name or category number from here on.

In LB IgM (Figure 5A), 13 (24%), 5 (9%), 3 (5%), 9 (17%), 2 (4%), and 22 (41%) positive responses were recorded for CMK categories 1, 2, 4, 5, 7, and 8, respectively. No positive responses were recorded for CMK categories 3, and 6. In SpA IgM (Figure 5B), 15 (28%), 1 (2%), 16 (29%), 6 (11%), 9 (17%), and 7 (13%) positive responses were recorded for CMK categories 1, 2, 4, 5, 7, and 8, respectively. No positive responses were recorded for CMK categories 3, and 6. In LB IgG (Figure 5C), 9 (17%), 7 (13%), 4 (7%), 8 (15%), 16 (30%), and 10 (18%) positive responses were recorded for CMK categories 2, 3, 4, 5, 7, and 8, respectively. No positive responses were recorded for CMK categories 1, and 6. In SpA IgG (Figure 5D), 1 (2%), 3 (6%), 4 (7%), 10 (18%), 26 (48%), and 10 (19%) positive responses were recorded for CMK categories 2, 3, 4, 5, 7, and 8, respectively. No positive responses were recorded for CMK categories 1, and 6. Evidently, no positive

responses were recorded for categories 3, and 1 both in LB and SpA patient groups with IgM and IgG antibody types, respectively. Also, no postive responses were recorded for category 6 in both patient groups and among both antibody types.

In Figure 5, pie of pie chart in each section also represent the percentages of positive responses observed towards all 8 CMK categories. They are only associated with the Nil response category, a category that is scored as 1 if a patient had not responded to any of the five antigens in either LB or SpA group for an antibody type. In order to obtain complete knowledge about truly negative serum samples or patients that have responded to the Nil response category for a particular antibody type, it is also important to record the same serum samples responses from the other antibody type. Pie of Pie charts has been presented with an intention to address this complete knowledge subject. For example, in Figure 3A, the main pie chart presents percentages of positive responses by all LB patients towards CMK categories with IgM, but the pie of pie chart shows percentages of positive responses only by LB patients that have responded to the Nil response category towards all CMK categories with IgG antibody type. Similarly, in Figure 3C, the main pie chart presents percentages of positive responses by all LB patients towards CMK categories with IgG, whereas the pie of pie chart presents percentages of positive responses only by LB patients that have responded to the Nil response category towards all CMK categories with IgM antibody type. The Same approach was applied to the SpA patient group.

In LB IgM pie of pie chart (Figure 5A), 22 LB patients had responded to CMK category 8 with IgM, of which 5 (23%), 4 (1%), 3 (14%), 2 (9%), 9 (41%) and 2 (9%) LB patients were recorded responding to CMK categories 2, 3, 4, 5, 7 and 8, respectively with IgG. No positive responses were recorded for CMK categories 1 and 6. In SpA IgM pie of pie chart (Figure 5B), 7 SpA patients had responded to CMK category 8 with IgM, of which 1 (14%), 3 (43%) and 3 (43%) SpA patients were recorded responding to CMK categories 4, 5 and 7, respectively with IgG. No positive responses were recorded for CMK categories 1, 2, 3, 6and 8. In LB IgG pie of pie chart (Figure 5C), 10 LB patients had responded to CMK category 8 with IgG, of which 3 (30%), 2 (20%), 1 (10%), 2 (20%) and 2 (20%) LB patients were recorded responding to CMK categories 1, 2, 4, 5 and 8, respectively with IgM. No positive responses were recorded for CMK categories 3, 6 and 7. In SpA IgG pie of pie chart (Figure 5D), 10 SpA patients had responded to CMK

category 8 with IgG, of which 4 (40%), 4 (40%) and 2 (20%) SpA patients were recorded responding to CMK categories 1, 4 and 7, respectively with IgM. No positive responses were recorded for CMK categories 2, 3, 5, 6 and 8. Seemingly, from LB IgM and IgG pie of pie charts it was clear that out of 54 LB serum samples 2 serum samples that were truly negative. In contrast, from SpA IgM and IgG pie of pie charts it was lucid that none of the serum samples were truly negative. Hence, the Nil response category represents patients immunological response frequencies towards CMK categories across the antibody types.

In general, with IgM, about a quarter of both patient groups were found responding to C1, but with IgG, both patient groups were found dominantly responding to the MTBDI C7. LB patients with IgM responded to C8, C1, MTBDI C5, C2, MTBDI C4, and MTBDI C7 in descending order. With IgG, LB patients responded to MTBDI C7, C8, C2, MTBDI C5, C3, and MTBDI C4 in descending order. Similarly, SpA patients with IgM responded to MTBDI C4, C1, MTBDI C7, C8, MTBDI C5, and C2 in descending order. With IgG, SpA patients responded to MTBDI C7, C8, MTBDI C5, MTBDI C4, C3, and C2 in descending order.

On comparing IgM and IgG responses of patients within LB group, it was observed that there was a 2 fold decrease in the number of patients responding to C8 from IgM to IgG, but there was a 2 fold and 8 fold increase in the number of patients that responded to C2 and MTBDI C7 from IgM to IgG. Two CMK categories that were relatively consistent across both antibody types were MTBDI C4 and MTBDI C5. On comparing IgM and IgG responses of patients within SpA group, it was observed that there was a 4 fold decrease in the number of patients responding to the MTBDI C4 from IgM to IgG, but there was a 3 fold increase in the number of patients that responded MTBDI C7 from IgM to IgG. Three CMK categories that were relatively consistent across both antibody types were C8, C2 and MTBDI C5. On comparing LB IgM and SpA IgM proportions, it was observed that there was a 3 fold decrease in the number of patients responding to C8 from LB IgM to SpA IgM, but there was a 4 fold increase in the number of patients that responded to C2 and MTBDI C7 from LB IgM to SpA IgM. Similarly, there was a 5 fold increase in the number of patients that responded to MTBDI C4 from LB IgM to SpA IgM. Two CMK categories that were relatively consistent across both patient groups were C1 and MTBDI C5. On comparing LB IgG and SpA IgG proportions, it was observed that there was an 8

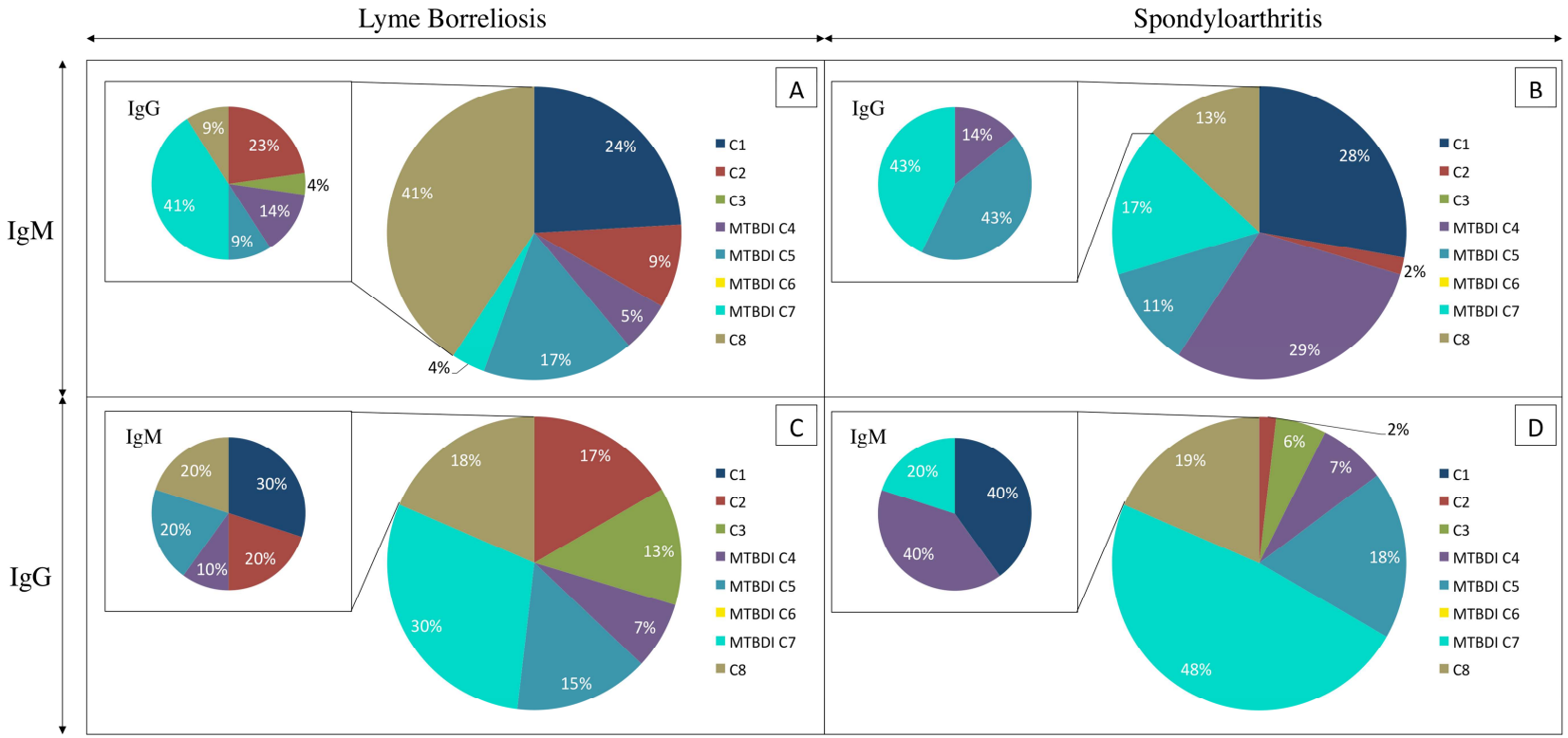

Figure 5: Graphical representation of positive IgM and IgG responses by LB and SpA patients to the 8 CMK categories independently. In every section, the pie of pie charts are solely associated with the sera samples that had positively responded to C8 category. The pie of pie charts presents percentages of positive responses to the 8 CMK categories only by respective C8 respondents across both antibody types. A) IgM immune responses by LB patients to all 8 CMK categories were recorded. B) IgM immune responses by SpA patients to all 8 CMK categories were recorded. C) IgG immune responses by LB patients to all 8 CMK categories were recorded. D) IgG immune responses by SpA patients to all 8 CMK categories were recorded. It was inferred that higher number of SpA patients across both antibody types had responded to the most severe CMK category (MTBDI C7) when compared with LB patients responses.

fold decrease in the number of patients responding to the C2 from LB IgG to SpA IgG, but there were about 2 fold increase in the number of patients that responded to C3 and MTBDI C7 from LB IgG to SpA IgG. Three CMK categories that were fairly consistent across both patient groups were C8, MTBDI C4, and MTBDI C5. As discussed earlier, MTBDI C7 is the most severe category among the 8 CMK categories. In descending order highest number of positive responses towards MTBDI C7 were recorded in SpA IgG, LB IgG, SpA IgM and LB IgM patient groups, respectively.

4.5.Statistical analysis between LB and SpA CMK categories binary data sets

In section 4.3, Fisher's exact test was used to compare individual antigen proportions between both patient groups. Likewise, in this section Fisher's exact test has been used to examine individual CMK categories between LB and SpA patient groups. Underlying concepts such as importance of both positive and negative immunological response frequencies, OBC, EXPC, φ_C value and three levels of dependencies remain the same for comparing individual CMK categories between both patient groups as shown in Appendix B, Table B. C8 was excluded from this statistical analysis because in the earlier section we recorded the category presenting immunological response frequencies across both antibody types that have already been accounted in the main pie charts. For example, in Figure 5A and 5C, immunological response frequencies towards CMK categories that are presented in pie of pie chart have already been accountant in the main IgG pie charts in both patients groups i.e. in Figure 5B and 5D, respectively. Thus, C8 was excluded to avoid redundancy. The remaining 7 CMK categories between both patient groups were compared against each other for recording dependent or independent associations. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG (Appendix B, Table B). In total, 98 Fisher's exact test proportions were tested of which 51 proportions were found significantly dependent i.e. 23 proportions were significantly dependent for IgM, and 28 proportions were significantly dependent for IgG. Among remaining 47 non-significant proportions, 37 were simply independent, 8 were Not Applicable (NA) and 2 were non-significant / independent (#). From Figure 5, we know that both patient groups did not respond to the C1, and C3 in accordance with IgG and IgM antibody types, respectively. NA Fisher's exact test proportions signify that both proportions that are being tested have negatives

counts that equal the population size (n = 54). Therefore, due to the absence of positive counts the IBM SPSS software equates OBC and EXPC for positives and negatives in both patient groups to the population size. For example, test for independence between C1 from both patient groups with IgG or between C3 from both patient groups are NA proportions. It can also be observed that NA proportions do not have φ_C value. Unlike the NA proportions, absolutely non-significant / independent Fisher's exact test proportions have φ_C value. They have a zero φ_C value because in both patient groups the positive and negative OBC exactly match their respective EXPC. Accordingly, test for independence between MTBDI C4 from both patient groups with IgG ideally represents an eminently independent group with zero φ_C value.

As discussed earlier, since graphical analysis of a particular binary data set provided an outlook with regards to only the positive immunological response frequencies, statistical analysis was implemented comprehensively to understand the difference between significantly dependent and independent proportions between both patients groups. In Appendix B, Table, B, highlighted in Yellow are the pseudo-relevant proportions that disregard the need for positive immunological response frequencies i.e. in either one of the patient groups zero positive OBC were recorded. For example, test for independence between C1 from LB and MTBD C4 from SpA for IgG is a pseudo-relevant group because no positive OBC were recorded for the former CMK category. Similarly, disregarding the need for positive immunological response frequencies was also witnessed in independent proportions that are highlighted in Blue. The term "pseudo-relevant proportion" will be used only to refer the Fisher's exact test proportions that are highlighted in Yellow. It is important to highlight pseudo-relevant proportions because they undermine the foundation of including both positive and negative immunological response frequencies for statistical analysis. Among 51 significantly dependent proportions, 28 were recorded as pseudo-relevant proportions.

Table 3 holistically represents all the details provided in Appendix B, Table B. All SpA CMK categories accommodate two columns which represent the Fisher's exact test results for two particular CMK categories, each from LB and SpA. The left column represents Fisher's exact test results for IgM antibody type and the right column represents Fisher's exact test results for IgG antibody type. Seemingly, as presented in Table 3

footnotes, each test result is colour coded to indicate significant dependence between two particular CMK categories, each from LB and SpA. Dark Blue and Dark Red colours represent test results for IgM and IgG, respectively. White / blank describes non-significant tests results for both antibody types. In addition, φ_C value range associated with dependent ($p \leq 0.05$) and independent Fisher's exact test proportions have been tabulated in the footnotes of Table 3. Pseudo-relevant proportions have been indicated with **Δ.** From Figure 2 (right side), we know that CMK categories correspond to a time gradient scale that would aid in positioning relevant or significantly dependent groups into a clinical context provided we have predictor variables. In order to obtain predictor variables between both patient groups binary logistic regression was performed only on significantly dependent groups as shown in Table 3 / Appendix C, Table C.

4.5.1. Binary logistic regression between LB and SpA significantly dependent CMK categories

Tabulated in Appendix C, Table C, are results of the Binary logistic regression that was performed only on significantly dependent Fisher's exact test proportions from Appendix B, Table B. The test was performed only on significantly dependent proportions because they signify potential correlation between the two CMK categories from both patient groups. In order to perform the test, a dependent / outcome and explanatory variables were required. The former variable is an outcome of the latter variable. Simply, explanatory variables act as predictor variables that would help in explaining an outcome / dependent variable. Through binary logistic regression, if an explanatory variable can construct a significant ($p \leq 0.05$) prediction model that describes a particular outcome variable, then that explanatory variable is considered a potential predictor (denoted by **#** in Appendix C Table C / by P in Table 3). In this study, since SpA in the form of LA is a chronic manifestation of LB, we try to record potential predictor variables between LB and SpA patient groups across both antibody types wherein SpA CMK categories are dependent / outcome variables and LB CMK categories are explanatory variables. To signify the difference between predictor variables that would likely or unlikely explain an outcome variable, two parameters have been tabulated in Appendix C, Table C i.e. Correct prediction percentage and the *p*-value. Former indicates overall predictability rate of a model between a particular dependent and explanatory variable and the latter indicates if

the model is statistically relevant ($p \leq 0.05$). The Correct prediction percentage is further divided into two blocks: 1) Block without a predictor means the no-hypothesis condition. It is a condition where the overall predictability rate of the model does not depend on any explanatory variable and 2) Block with a predictor variable where the overall predictability rate of the model depends on a particular explanatory variable. Ideally, overall predictability of a model should increase from a block with no explanatory variable to a block with explanatory variables. In the last column of Appendix C, Table C, p values $\leq$ 0.05 indicate that increase in overall predictability percentage has been significant enough, and if an explanatory variable is a potential predictor variable for an outcome variable.

Table 3: Holistic representation of Appendix B, Table B wherein Fisher's exact test was used to statistically compare immunological response frequencies (binary data set) of CMK categories C1, C2, C3, MTBDI C4, MTBDI C5, MTBDI C6, and MTBDI C7 between both patient groups against each other for significant or non-significant association. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG. All SpA CMK categories accommodate two columns that represent the Fisher's exact test results for two particular category types, each from LB and SpA. The left column represents Fisher's exact test results for IgM antibody type, and the right column represents Fisher's exact test results for IgG antibody type. Dark Blue and Dark Red colours represent significantly dependent Fisher's exact test proportions for IgM and IgG respectively. White / blank represents non-significant / independent tests results for both antibody types. In addition, φ_C value range associated with dependent ($p \leq 0.05$) and independent Fisher's exact test proportions have been tabulated in the footnotes. The binary logistic regression was performed only on significantly dependent Fisher's exact test proportions from Appendix B, Table B. Three predictor variables [P (P 1, P2, and P3)] and seven indicator variables [I (I1, I2, I3, I4, I5, I6, and I7)] that resulted from the binary logistic regression have been tabulated below.

Patient Type	Spondyloarthritis													
Lyme Borreliose	Chronic Manifestation Knot (CMK) Categories	C 1		C 2		C 3		MTBDI C4		MTBDI C5		MTBDI C6		MTBDI C7	
	C 1								Δ		Δ	Δ			Δ
	C 2		Δ			Δ		P1				Δ	Δ		I3
	C 3	Δ	Δ					Δ		Δ			Δ	Δ	I4
	MTBDI C4	I1	Δ					P2							I5
	MTBDI C5		Δ			Δ						Δ	Δ		I6
	MTBDI C6	Δ						Δ	Δ	Δ	Δ			Δ	Δ
	MTBDI C7	I2	Δ					P3					Δ		I7
	Antibody Type*	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG

* Color code for IgM and IgG associated with P and Cramer's V value are as follows

Parameter / P Value	IgM	IgG	Cramer's V Value (φ_C) range
P ≤ 0.05			0.177 – 0.563
Independent proportions			0.000 – 0.169

Δ = Statistically significant Fisher's exact test proportions with a zero number of positive counts either in LB or SpA group (or) pseudo-relevant proportions.
P = Significant prediction model between the dependent and predictor where P ≤ 0.05
I = Non-significant prediction model but with increase in correct prediction percentage between block without covariates and with covariates

At the outset, from Appendix B, Table B it was noted that all pseudo-relevant Fisher's exact test proportions (28) from Appendix B Tables B were recorded as Not Applicable (NA) for binary logistic regression. The proportions were NA because the total number of OBC either in the dependent or outcome variable were entirely negative. Among remaining 23 significant proportions, 3 predictor variables (#) were recorded explaining MTBDI C4 from SpA patient group. All 3 predictor variables from LB patient group i.e. C1, MTBDI C4, and MTBDI C7 were observed predicting MTBDI C4 from SpA with IgM antibody type. In addition, 7 indicator variables (°) were recorded explaining C1 and MTBDI C7 from SpA patient groups with IgM and IgG antibody types, respectively (Appendix C, Table C). Indicator variables differ from predictor variables in the way that the increase in overall predictability percentage was recorded, but the *p* value was not statistically relevant enough. Two indicator variables from LB i.e. MTBDI C4, and MTBDI C7 were observed predicting the C1 from SpA with IgM antibody type. Next, 5 indicator variables from LB i.e. C2, C3, MTBDI C4, MTBDI C5, and MTBDI C7 were observed predicting the MTBDI C7 from SpA with IgG antibody type. Both predictor [P (P1, P2, and P3)] and indicator [I (I1, I2, I3, I4, I5, I6, and I7)] variables have been tabulated in Table 3.

5. Discussion

In addition to *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii*, and *Ehrlichia chaffeensis* (Hermansen *et al*., 2015), immune response by SpA patients to *Borrelia burgdorferi* RB was also noticeably elevated when compared with the 40 healthy controls (Figure 3A and 3B). Elevated immune response to *Borrelia burgdorferi* RB and *Borrelia burgdorferi* spirochete by LB (Thammasri *et al*., 2015) and SpA (Hermansen *et al*., 2015) patients, indicates that the outer surface membrane proteins of *Borrelia burgdorferi* RB (Meriläinen *et al*., 2015; Thammasri *et al*., 2015) are distinctive when compared to *Borrelia burgdorferi* spirochete (Templeton, 2004). This divergence between the outer surface membrane proteins of *Borrelia burgdorferi* RB and *Borrelia burgdorferi* spirochete may be pertinent between the RBs and the spirochetes in all *Borrelia* strains (Templeton, 2004; Miklossy *et al*., 2008; Meriläinen *et al*., 2015; Thammasri *et al*., 2015). In order to precisely conclude that the outer surface membrane proteins of all *Borrelia* RB strains are divergent when compared to their respective spirochete equivalents, immune

response by acute and chronic LB patients to relevant *Borrelia* RBs and *Borrelia* spirochetes is required. Divergent antigenic properties across both *Borrelia* morphologies could conceivably contribute towards immune evasive and persistent characteristics of *Borrelia* species (Domingue and Woody, 1997; Justice *et al*., 2008; Miklossy *et al*., 2008; Embers *et al*., 2012; Meriläinen *et al*., 2015; Thammasri *et al*., 2015).

Interestingly, immune response by both patient groups to European *Borrelia burgdorferi* sensu lato genospecies [i.e. *Borrelia afzelii*, and *Borrelia garinii* (Figure 4)] and American *Borrelia burgdorferi* sensu lato genospecies [i.e. *Borrelia burgdorferi* sensu stricto (Figure 4)] indicates consensus with preceding reports, that emphasize on global prevalence of several *Borrelia burgdorferi* sensu lato genospecies (Norman *et al*., 1996; Piesman and Gern, 2004; Aguero-Rosenfeld *et al*., 2005; Rauter and Hartung, 2005). In addition, IgM immune response by LB and SpA patient groups to *Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia garinii* (Figure 4A and 4B) reflects high prevalance of *Borrelia garinii* in Germany (Piesman and Gern, 2004; Rauter and Hartung, 2005; Strle *et al*., 2006) in contrast to relatively equivalent prevalance of all three *Borrelia burgdorferi* sensu lato genospecies in Denmark (Piesman and Gern, 2004; Vennestrøm *et al*., 2008), respectively. The graphical representation of immune responses to individual antigens (Figure 4), sheds light on national (Strle *et al*., 2006; Vennestrøm *et al*., 2008) and global (Piesman and Gern, 2004; Rauter and Hartung, 2005) prevalance of different *Borrelia burgdorferi* sensu lato genospecies, but it fails to address the difference in severity of symptoms between LB and SpA patients. This is because, unlike the graphical representation of immune responses to the eight CMK categories (Figure 5), individual antigens (Figure 4) cannot assist in quantifying the number of patients responding either to an individual antigen infection type (C1, C2, or C3) or to a multiple antigen infection type (MTBDI C4, MTBDI C5, MTBDI C6, or MTBDI C7).

The eight CMK categories are independent of each other (section 3.7). Therefore, graphical presentation of immune responses by both patient groups to the CMK categories (Figure 5) offers a unique benefit to clearly appreciate and visualize quantitatively and qualitatively the number of LB, and SpA patients that have responded to different infection type categories [individual infection type or multiple infection type]. Segregation of IgM, and IgG immune responses by both patient groups among the five antigens (Figure 4)

relates to the segregation of IgM, and IgG immune responses by both patient groups among the eight CMK categories (Figure 5). The descending order of uniform segregation among the individual antigens (Figure 4) and the descending order for the most number of responses towards MTBDI C7 CMK category (Figure 5) is the same. Thus, implying that higher the segregation uniformity is among individual antigens, higher is the number of responses towards the most severe CMK category [MTBDI C7 (Figure 5)]. Therefore, graphical representation of immune responses to CMK categories across identical antibody types (Figure 5), clearly indicates that the SpA patient group (Figure 5B and 5D) is exceedingly inclined towards developing / experiencing chronic conditions (Barthhold, 1996; Jaulhac *et al*., 1996; Kim *et al*., 2005) when compared with the LB patient group (Figure 5A and 5C). Further, unlike the immune responses by LB patient group specifically to C8 CMK category(Figure 5A and 5C), the immune responses by SpA patient group to C8 CMK category (Figure 5B and 5D) are majorly engaged with the multiple infection type categories across both antibody types, and do not contain any truly negative SpA patients. Therefore, immune responses by both patients groups specifically to the C8 / Nil response category (Figure 5) consolidates the fact that SpA patients are exceptionally inclined towards developing / experiencing chronic conditions (Kim *et al*., 2005).

Distinctive immune responses by both patient groups to C1, and C2 CMK categories (Figure 5), evidently reiterates two fold. Firstly, the fact that the outer surface membrane proteins of *Borrelia burgdoferi* RB are divergent when compared to all three *Borrelia* strain spirochetes. Secondly, the necessity to further study immune responses by acute and chronic LB patients to relevant *Borrelia* RBs and *Borrelia* spirochetes. This will help in precisely concluding if divergent antigenic properties across both *Borrelia* morphologies contribute towards immune evasive and persistent characteristics of *Borrelia* species. Interestingly, the graphical representation (Figure 5) of immune responses with the help of CMK categories is completely unbiased of the presumed clinical indications (Figure 2, right side). The IgM immune responses by LB (Figure 5A) and SpA (Figure 5B) patient groups were not only positive for C1, C2, and MTBDI C4 CMK categories, but were also positive for MTBDI C5, and MTBDI C7 CMK categories that were specifically applicable to IgG immune responses (Figure 2, right side). Thus graphical presentation of IgM immune responses by LB (Figure 5A) and SpA (Figure 5B) patient groups with the help of

CMK categories clearly indicates the IgM dysfunction (Kalish *et al*., 2001; Horowitz, 2013) in both patients groups. Typically, the IgM antibody is an indicative of early stage infection events (C1, C2, and MTBDI C4) because IgM antibody does not require numerous weeks to build up, but an IgM antibody titer may prevail only for several weeks (Figure 1 and 2). Further, the IgG antibody is not only indicative of memory from early stage infection, but also late stage infection (all eight CMK categories), because seroconversion and production of an appropriate IgG immune response could take several weeks that would then prevail for years (Figure 1 and 2). The IgM dysfunction is an anomaly, wherein IgM immune response is not an indicative of only early stage infection because, IgM antibody titers were reported positive even after 10-20 years of a *Borrelia* infection in LB patients (Kalish *et al*., 2001; Horowitz, 2013). Thus, an atypical prevalance of IgM antibody response in LB patients for years (IgM dysfunction) probably elucidates the reason behind IgM immune response to MTBDI C5, and MTBDI C7 CMK categories (Figure 5).

Implementation of statistical modelling between LB and SpA patient groups to discover / predict, key MTBDI combination(s) that may play an imperative role in inducing chronic conditions has been performed for the first time in this study. At the outset, discovering predictor and indicator variables (Appendix B, Table B and Appendix C, Table C / Table 3) between both the patient groups would have been impossible even if binary logistic regression was performed between individual antigens (Appendix A, Table A / Table 2). This is simply because individual antigens completely fail to include MTBDI combinations such as MTBDI C4, MTBDI C5, MTBDI C6 and MTBDI C7 that are only addressed by the CMK model (Appendix B, Table B and Appendix C, Table C / Table 3). The three predictor variables (P1, P2, and P3) and the first two indicator variables (I1 and I2) are implying that *Borrelia burgdorferi* RB can assist *Borrelia* spirochetes in inducing multiple overlapping chronic etiologies (Appendix C, Table C / Table 3). In order to interpret the statistical modelling results (Appendix C, Table C / Table 3) from a clinical perspective, mutual primary ends (C1, C2, and C3) among MTBDI categories (MTBDI C4, MTBDI C5, MTBDI C6 and MTBDI C7) need to be taken into consideration (Figure 2).

A mutual primary end between C2 and MTBDI C4 is the C2 / only *Borrelia* Round Body category. Similarly, a mutual primary end between C1 and MTBDI C4 is the C1 / only *Borrelia* spirochete category (Figure 2). Also, a mutual CMK category between P1, and I1 (Table 3) is MTBDI C4 (only *Borrelia* spirochete and Round Body). The C2 category from LB patient type, predicts (P1) the MTBDI C4 from SpA patient type (Table 3), and the MTBDI C4 from LB patient type, indicates (I1) towards the C1 category from SpA patient type (Table 3). The P1 and I1 variables suggest that *Borrelia* spirochetes (C1) may progress through early stages (IgM) of LB in the form of *Borrelia* RB (C2). The MTBDI C4 from LB patient type, predicts (P2) the MTBDI C4 from SpA patient type. The P2 variable further consolidates the idea provided by P1 and I1 variables that, *Borrelia* spirochetes (C1) may progress through early stages (IgM) of LB in the form of *Borrelia* RB (C2).

A mutual primary end between C2 and MTBDI C7 (*Borrelia* spirochete, *Borrelia* RB, and *Ehrlichia*) is the C2 category. Similarly, mutual primary ends among MTBDI C4 and MTBDI C7 are the C1 and C2 categories (Figure 2). The MTBDI C4 is the result of an interlace between C1 and C2 categories (Figure 2). Therefore, MTBDI C4 is a mutual MTBDI combination between MTBDI C4 and MTBDI C7. Thus, MTBDI C4 is also a common MTBDI combination (Table 3) for P3 and I5. Interestingly, the C2, and MTBDI C4 from LB patient type, indicates (I3, and I5) towards MTBDI C7 from SpA patient type. The I3 and I5 variables suggest that *Borrelia*'s pleomorphic (Meriläinen *et al*., 2015) ability (C2 and MTBDI C4) may play a crucial role for *Borrelia* spirochete to progress through the early (IgM) to late stages (IgG) of LB to induce chronic conditions. Hence, MTBDI C4 (*Borrelia*'s pleomorphic ability) is the key MTBDI combination that may play an imperative role in inducing multiple overlapping chronic etiologies in LB patients.

From an experimental standpoint, MTBDI C4 might evidently be the key MTBDI combination because, *Borrelia* spirochete (C1), especially in human serum, can switch its morphology to *Borrelia* RB (C2) when exposed to unfavorable environmental conditions (Miklossy *et al*., 2008; Meriläinen *et al*., 2015). Further, *Borrelia* RB (C2) can revert to its native morphology (C1) when exposed to favorable environmental conditions (Miklossy *et al*., 2008; Meriläinen *et al*., 2015). Therefore, statistically (Appendix C, Table C / Table 3) and experimentally (Miklossy *et al*., 2008; Meriläinen *et al*., 2015) *Borrelia* spirochete

may utilize the most out of it pleomorphic ability (i.e. MTBDI C4) to survive through the early (IgM) to late (IgG) stages of LB to induce chronic conditions like arthritis (SpA). The MTBDI C7 from LB patient type, predicts (P3) the MTBDI C4 from SpA patient type (Table 3), and the MTBDI C7 from LB patient type, indicates towards the C1 (I2) and MTBDI C7 (I7) from SpA patient type (Table 3). The P3, I2, and I7 variables reiterate the notion that MTBDI C4 (P3) may play a crucial role for *Borrelia* spirochete (C1) to progress through the early (I2) to late stages (I7) of LB to induce chronic conditions like arthritis together with coinfections (such as *Ehrlichia*).

The statistical (Appendix C, Table C / Table 3) and experimental (Miklossy *et al*., 2008; Meriläinen *et al*., 2015) evidence that *Borrelia* spirochete may utilize the most out of it pleomorphic ability to survive through unfavourable circumstances to later induce chronic conditions can be mapped on and around the Yin-Yang model (Zhang, 2014), respectively. Briefly, Yin-Yang model (Figure 6) is the most recent and up-to-date model for persisters and persistent infections (Zhang, 2014). The Yin-Yang model addresses major issues with persisters and persistent infections such as heterogeneity among persisters, age of a bacterial culture, antibiotic type, dosage, and duration for an effective treatment against the bacterial infection, and inclusion of viable, but non-culturable / dormant bacteria that grow only in distinct conditions (Zhang, 2014). The Yin-Yang model presents multifaceted characteristics of hererogeneous bacterial population consisting of non-growing persistent bacterial cells [Yin (Latent phase) / in Red (Figure 6)] and growing bacterial cells [Yang (Active phase) / in Blue (Figure 6)]. The Red and Blue colour gradients in Yin and Yang, respectively, indicate that the non-growing and growing (Figure 6) bacterial cells are in fluctuating growth and metabolic states throughout perpetuity. The Yin-Yang model suggests that interconversion between the growing and non-growing bacterial cells [from Yang to Yin (P) and Yin to Yang (R)] can be realized *In-vitro or In-vivo* when exposed to unfavorable environmental conditions (antibiotics, drugs, host's immunity, and etc) (Zhang, 2014). Initially, in the active phase of a disease, a bacterial culture with pleomorphic ability reaches a certain age and density alongside a very small population of non-growing / slowly growing / persister bacterial cells. Further, the interconversion from Yang to Yin [P (Figure 6)] may occur because of the unfavourable conditions established either by antibiotics or host's immune response (pre-antibiotic). Later, in the latent phase of the disease, only the non-growing yet viable cells

persist. A reversion from Yin to Yang [R (Figure 6)] may occur when the non-growing / persistent bacterial cells have survived through the unfavourable conditions (post-antibiotic) to relapse their active phase pathogenesis (Zhang, 2014).

In the case of *Borrelia* bacteria, the pleomorphic form (*Borrelia* RB) has been recorded to be metabolically (ATP) inactive when compared to *Borrelia* spirochetes (Meriläinen *et al*., 2015). Therefore, if *Borrelia* spirochetes (C1) are the growing bacterial cells [Active phase (Figure 6)] and *Borrelia* Round Body (C2) are the non-growing / persistent bacterial cells [Latent phase (Figure 6)], then the cycle of interconversion and reversion between C1 and C2 [represented by a White line between Yin and Yang (Figure 6)] can indeed be denoted as the MTBDI C4. Although, the cycle of interonversion and reversion between C1 and C2 is plausible because *Borrelia* bacteria is both, pre-antibiotic and post-antibiotic (Barthold *et al*., 1993; Barthhold, 1996; Miklossy *et al*., 2008; Embers *et al*., 2012; Meriläinen *et al*., 2015), the precise number of months or years that *Borrelia* bacteria specifically depends on its pleomorphic ability to induce chronic conditions alongside other infections are still unknown. Around the active and latent phases of the Yin-Yang model, EM micrographs of *Borrelia burgdoferi* spirochete (Figure 6A) and *Borrelia burgdoferi* RB (Figure 6B) from Meriläinen, L and colleagues (2015) have been positioned. The EM micrographs around the Yin-Yang model emphasize that MTBDI C4 is not only a relevant MTBDI combination from an experimental outlook (Miklossy *et al*., 2008; Meriläinen *et al*., 2015), but also from the most recent model's (Yin-Yang model) perspective that is applicable to numerous other bacterias (Zhang, 2014).

The C3, and MTBDI C5 from LB patient type, indicates (I4, and I6) towards MTBDI C7 from SpA patient type (Table 3). The I4 and I6 variables suggest the mode of infections in the later stages of LB, for diseases such as arthritis. The I4 variable suggests that the reason for a patient's intensified symptoms could be solely because of coinfection(s) [such as *Ehrlichia* (C3)] that dominantly exhibit their pathogenesis (Berghoff, 2012) when compared to the primary infection (*Borrelia* infection). In contrarst, I6 variable suggests that the reason for a patient's intensified symptoms could be because the coinfections may accompany the primary infection (MTBDI C5) to simultaneously induce chronic conditions (Jaulhac *et al*., 1996).

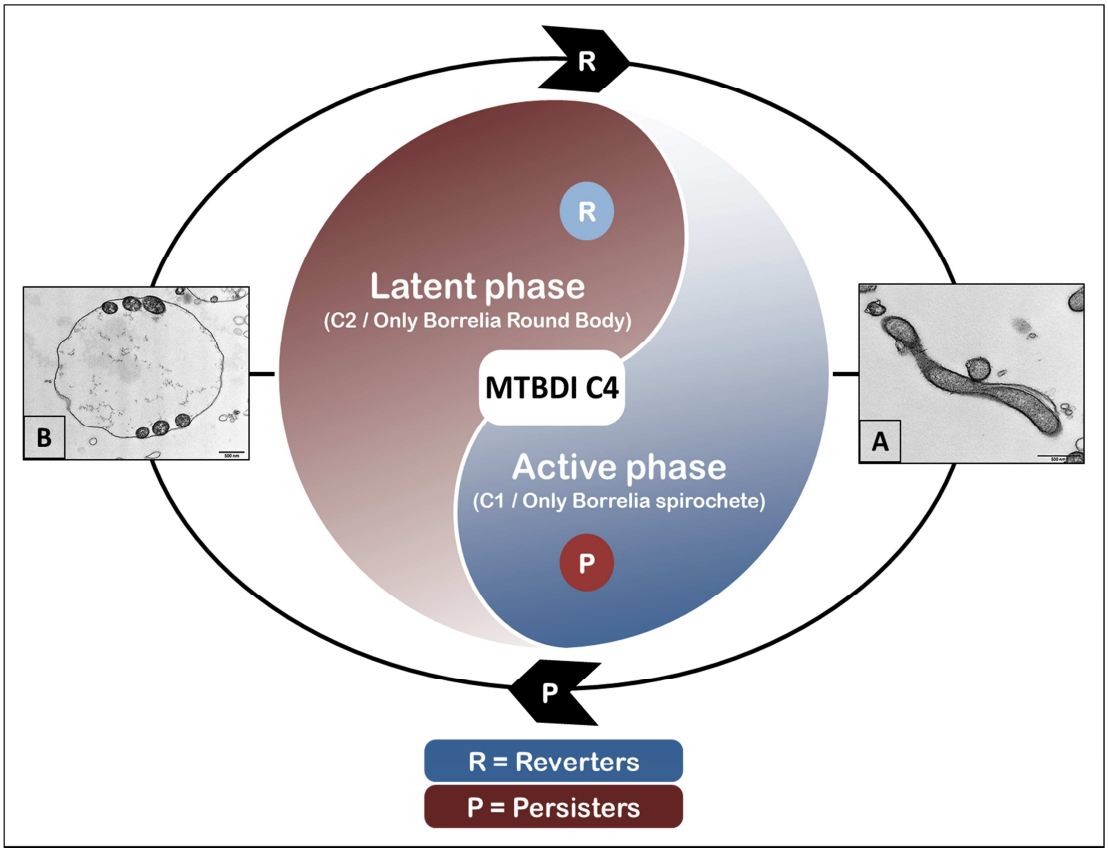

Figure 6: Modified Yin-Yang model (Zhang, 2014) surrounded by EM micrographs of (A) *Borrelia burgdoferi* spirochete, and (B) *Borrelia burgdoferi* RB (Meriläinen *et al.*, 2015). The interconversion between C1 and C2 evidently illustrates that *Borrelia* spirochete may utilize the most out of it pleomorphic ability (i.e. MTBDI C4) to survive through unfavourable conditions to induce chronic conditions in later stages of LB.

Application of the CMK model is neither restricted only to immunological response frequencies nor only to TBDs. Creation of binary data sets merely based on scoring methodology that can be accomplished with different techniques such as PCR, bacterial culturing, and many others. For example, positive or negative PCR test results for different bacterium would lead to creating "genetic response frequencies" binary data sets, and positive or negative culturing of different bacterium would result in creating "growth response frequencies" binary data sets. Further, the CMK model may also be applicable to different vector-borne diseases (Chan, 2014) or even specific diseases such as Tuberculosis, Syphilis, Brucellosis, various biofilm infections, Endocarditis, Sepsis, Otitis media, and numerous others that well known for coexistence of various primary (C1), persistent (C2), and other (C3) infections (Zhang, 2014).

6. Conclusion

The CMK model is a holistic, unified, and an unbiased method to facilitate graphical and statistical analysis in order to discover and comprehend different developmental aspects of a disease at the microorganism(s) level. Two core benefits dictate the necessity for utilizing the CMK model over individual antigens for graphical and statistical analysis of immune response frequencies, genetic response frequencies or growth response frequencies. Firstly, unlike graphical analysis among individual antigens, the CMK model from an end-users perspective clearly addresses the need to visualize quantitatively and qualitatively the number of patients that have responded to different infection type categories [individual infection type (C1, C2, or C3) or multiple infection type (MTBDI C4, MTBDI C5, MTBDI C6, or MTBDI C7)]. Secondly, the CMK model evidently addresses multiple infection type combinations for statistical analysis that the individual antigens absolutely fail to address. The graphical and statistical analysis between LB and SpA patient groups with the help of the CMK model reiterates the idea that divergent antigenic properties of *Borrelia* RBs could conceivably contribute towards immune evasive and persistent characteristics of *Borrelia* species. Also, the pleomorphic ability of *Borrelia* spirochetes may help *Borrelia* to progress through the early to late stages of LB to induce chronic conditions like arthritis together with coinfections. It is of paramount importance that the ongoing diagnostic tools consider reforming their detection efficiency by including different *Borrelia* RB strains for diagnosing prospective TBD sufferers.

References

Aguero-Rosenfeld, M.E., G. Wang, I. Schwartz, and G.P. Wormser. 2005. Diagnosis of Lyme Borreliosis. *Clin Microbiol Rev*. 18:484-509.

Arnold, M.M., and F. McKenna. 1994. Reactive arthritis in a patient with cat-scratch disease. *Postgrad.Med.J.* 70:147-147.

Aucott, J., C. Morrison, B. Munoz, P. Rowe, A. Schwarzwalder, and S. West. 2009. Diagnostic challenges of early Lyme disease: Lessons from a community case series. *BMC Infect Dis.* 9:79.

Barbour, A.G., and S.F. Hayes. 1986. Biology of *Borrelia* species. *Microbiol.Rev.* 50:381-400.

Barker, S.C., and A. Murrell. 2004. Systematics and evolution of ticks with a list of valid genus and species names. Int J *Parasitol.* 129:S15-S36.

Barthhold, S.W. 1996. Lyme borreliosis in the laboratory mouse. *J. Spirochet. Tick-Borne*. 3:22-44.

Barthold, S.W., de Souza, M.S., Janotka, J.L., Smith, A.L., and Persing, D.H.. 1993. Chronic Lyme borreliosis in the laboratory mouse. *Am J Pathol.* 143:959-971.

Benjamin, R., and Parham, P. 1990. Guilt by association: HLA-B27 and ankylosing spondylitis. *Immunol.Today*. 11:137-142.

Berghoff, W. 2012. Chronic Lyme Disease and Co-infections: Differential Diagnosis. *Open Neurol J*. 6:158-178.

Blindheim, M. 1985. Graffiti in Norwegian Stave Churches, C. 1150-C. 1350. -*Scand Uni Pres, Michigan*. 158 pp.

Braun, J., Kingsley, G., Van der Heijde, D., and Sieper, J. 2000. On the difficulties of establishing a consensus on the definition of and diagnostic investigations for reactive arthritis. Results and discussion of a questionnaire prepared for the 4th International Workshop on Reactive Arthritis, Berlin, Germany, July 3-6, 1999. *J Rheumatol.* 9:2185-2192.

Braun, J., Tuszewski, M., Eggens, U., Mertz. A., Schauer-Petrowskaja, C., Döring, E., Laitko, S., Distler, A., Sieper, J., and Ehlers, S. 1997. Nested polymerase chain reaction strategy simultaneously targeting DNA sequences of multiple bacterial species in inflammatory joint diseases. I. Screening of synovial fluid samples of patients with spondyloarthropathies and other arthritides. *J Rheumatol.* 6:1092-1100.

Brown, M.A., Kennedy, L.G., Darke, C., Gibson, K., Pile, K.D., Shatford, J.L., Taylor, A., Calin, A., and Wordsworth, B.P. 1998. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. *Arthritis & Rheumatism.* 41:460-465.

Brown, W.C., McGuire, T.C., Zhu, D., Lewin, H.A., Sosnow, J., and Palmer, G.H. 2001. Highly Conserved Regions of the Immunodominant Major Surface Protein 2 of the Genogroup II Ehrlichial Pathogen Anaplasma marginale Are Rich in Naturally Derived CD4+ T Lymphocyte Epitopes that Elicit Strong Recall Responses. *J Immunol.* 166:1114-1124.

Burchill, M.A., Nardelli, D.T., England, D.M., DeCoster, D.J., Christopherson, J.A., Callister, S.M., and Schell, R.F. 2003. Inhibition of Interleukin-17 Prevents the Development of Arthritis in Vaccinated Mice Challenged with *Borrelia burgdorferi*. *Infect Immun.* 71:3437-3442.

Burgdorfer, W., Barbour, A.G., Hayes, S., Benach, J.L., Grunwaldt, E., and Davis, J. 1982. Lyme Disease-a tick-borne spirochetosis? *Sci.* 216:1317-1319.

Busch, U., Hizo-Teufel, C., Boehmer, R., Fingerle, V., Nitschko, H., Wilske, B., and Preac-Mursic, V. 1996. Three species of *Borrelia burgdorferi* sensu lato (*B. burgdorferi* sensu stricto, *B afzelii*, and *B. garinii*) identified from cerebrospinal fluid isolates by pulsed-field gel electrophoresis and PCR. *J Clin Microbiol.* 34:1072-1078.

Butrimiene, I., Ranceva, J., and Griskevicius, A. 2006. Potential triggering infections of reactive arthritis. *Scand.J.Rheumatol.* 35:459-462.

Carter, J.D., Espinoza, L.R., Inman, R.D., Sneed, K.B., Ricca, L.R., Vasey, F.B., Valeriano, J., Stanich, J.A., Oszust, C., Gerard, H.C., and Hudson, A.P. 2010. Combination antibiotics as a treatment for chronic Chlamydia-induced reactive arthritis: A double-blind, placebo-controlled, prospective trial. *Arthritis Rheum.* 62:1298-1307.

Carter, J.D., and Hudson, A.P. 2009. Reactive Arthritis: Clinical Aspects and Medical Management. *Rheum Dis Clin North Am.* 35:21-44.

Chan, M. 2014. A global brief on vector-borne diseases. *WHO.* WHO/DCO/WHD/2014.1.

Christopherson, J.A., Munson, E.L., England, D.M., Croke, C.L., Remington, M.C., Molitor, M.L., DeCoster, D.J., Callister, S.M., and Schell, R.F. 2002. Destructive Arthritis in Vaccinated Interferon Gamma-Deficient Mice Challenged with *Borrelia burgdorferi*: Modulation by Tumor Necrosis Factor Alpha. *Clin.Diagn.Lab.Immunol.* 10:44-52.

Colmegna, I., Cuchacovich, R., and Espinoza, L.R. 2004a. HLA-B27-Associated Reactive Arthritis: Pathogenetic and Clinical Considerations. *Clin Microbiol Rev.* 17:348-369.

Diza, E., Papa, A., Vezyri, E., Tsounis, S., Milonas, I., and Antoniadis, A. 2004. *Borrelia valaisiana* in Cerebrospinal Fluid. *Emerging Infect.Dis.* 10:1692-1693.

Domingue, G.J., and Woody, H.B. 1997. Bacterial persistence and expression of disease. *Clin Microbiol Rev.* 10:320-344.

Dumler, J.S., and Bakken, J.S. 1995. Ehrlichial Diseases of Humans: Emerging Tick-Borne Infections. *Clin Infect Dis.* 20:1102-1110.

Embers, M.E., Barthold, S.W., Borda, J.T., Bowers, L., Doyle, L., Hodzic, E., Jacobs, M.B., Hasenkampf, N.R., Martin, D.S., Narasimhan, S., Phillippi-Falkenstein, K., Purcell, J.E., Ratterree, M.S., and Philipp, M.T. 2012. Persistence of *Borrelia burgdorferi* in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection. *PLoS One.* 7:e29914.

Eskow, E., Rao, R.S., and Mordechai, E. 2001. Concurrent Infection of the Central Nervous System by *Borrelia burgdorferi* and *Bartonella henselae*: Evidence for a Novel Tick-borne Disease Complex. *Arch Neurol.* 58(9):1357-1363.

Falkenhorst, G., Ceper, T.H., Strid, M.A., Mølbak, K., and Krogfelt, K.A. 2013. Serological follow-up after non-typhoid salmonella infection in humans using a mixed lipopolysaccharide ELISA. *Int J Med Microbiol.* 303:533-538.

Field, A. 2013. Discovering Statistics using IBM SPSS Statistics. Sage publications, Canada. 908 pp.

Fries, J.F., Spitz, P., Kraines, R.G., and Holman, H.R. 1980a. Measurement of patient outcome in arthritis. *Arthritis Rheum.* 23:137-145.

Fukuda, K., Straus, S.E., Hickie, I., Sharpe, M.C., Dobbins, J.G., and Komaroff, A. 1994. The Chronic Fatigue Syndrome: A Comprehensive Approach to Its Definition and Study. *Ann Intern Med.* 121:953-959.

Girschick, H., Morbach, H., and Tappe, D. 2009. Treatment of Lyme borreliosis. *Arthritis Res Ther.* 11:258.

Granfors, K., Merilahti-Palo, R., Luukkainen, R., Möttönen, T., Lahesmaa, R., Probst, P., Märker-Hermann, E., and Toivanen, P. 1998. Persistence of *Yersinia* antigens in peripheral blood cells from patients with *Yersinia enterocolitica* O:3 infection with or without reactive arthritis. *Arthritis Rheum.* 41:855-862.

Hermansen, L., Christiansen, A., Garg, K., Karvonen, K., Gilbert, L., Loft, A.G., Manniche, C., Jensen, T.S., Arnbak, B., Østergaard, M., Pedersen, S.J., Jurik, A.G., Weber, U., Hørslev-Petersen, K., and Hendricks, O. 2015. Is there an association between spondyloarthritis on the basis of ASAS classification criteria and antibodies towards *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia garinii*, *Ehrlichia*, *Chlamydia trachomatis*, and *Chlamydia pneumoniae*? (manuscript).

Hill, A.V., Jepson, A., Plebanski, M., and Gilbert, S.C. 1997. Genetic analysis of host-parasite coevolution in human malaria. *Philos T R Soc B.* 352:1317-1325.

Horowitz, R. 2013. Why can't I Get Better? Solving the Mystery of Lyme and Chronic Diseases. St. Martin's Press, New York City. 544 pp.

Hunfeld, K.P. 2002. *Granulocytic Ehrlichia*, *Babesia*, and spotted fever *Rickettsia*. Not yet widely known tick-borne pathogens of considerable concern for humans at risk in Europe. *Bio Bull.* 6:321-344.

Jaulhac, B., Chary-Valckenaere, I., Sibilia, J., Javier, R., Piémont, Y., Kuntz, J., Monteil, H., and Pourel, J. 1996. Detection of *Borrelia burgdorferi* by DNA amplification in synovial tissue samples from patients with lyme arthritis. *Arthritis Rheum.* 39:736-745.

Justice, S.S., Hunstad, D.A., Cegelski, L., and Hultgren, S.J. 2008. Morphological plasticity as a bacterial survival strategy. *Nat Rev Micro.* 6:162-168.

Kalish, R.A., McHugh, G., Granquist, J., Shea, B., Ruthazer, R., and Steere, A.C. 2001. Persistence of Immunoglobulin M or Immunoglobulin G Antibody Responses to *Borrelia* burgdorferi 10–20 Years after Active Lyme Disease. *Clin Infect Dis.* 33:780-785.

Kim, T., Uhm, W., and Inman, R.D. 2005. Pathogenesis of ankylosing spondylitis and reactive arthritis. *Curr Opin Rheumatol.* 17:400-405.

Kovalchuka, L., Eglite, J., Lucenko, I., Zalite, M., Viksna, L., and Krumina, A. 2012. Associations of HLA DR and DQ molecules with Lyme borreliosis in Latvian patients. *BMC Res Notes.* 5:438.

Krause, P.J., Narasimhan, S., Wormser, G.P., Rollend, L., Fikrig, E., Lepore, T., Barbour, A., and Fish, D. 2013. Human *Borrelia miyamotoi* Infection in the United States. *N.Engl.J.Med.* 368:291-293.

Liebling, M.R., Arkfeld, D.G., Michelini, G.A., Nishio, M.J., Eng, B.J., Jin, v, and Louie, J.S. 1994. Identification of neisseria gonorrhoeae in synovial fluid using the polymerase chain reaction. *Arthritis Rheum.* 37:702-709.

Mayne, P. 2014. Clinical determinants of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort. *Int J Gen Med.* 2015:8:15-26.

Meriläinen, L., Herranen, A., Schwarzbach, A., and Gilbert, L. 2015. Morphological and biochemical features of *Borrelia burgdorferi* pleomorphic forms. *Microbiol.* 161:516-527.

Miklossy, J., Kasas, S., Zurn, A., McCall, S., Yu, S., and McGeer, P. 2008. Persisting atypical and cystic forms of *Borrelia burgdorferi* and local inflammation in Lyme neuroborreliosis. *J Neuroinflammation.* 5:40.

Milicic, A., Lindheimer, F., Laval, S., Rudwaleit, M., Ackerman, H., Wordsworth, P., Hohler, T., and Brown, M.A. 2000. Interethnic studies of TNF polymorphisms confirm the likely presence of a second MHC susceptibility locus in ankylosing spondylitis. *Genes Immun.* 1:418-422.

Mitchell, P.D., Reed, K.D., and Hofkes, J.M. 1996. Immunoserologic evidence of coinfection with *Borrelia burgdorferi, Babesia microti,* and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *Journal Clin Microbiol.* 34:724-727.

Mohamed Zahidi, J., Bee Yong, T., Hashim, R., Mohd Noor, A., Hamzah, S.H., and Ahmad, N. 2015. Identification of *Brucella spp.* isolated from human brucellosis in Malaysia using high-resolution melt (HRM) analysis. *Diagn.Microbiol.Infect.Dis.* 81:227-233.

Nardelli, D.T., Cloute, J.P., Luk, K.H.K., Torrealba, v, Warner, T.F., Callister, S.M., and Schell, R.F. 2005. CD4+ CD25+ T Cells Prevent Arthritis Associated with *Borrelia* Vaccination and Infection. *Clin Diagn Lab Immun.* 12:786-792.

Norman, G.L., Antig, J.M., Bigaignon, G., and Hogrefe, W.R. 1996. Serodiagnosis of Lyme borreliosis by *Borrelia* burgdorferi sensu stricto, *B. garinii*, and *B. afzelii* western blots (immunoblots). *J Clin Microbiol.* 34:1732-1738.

Panush, R.S., Wallace, D.J., Dorff, R.E.N., and Engleman, E.P. 2007. Retraction of the suggestion to use the term ?Reiter's syndrome? sixty-five years later: The legacy of Reiter, a war criminal, should not be eponymic honor but rather condemnation. *Arthritis Rheum.* 56:693-694.

Peterson, S.H., Nardelli, D.T., Warner, T.F., Callister, S.M., Torrealba, J.R., and Schell, R.F. 2007. Anti-p19 Antibody Treatment Exacerbates Lyme Arthritis and Enhances *Borrelia*cidal Activity. *Clin Vaccine Immunol.* 14:510-517.

Piesman, J., and Gern, L. 2004. Lyme borreliosis in Europe and North America. Int J *Parasitol.* 129:Suppl:S 191-220.

Radolf, J.D., Caimano, M.J., Stevenson, B., and Hu, L.T. 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Micro.* 10:87-99.

Rauter, C., and Hartung, T. 2005. Prevalence of *Borrelia burgdorferi* Sensu lato Genospecies in Ixodes ricinus Ticks in Europe: a Metaanalysis. *Appl.Environ.Microbiol.* 71:7203-7216.

Rudenko, N., Golovchenko, M., Grubhoffer, L., and Oliver Jr, J.H. 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. *Ticks Tick Borne Dis.* 2:123-128.

Rudwaleit, M., Van der Heijde, D., Khan, M.A., Braun, J., and Sieper, J. 2004. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis.* 63:535-543.

Saebø, A., and Lassen, J. 1994. *Yersinia enterocolitica*: an inducer of chronic inflammation. *Int J Tissue React.* 16(2):51-57.

Steere, A.C. 2001. Lyme Disease. *N.Engl.J.Med.* 345:115-125.

Strle, F., Ružic-Sabljic, E., Cimperman, J., Lotric-Furlan, S., and Maraspin, V. 2006. Comparison of Findings for Patients with *Borrelia garinii* and *Borrelia afzelii* Isolated from Cerebrospinal Fluid. *Clin Infect Dis.* 43:704-710.

Swanson, S.J., Neitzel, D., Reed, K.D., and Belongia, E.A. 2006. Coinfections Acquired from Ixodes Ticks. *Clin Microbiol Rev.* 19:708-727.

Templeton, T.J. 2004. *Borrelia* Outer Membrane Surface Proteins and Transmission Through the Tick. *J.Exp.Med.* 199:603-606.

Thammasri, K., Campillo, A., Meriläinen, L., Schwarzbach, A., Garcia-Nogales, P., and Gilbert, L. 2015. Advance ELISA with multiplex antigen array for the diagnosis of Tick-borne diseases. (manuscipt).

Thomas, V., Anguita, J., Barthold, S.W., and Fikrig, E. 2001. Coinfection with *Borrelia burgdorferi* and the Agent of Human Granulocytic Ehrlichiosis Alters Murine Immune Responses, Pathogen Burden, and Severity of Lyme Arthritis. *Infect Immun.* 69:3359-3371.

Vennestrøm, J., Egholm, H., and Jensen, P.M. 2008. Occurrence of multiple infections with different *Borrelia burgdorferi* genospecies in Danish Ixodes ricinus nymphs. *Parasitol.Int.* 57:32-37.

Wang, G., Van Dam, A.P., Schwartz, I., and Dankert, J. 1999. Molecular typing of *Borrelia burgdorferi* sensu lato: Taxonomic, epidemiological, and clinical implications. *Clin.Microbiol.Rev.* 12:633-653.

Wormser, G.P., Dattwyler, R.J., Shapiro, E.D., Halperin, J.J., Steere, A.C., Klempner, M.S., Krause, P.J., Bakken, J.S., Strle, F., Stanek, G., Bockenstedt, L., Fish, D., Dumler, J.S., and Nadelman, R.B. 2006. The Clinical Assessment, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: Clinical Practice Guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 43:1089-1134.

Zhang, Y. 2014. Persisters, persistent infections and the Yin-Yang model. *Emerg Microbes Infect.* 3:e3.

Appendix A

Table A: Fisher's exact test was used to statistically compare immunological response frequencies (binary data set) of *Borrelia burgdorferi*, *Borrelia burgdorferi* RB, *Borrelia afzelii*, *Borrelia garinii* and *Ehrlichia chaffeensis* between both patient groups against each other for significant or non-significant association. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG. The number of negative and positive counts observed in the binary data set corresponding to a particular Fisher's exact test proportion has been tabulated in the Observed count (OBC) columns. Counts in the Expected count (EXPC) count column are statistically computed value that assumes no-hypothesis condition. Cramer's V (φ_C) value in the last column signifies effect size between the proportions being tested. In order for a Fisher's exact test proportion to be significantly dependent, difference between positive and negative OBC and EXPC pairs should be diverse enough for the proportion to be significantly dependent at $p \leq 0.05$ (denoted with * in the last column beside φ_C value). In total, 50 Fisher's exact test proportions were tested, of which 40 proportions were found significantly dependent i.e. 21 proportions were significantly dependent for IgM, and 19 proportions were significantly dependent for IgG. It was inferred that more than ¾ Fisher's exact test proportions were found to be significantly dependent, signifying that the immunological response frequencies of antigens across both antibody types between LB and SpA groups statistically correlate at 80%.

Fisher's Exact test proportions		Antibody Type	Lyme Borreliosis				Spondyloarthritis				Cramer's V Value (φ_C)
			Negative		Positive		Negative		Positive		
Lyme Borreliosis	Spondylo-arthritis		OBC	EXPC	OBC	EXPC	OBC	EXPC	OBC	EXPC	
Borrelia burgdorferi	*Borrelia burgdorferi*	IgM	50	31	4	23	12	31	42	23	0.712*
		IgG	26	20	28	34	14	20	40	34	0.230*
	Borrelia burgdorferi RB	IgM	50	38.5	4	15.5	27	38.5	27	15.5	0.471*
		IgG	26	23.5	28	30.5	21	23.5	33	30.5	0.093
	Borrelia afzelii	IgM	50	40.5	4	13.5	31	40.5	23	13.5	0.406*
		IgG	26	22.5	28	31.5	19	22.5	35	31.5	0.131
	Borrelia garinii	IgM	50	38.5	4	15.5	27	38.5	27	15.5	0.471*
		IgG	26	21	28	33	16	21	38	33	0.190*
	Ehrlichia chaffeensis	IgM	50	44.5	4	9.5	39	44.5	15	9.5	0.267*
		IgG	26	20.5	28	33.5	15	20.5	39	33.5	0.210*
Borrelia Round Body	*Borrelia burgdorferi*	IgM	45	28.5	9	25.5	12	28.5	42	25.5	0.612*
		IgG	25	19.5	29	34.5	14	19.5	40	34.5	0.212*
	Borrelia burgdorferi RB	IgM	45	36	9	18	27	36	27	18	0.354*
		IgG	25	23	29	31	21	23	33	31	0.075
	Borrelia afzelii	IgM	45	38	9	16	31	38	23	16	0.284*
		IgG	25	22	29	32	19	22	35	32	0.113
	Borrelia garinii	IgM	45	36	9	18	27	36	27	18	0.354*
		IgG	25	20.5	29	33.5	16	20.5	38	33.5	0.172*
	Ehrlichia chaffeensis	IgM	45	42	9	12	39	42	15	12	0.134
		IgG	25	20	29	34	15	20	39	34	0.192*
Borrelia afzelii	*Borrelia burgdorferi*	IgM	50	31	4	23	12	31	42	23	0.712*
		IgG	37	25.5	17	28.5	14	25.5	40	28.5	0.427*
	Borrelia burgdorferi RB	IgM	50	38.5	4	15.5	27	38.5	27	15.5	0.471*
		IgG	37	29	17	25	21	29	33	25	0.297*
	Borrelia afzelii	IgM	50	40.5	4	13.5	31	40.5	23	13.5	0.406*
		IgG	37	28	17	26	19	28	35	26	0.334*
	Borrelia garinii	IgM	50	38.5	4	15.5	27	38.5	27	15.5	0.471*
		IgG	37	26.5	17	27.5	16	26.5	38	27.5	0.389*
	Ehrlichia chaffeensis	IgM	50	44.5	4	9.5	39	44.5	15	9.5	0.267*
		IgG	37	26	17	28	15	26	39	28	0.408*
	Borrelia burgdorferi	IgM	28	20	26	34	12	20	42	34	0.307*
		IgG	38	26	16	28	14	26	40	28	0.445*
	Borrelia	IgM	28	27.5	26	26.5	27	27.5	27	26.5	0.19

Borrelia garinii	*burgdorferi* RB	IgG	38	29.5	16	24.5	21	29.5	33	24.5	0.316*
	Borrelia afzelii	IgM	28	29.5	26	24.5	31	29.5	23	24.5	0.019
		IgG	38	28.5	16	25.5	19	28.5	35	25.5	0.407*
	Borrelia garinii	IgM	28	27.5	26	26.5	27	27.5	27	26.5	0.210*
		IgG	38	27	16	27	16	27	38	27	0.426*
	Ehrlichia chaffeensis	IgM	28	33.5	26	20.5	39	33.5	15	20.5	0.210*
		IgG	38	26.5	16	27.5	15	26.5	39	27.5	0.426*
Ehrlichia chaffeensis	*Borrelia burgdorferi*	IgM	43	27.5	11	26.5	12	27.5	42	26.5	0.574*
		IgG	25	19.5	29	34.5	14	19.5	40	34.5	0.212*
	Borrelia burgdorferi RB	IgM	43	35	11	19	27	35	27	19	0.310*
		IgG	25	23	29	31	21	23	33	31	0.075
	Borrelia afzelii	IgM	43	37	11	17	31	37	23	7	0.239*
		IgG	25	22	29	32	19	22	35	32	0.113
	Borrelia garinii	IgM	43	35	11	19	27	35	29	19	0.310*
		IgG	25	20.5	29	33.5	16	20.5	38	33.5	0.172*
	Ehrlichia chaffeensis	IgM	43	41	11	13	39	41	15	13	0.087
		IgG	25	20	29	34	15	20	39	34	0.192*

*$P \leq 0.05$
OBC: Observed counts.
EXPC: Expected counts.

Appendix B

Table B: Fisher's exact test was used to statistically compare immunological response frequencies (binary data set) of CMK categories C 1, C 2, C 3, MTBDI C4, MTBDI C5, MTBDI C6 and MTBDI C7 between both patient groups against each other for significant or non-significant association. LB IgM immunological response frequencies were compared with SpA IgM, and LB IgG immunological response frequencies were compared with SpA IgG. The number of negative and positive counts observed in the binary data set corresponding to a particular Fisher's exact test proportion has been tabulated in the Observed count (OBC) columns. Counts in the Expected count (EXPC) count column are statistically computed value that assumes no-hypothesis condition. Cramer's V (φ_C) value in the last column signifies effect size between the proportions being tested. In order for a Fisher's exact test proportion to be significantly dependent, difference between positive and negative OBC and EXPC pairs should be diverse enough for the proportion to be significantly dependent at $p \leq 0.05$ (denoted with * in the last column beside φ_C value). In total, 98 Fisher's exact test proportions were tested of which 51 proportions were found significantly dependent i.e. 23 proportions were significantly dependent for IgM, and 28 proportions were significantly dependent for IgG. Among remaining 47 non-significant proportions, 37 were simply independent, 8 were Not Applicable (NA) and 2 were non-significant/independent (#). Highlighted in Yellow are statistically significant Fisher's exact test proportions with a zero number of positive counts either in LB or SpA group or pseudo-relevant proportions. Highlighted in Blue are statistically insignificant Fisher's exact test proportions with a zero number of positive counts either in LB or SpA group. By all of 51 significantly dependent proportions, 28 were recorded as pseudo-relevant proportions.

Fisher's Exact test proportions		Antibody Type	Lyme Borreliosis				Spondyloarthritis				Cramer's V Value (φ_C)
			Negative		Positive		Negative		Positive		
Lyme Borreliosis	Spondyloarthritis		OBC	EXPC	OBC	EXPC	OBC	EXPC	OBC	EXPC	
C 1	C 1	IgM	41	40	13	14	39	40	15	14	0.042
		IgG	54	54	54	54	54	54	54	54	NA
	C 2	IgM	41	47	13	7	53	47	1	7	0.331*
		IgG	54	53.5	0	0.5	53	53.5	1	0.5	0.097
	C 3	IgM	41	47.5	13	6.5	54	47.5	0	6.5	0.370*
		IgG	54	52.5	0	1.5	51	52.5	3	1.5	0.169
	MTBDI C4	IgM	41	39.5	13	14.5	38	39.5	16	14.5	0.063
		IgG	54	52	0	2	50	52	4	2	0.196*
	MTBDI C5	IgM	41	44.5	13	9.5	48	44.5	6	9.5	0.170
		IgG	54	49	0	5	44	49	10	5	0.319*
	MTBDI C6	IgM	41	47.5	13	6.5	54	47.5	0	6.5	0.370*
		IgG	54	54	54	54	54	54	54	54	NA
	MTBDI C7	IgM	41	43	13	11	45	43	9	11	0.092
		IgG	54	41	0	13	28	41	26	13	0.563*
C 2	C 1	IgM	49	44	5	10	39	44	15	10	0.238*
		IgG	45	49.5	9	4.5	54	49.5	0	4.5	0.302*
	C 2	IgM	49	51	5	3	53	51	1	3	0.162
		IgG	45	49	9	5	53	49	1	5	0.256*
	C 3	IgM	49	51.5	5	2.5	54	51.5	0	2.5	0.220*
		IgG	45	48	9	6	51	48	3	6	0.177
	MTBDI C4	IgM	49	43.5	5	10.5	38	43.5	16	10.5	0.257*
		IgG	45	47.5	9	6.5	50	47.5	4	6.5	0.142
	MTBDI C5	IgM	49	48.5	5	5.5	48	48.5	6	5.5	0.031
		IgG	45	44.5	9	9.5	44	44.5	10	9.5	0.024
	MTBDI C6	IgM	49	51.5	5	2.5	54	51.5	0	2.5	0.220*
		IgG	45	49.5	9	4.5	54	49.5	0	4.5	0.302*
	MTBDI C7	IgM	49	47	5	7	45	47	9	7	0.110
		IgG	45	36.5	9	17.5	28	36.5	26	17.5	0.336*
C 3	C 1	IgM	54	46.5	0	7.5	39	46.5	15	7.5	0.402*
		IgG	47	50.5	7	3.5	54	50.5	0	3.5	0.263*
	C 2	IgM	54	53.5	0	0.5	53	53.5	1	0.5	0.097
		IgG	47	50	7	4	53	50	1	4	0.212*
	C 3	IgM	54	54	54	54	54	54	54	54	NA
		IgG	47	49	7	5	51	49	3	5	0.128
	MTBDI C4	IgM	54	46	0	8	38	46	16	8	0.417*

		IgG	47	48.5	7	5.5	50	48.5	4	5.5	0.092
	MTBDI C5	IgM	54	51	0	3	48	51	6	3	0.243*
		IgG	47	45.5	7	8.5	44	45.5	10	8.5	0.076
	MTBDI C6	IgM	54	54	54	54	54	54	54	54	NA
		IgG	47	50.5	7	3.5	54	50.5	0	3.5	0.263*
	MTBDI C7	IgM	54	49.5	0	4.5	45	49.5	9	4.5	0.302*
		IgG	47	37.5	7	16.5	28	37.5	26	16.5	0.382*
MTBDI C4	C 1	IgM	51	45	3	9	39	45	15	9	0.298*
		IgG	50	52	4	2	54	52	0	2	0.196*
	C 2	IgM	51	52	3	2	53	52	1	2	0.098
		IgG	50	51.5	4	2.5	53	51.5	1	2.5	0.132
	C 3	IgM	51	52.5	3	1.5	54	52.5	0	1.5	0.169
		IgG	50	50.5	4	3.5	51	50.5	3	3.5	0.038
	MTBDI C4	IgM	51	44.5	3	9.5	38	44.5	16	9.5	0.316*
		IgG	50	50	4	4	50	50	4	4	0.000
	MTBDI C5	IgM	51	49.5	3	4.5	48	49.5	6	4.5	0.101
		IgG	50	47	4	7	44	47	10	7	0.165
	MTBDI C6	IgM	51	52.5	3	1.5	54	52.5	0	1.5	0.169
		IgG	50	52	4	2	54	52	0	2	0.196
	MTBDI C7	IgM	51	48	3	6	45	48	9	6	0.177*
		IgG	50	39	4	15	28	39	26	15	0.455*
MTBDI C5	C 1	IgM	45	42	9	12	39	42	15	12	0.134
		IgG	46	50	8	4	54	50	0	4	0.283*
	C 2	IgM	45	49	9	5	53	49	1	5	0.256*
		IgG	46	49.5	8	4.5	53	49.5	1	4.5	0.253*
	C 3	IgM	45	49.5	9	4.5	54	49.5	0	4.5	0.302*
		IgG	46	48.5	8	5.5	51	48.5	3	5.5	0.153
	MTBDI C4	IgM	45	41.5	9	12.5	38	41.5	16	12.5	0.154
		IgG	46	48	8	6	50	48	4	6	0.118
	MTBDI C5	IgM	45	46.5	9	7.5	48	46.5	6	7.5	0.080
		IgG	46	45	8	9	44	45	10	9	0.050
	MTBDI C6	IgM	45	49.5	9	4.5	54	49.5	0	4.5	0.302*
		IgG	46	50	8	4	54	50	0	4	0.283*
	MTBDI C7	IgM	45	45	9	9	45	45	9	9	0.000
		IgG	46	37	8	17	28	37	26	17	0.359*
MTBDI C6	C 1	IgM	54	46.5	0	7.5	39	46.5	15	7.5	0.402*
		IgG	54	54	54	54	54	54	54	54	NA
	C 2	IgM	54	53.5	0	0.5	53	53.5	1	0.5	0.097
		IgG	54	53.5	0	0.5	53	53.5	1	0.5	0.097
	C 3	IgM	54	54	54	54	54	54	54	54	NA
		IgG	54	52.5	0	1.5	51	52.5	3	1.3	0.169
	MTBDI C4	IgM	54	46	0	8	38	46	16	8	0.417*
		IgG	54	52	0	2	50	52	4	2	0.196*
	MTBDI C5	IgM	54	51	0	3	48	51	6	3	0.243*
		IgG	54	49	0	5	44	49	10	5	0.319*
	MTBDI C6	IgM	54	54	54	54	54	54	54	54	NA
		IgG	54	54	0	54	54	54	54	54	NA
	MTBDI C7	IgM	54	49.5	0	4.5	45	49.5	9	4.5	0.302*
		IgG	54	41	0	13	28	41	26	13	0.563*
MTBDI C7	C 1	IgM	52	45.5	2	8.5	39	45.5	15	8.5	0.331*
		IgG	38	46	16	8	54	46	0	8	0.417*
	C 2	IgM	52	52.5	2	1.5	53	52.5	1	1.5	0.056
		IgG	38	45.5	16	8.5	53	45.5	1	8.5	0.381*
	C 3	IgM	52	53	2	1	54	53	0	1	0.137
		IgG	38	44.5	16	9.5	51	44.5	3	9.5	0.316*
	MTBDI C4	IgM	52	45	2	9	38	45	16	9	0.348*

		IgG	38	44	16	10	50	44	4	10	0.286*
	MTBDI C5	IgM	52	50	2	4	48	50	6	4	0.141
		IgG	38	41	16	13	44	41	10	13	0.130
	MTBDI C6	IgM	52	53	2	1	54	53	0	1	0.137
		IgG	38	46	16	8	54	46	0	8	0.417*
	MTBDI C7	IgM	52	48.5	2	5.5	45	48.5	9	5.5	0.214*
		IgG	38	33	16	21	28	33	26	21	0.190*

*$P \leq 0.05$
OBC: Observed counts.
EXPC: Expected counts.
[#] in both patient groups the positive and negative observed counts exactly matches their respective expected counts.
NA: Not Applicable (where both proportions being tested for fisher's exact test significance have the number of negative counts that equals to the population size (54)).
Values highlighted in Yellow: Statistically significant Fisher's exact test proportions with a zero number of positive counts either in LB or SpA group or pseudo-relevant proportions.
Values highlighted in Blue: Statistically insignificant Fisher's exact test proportions with a zero number of positive counts either in LB or SpA group.

Appendix C

Table C: Binary logistic regression was performed only on significantly dependent Fisher's exact test proportions from appendix B Table B. The test was performed between two variables i.e. a dependent variable and an explanatory variable. Dependent variable is an outcome of the explanatory variable. SpA CMK categories are dependent variables and LB CMK categories are the explanatory/predictor variables. Correct prediction percentage and the p-value signify the difference between predictor variables that would likely or unlikely explain an outcome variable. Correct prediction percentage indicates overall predictability rate of a model between a particular dependent and explanatory variable and P value indicates if the model is statistically relevant at $p \leq 0.05$.Correct prediction percentage consists of Block without a predictor and Block with a predictor. Overall predictability rate of the model in the former does not depend on any LB explanatory variable, whereas in the latter Overall predictability rate of the model depends on respective LB explanatory variable. Overall predictability of a model should increase from a block with no explanatory variable to a block with explanatory variables. In the last column, p-values ≤ 0.05 indicate that increase in overall predictability percentage has been significant enough, and if an explanatory variable is a potential predictor variable for an outcome variable. All 28 pseudo-relevant Fisher's exact test proportions from appendix B Tables B were recorded as Not Applicable (NA) for binary logistic regression. Among remaining 23 significant proportions, 3 predictor variables (#) were recorded explaining MTBDI C4 from SpA patient group and 7 indicator variables (°) were recorded explaining C 1 and MTBDI C7 from SpA patient groups with IgM and IgG antibody types respectively. Indicator variables differ from predictor variables in the way that the increase in overall predictability percentage was recorded, but the p value was not statistically relevant enough.

Binary Logistic Regression parameters		Antibody Type	Correct Prediction Percentage		*P* Value for the Model
Dependent variable: Spondyloarthritis	Explanatory/Predictor variables: Lyme Borreliosis		Block without a predictor (%)	Block with a predictor (%)	
C 1	C 2	IgM	72.2	72.2	0.063
		IgG	NA		
	C 3	IgM	NA		
		IgG	NA		
	MTBDI C4	IgM	72.2	74.1	0.074°
		IgG	NA		
	MTBDI C5	IgG	NA		
	MTBDI C6	IgM	NA		
	MTBDI C7	IgM	72.2	74.1	0.087°
		IgG	NA		
C 2	C 1	IgM	98.1	98.1	0.456
	C 2	IgG	98.1	98.1	0.544
	C 3	IgG	98.1	98.1	0.121
	MTBDI C5	IgM	98.1	98.1	0.589
		IgG	98.1	98.1	0.239
	MTBDI C7	IgG	98.1	98.1	0.377
C 3	C 1	IgM	NA		
	C 2	IgM	NA		
	MTBDI C5	IgM	NA		
	MTBDI C7	IgG	94.4	94.4	0.140
MTBDI C4	C 2	IgG	NA		
	C 2	IgM	70.4	75.9	0.014#
	C 3	IgM	NA		
	MTBDI C4	IgM	70.4	75.9	0.020#
	MTBDI C6	IgM	NA		
		IgG	NA		
	MTBDI C7	IgM	70.4	75.9	0.039#
		IgG	92.6	92.6	0.086
MTBDI C5	C1	IgG	NA		
	C 3	IgM	NA		
	MTBDI C6	IgM	NA		
		IgG	NA		
MTBDI C6	C 1	IgM	NA		
	C 2	IgM	NA		
		IgG	NA		

	C 3	IgG	NA		
	MTBDI C5	IgM	NA		
		IgG	NA		
	MTBDI C7	IgG	NA		
MTBDI C7	C 1	IgG	NA		
	C 2	IgG	51.9	57.4	0.080°
	C 3	IgM	NA		
		IgG	51.9	57.4	0.211°
	MTBDI C4	IgM	83.3	83.3	0.288
		IgG	51.9	57.4	0.255°
	MTBDI C5	IgG	51.9	64.8	0.149°
	MTBDI C6	IgM	NA		
		IgG	NA		
	MTBDI C7	IgM	83.3	83.3	0.382
		IgG	51.9	64.8	0.209°

[#] Predictors: Significant prediction model observed between the dependent and covariate where $P \leq 0.05$.
° Indicators: Non-significant prediction model, but with increase in Correct Prediction Percentage between Block without predictor and with predictor.
NA: Not Applicable (where the total number of observed counts in either the dependent or explanatory variable is completely negative).