UNORTHODOX AND UNVALIDATED LABORATORY TESTS IN THE DIAGNOSIS OF LYME BORRELIOSIS AND IN RELATION TO MEDICALLY UNEXPLAINED SYMPTOMS

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Background

There has been an increasing trend in recent years for some medical practitioners to make a diagnosis of Lyme borreliosis in patients with a range of serious but non-specific symptoms based on unorthodox and unvalidated laboratory tests. The consequences of this over-diagnosis included recommendations for a range of potentially hazardous treatments such as prolonged courses of parenteral cephalosporins and other antibiotics and the possibility that other medically significant causes of the patients' symptoms were being overlooked.

Borrelia burgdorferi infection and Lyme borreliosis

Lyme borreliosis is a zoonosis caused by several closely related genospecies of the spirochaete *Borrelia burgdorferi sensu lato*. The spirochaetes' main natural hosts are small mammals and birds, and infection is transmitted by bites of ticks of the *Ixodes ricinus* complex. Ticks become infected through taking blood meals from infected hosts in the first and second stages of their three-stage life cycle. They usually feed on larger mammals, especially deer, at the third (reproductive) stage. Human being may be incidental feeding hosts at any stage in the life cycle. Clinical features of what is now known as Lyme borreliosis were described in the late nineteenth century in Europe. It was named Lyme disease after a cluster of cases of oligoarthritis and erythema migrans occurred in the Old Lyme area of Connecticut, USA, in the mid-1970s. The aetiological agent, *B. burgdorferi* was described by Burgdorfer in 1982. The three genospecies mainly associated with human disease are *B. burgdorferi sensu stricto, B. garinii*, and *B.afzelii*.

Lyme disease is a multi-system infection. Many of its symptoms are common to other human diseases and also overlap with less specific syndromes for which there are currently few diagnostic or pathological explanations such as the clinical complexes of chronic or post-viral fatigue syndromes.

The initial specific feature of Lyme disease is generally erythema migrans (3-30 days after exposure) but this can be unrecognised, atypical or even absent in up to 30% of cases. It is an erythematous rash, spreading from the site of a preceding (often unrecognised) tick bite. Rashes usually resolve promptly with appropriate antibiotic treatment, which should also prevent the development of later complications.

The organism may spread to other organs and tissues through the bloodstream and lymphatics and cause more serious illness. Systemic symptoms may then include fatigue, myalgia, arthralgia, headache, fever or chills, stiff neck, and regional lymphadenopathy. The most common complications arise from damage to the nervous and musculoskeletal systems, but other organs and tissues, including the heart

and eye, can be affected. Untreated or inadequately treated patients may develop joint pains, stiffness and frank arthritis. Cranial nerve abnormalities, 'viral type' meningitis and painful radiculpathy may occur within a few weeks or several months after infection.

Untreated or inadequately treated patients may develop late or persistent disease months or years after the initial infection. These symptoms of late disease include migratory musculoskeletal pains in joints, bursae, tendons and muscles which may develop into intermittent, then persistent, arthritis with marked synovitis, usually affecting the knee. Chronic neurological presentations include a subacute encephalopathy, peripheral neuropathy and, rarely, encephalomyelitis including spastic paraparesis, ataxia and cognitive impairment. Acrodermatitis chronica atrophicans in an uncommon late stage skin manifestation, and may be accompanied by peripheral neuropathy affecting the same part of the body as the skin lesion, usually an exposed area of a limb.

A small percentage of patients have a spectrum of non-specific symptoms similar to those of chronic or post-viral fatigue syndrome despite apparently adequate treatment and lack of objective evidence of continuing infection activity. The results of long-term case control studies suggest that the overall outcomes of adequately treated patients is good, with no excess of morbidity over matched control groups..

Epidemiology of human Lyme disease

Lyme disease is endemic in large parts of the northern hemisphere. Approximately 20,000 cases are reported annually in the USA. It occurs in most European countries where tens of thousands of cases occur annually, but registration is not systematic. Human disease is associated with rural and forest areas where contact with ticks that also feed on deer is likely to occur. Although infected ticks are abundant in many parts of the UK, human infections are relatively uncommon with specific foci of endemic infection in forest areas that include, in particular, the New Forest, Thetford Forest, Exmoor, Savernake Forest, the Lake District and the Scottish Highland and Islands.

Diagnosis

Direct detection methods for *B. burgdorferi* , including culture and DNA detection, are available, but have limited value in routine diagnosis. Isolation of *B. burgdorferi sensu lato* from clinical samples is slow, taking two to six weeks, requires special media, and is notoriously unreliable as a diagnostic tool. Culture of skin biopsy specimens from erythema migrans lesions provides the highest yield. Early in the disease, *B. burgdorferi* can be isolated from the blood but because spirochaetaemia occurs intermittently and with a low number of organisms only 2-7% of specimens are culture positive.

PCR tests have been developed for *B. burgdorferi*, and are useful in certain circumstances. Borrelial DNA is detectable in about 70% of well taken skin biopsies from patients with untreated erythema migrans and in over 90% of samples from patients with untreated acrodermatitis chronica atrophanicans. It is also useful in testing synovial fluid from patients with chronic Lyme arthritis, and may be helpful in assessing the need for further antibiotic treatment in those with refractory arthritis,

where an autoimmune component may be a major contributing factor to the inflammatory arthritis. There have been serious cross-contamination problems in some laboratories performing these tests leading to false-positive results. PCR is not yet a routine diagnostic test.

Antibody detection remains the mainstay of laboratory support for a clinical diagnosis. It is insensitive in early infection as an IgG response takes some weeks to develop. The great majority of patients with established later stage infection are seropositive.

Borreliae share many antigens with other bacteria and cross-reactions in EIA and IFA tests may be problematic. False-positive results may also occur in EIA and IFA tests in patients with conditions such as glandular fever, rheumatoid arthritis or other autoimmune conditions. Difficulties with specificity are accentuated if tests are applied in clinical situations where there is a low likelihood of Lyme borreliosis and thus the predictive value of a positive result if low.

Internationally recognised criteria for the diagnosis of Lyme borreliosis are based upon stringent interpretation of serological tests for specific antibodies to *B. burgdorferi sensu lato.*. The criteria recommended in the USA (from the Centers for Disease Control and Prevention), Europe (i.e. MiQ 20-00 Germany) and the UK are:

- Serum samples for the detection of antibodies to *B. burgdorferi* should be analysed by a two-test procedure:
    - a sensitive screening test (e.g. ELISA or IFA). All samples judged to be reactive or equivocal in the screening test should then be confirmed by
    - a Western blot for antibodies to specific *B. burgdorferi* antigens. The Western blot should only be used in succession with an ELISA or IFA test. Detailed interpretive criteria for Western blots differ between Europe and the USA, to take into account differences in the geographic distribution of the infecting genospecies.

These serological criteria are used for the laboratory diagnosis of Lyme borreliosis by the HPA Lyme Borreliosis Specialist Diagnostic Service at the HPA South-East Regional Laboratory, Southampton.

Treatment

Patients with early Lyme disease respond well to treatment with doxycycline or amoxicillin. Cerfuroxime axetil is a useful oral alternative. Macrolides are less effective but can be used for patients who cannot be treated with the other fist line agents. Parenteral treatment is generally recommended for patients with neurological complications. Ceftriaxone is the most commonly used parenteral agent, because of its one-daily dosing regimen. Treatment duration is of moderate length – 14-30 days depending on the stage and severity of the disease. There is currently no scientific evidence to support longer term therapy in the absence of objective evidence for continuing active infection. The outcome for most appropriately treated patients is excellent, but patients with longstanding infection and significant tissue damage may be slow to respond and their recovery may be incomplete, depending on the severity of their illness before treatment.

Unorthodox approaches in the Lyme disease controversy

Over the past decade in the USA a campaign has developed increasing momentum that Lyme borreliosis is significantly under-diagnosed and that many (thousands) of patients with general, often debilitating but non-specific syndromes, are in fact suffering from chronic Lyme borreliosis. It is claimed that their infections are not detected by the internationally accepted laboratory criteria. In effect, this view has become that undiagnosed Lyme borreliosis is the major cause of chronic fatigue syndrome. The proponents of this view comprise some clinicians who do not support the evidence-based majority opinion, supported by vociferous patient support groups. Certain laboratories provide a range of unvalidated tests that claim to show evidence of *B. burgdorferi* infection in a very high proportion of these patients. In fact, tests such as those provided by the Bowen Institute in Florida appear to give positive results on all patient samples submitted regardless of validated evidence of *B. burgdorferi* infection. The use of these laboratories is heavily promoted by many of the support groups. The campaign is vociferous and acrimonious with serious physical and legal threats against clinicians who maintain the conventional and evidence-based approach to Lyme disease diagnosis. Over the last few years the unorthodox views have gained some support in the UK from patient groups and a small number of medical practitioners who have taken up the unorthodox approach.

Some UK patients with significant symptoms that are currently unexplained in terms of current medical knowledge or understanding have consulted these practitioners in private practice and been diagnosed as suffering from chronic Lyme borreliosis. The clinical impact can be the prescription of extensive courses of antibiotics plus a range of other marginal (complementary medicine) products. If the patients are referred back to NHS practitioners, in primary or secondary care, those practitioners may have severe misgivings about the treatment recommendations that can put the doctor-patient relationship under severe strain.

At the same time, patients lose faith in the standardised, quality controlled and licensed diagnostic tests provided principally by the HPA Specialist Diagnostic Unit, the Unit may be accused of producing false-negative results, and the specialist advice of the Unit is denigrated.

All of this can have serious implications for the care of patients – both those who genuinely are suffering from Lyme borreliosis and require appropriate treatment, and those who are led to believe that they have Lyme borreliosis and demand treatment that is inappropriate and may be potentially harmful.

The unorthodox tests

The unconventional diagnostic tests used to confirm a clinical diagnosis of chronic Lyme borreliosis in patients with a range of chronic fatigue and neurological symptoms have included:

1. Direct microscopy of whole blood.
2. Culture of blood for *Micrococcus* and *Staphylococcus* species.
3. Stool parasitology examination.
4. Fluorescent antibody tests and microscopy of blood samples sent to the Bowen Research and Training Institute Inc, Palm Harbour, Florida.

Microscopy of whole blood

It has been claimed that chronic Lyme borreliosis can be diagnosed on the basis of seeing spirochaetes in the blood of patients by high power (on-screen magnification reported to be X 10,000) 'live' microscopy of blood. A drop of blood is placed on a microscopy slide, covered with a cover slip, and then left to stand for a period of at least 6 hours and up to 24 hours in a moist chamber. The film is then examined by dark field and phase contrast microscopy. It is claimed that spirochaete forms can be seen emerging from red and white cells in these blood films. They have been described as being 'intracellular L-forms' that can be seen emerging from blood cells.

Patients and medical practitioners have been told that this test for Lyme borreliosis is positive in chronic fatigue syndrome patients, showing that CFS is caused by chronic *B. burgdorferi* infection.

However, these tests are not being performed by medical practitioners or clinical/biomedical trained or qualified in laboratory medicine specialties such as microbiology, parasitology or haematology that would include specific training in light microscopy. They are not performed in laboratories accredited for clinical pathology testing.

Culture of blood for *Micrococcus* and *Staphylococcus* species

An association has been claimed between bloodstream infection with *Micrococcus luteus* and *Staphylococcus cohnii* and chronic fatigue syndrome. Blood culture is performed by a method described as including 'ultra filtration of the blood through 0.4 micron filters to detect small forms rather than straight culture'. Approximately half of these tests have been reported to be positive for one or other of *M. luteus* or *S. cohnii.* No evidence has been provided of the validation of the identity of the isolates. These test are not done in a laboratory accredited for clinical pathology testing by registered healthcare scientists.

Stool parasitology

A link has also been claimed between intestinal infection with parasites, specifically *Blastocystis hominis*, and chronic fatigue syndrome. Tests for these are provided by the University of Leeds Teaching Hospital. This laboratory is a recognised laboratory for parasitology testing and is accredited by Clinical Pathology Accreditation (CPA) UK Ltd.

Fluorescent antibody test

EDTA whole blood samples have been sent to the Bowen Research and Training Institute Inc, Palm Harbour, Florida, for their Q-RIBb test (a direct fluorescent antibody method for quantitative rapid identification of *B. burgdorferi*). This test gives a result as a serial dilution factor to indicate the strength of positivity in the sample. The Bowen Institute also examines buffy coat smears for *Babesia* and *Ehrlichia*. The Bowen Institute claims a very high positivity rate in blood samples examined by the Q-RIBb test. However, the comparative tests claimed to confirm

their findings have themselves been discredited and shown not to detect *B. burgdorferi*.

The Bowen Institute was inspected by a team of Inspectors from the Florida Agency for Health Care Administration and the Centers for Disease Control and Prevention, Atlanta (Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases). A warning was published in MMWR, 11 February 2005 advising caution regarding many commercially promoted tests for Lyme borreliosis and restating the internationally accepted criteria for diagnosis. Barbara Johnston, PhD, of the Bacteria Zoonoses Branch at CDC, who was a member of the inspection team has also provided the following statement:

'The Florida Agency for Healthcare Administration (AHCA) has investigated Bowen Research Institute of Palm Harbour, Florida, which offers the Q-RIBb test. As a result of this investigation, AHCA has denied licensure to the Bowen Research Institute. This laboratory is also not certified under the Clinical Laboratory Improvement Amendments (CLIA). The CLIA programme is administered by the Centers for Medicare and Medicaid Services (CMS), an agency of the Department of Health and Human Services. CLIA certification is required for all laboratories performing clinical laboratory testing.

If you need further information, I refer you to Ms Patricia James of the Florida Agency for Healthcare Administration.

CDC advises clinicians to use laboratory tests that are FDA cleared or ones that have satisfactory performance characteristics documented in the peer-reviewed scientific literature. The Q-RIBb test does not meet these criteria.'

The Inspectors found that there was no proof in support of the statement by the Bowen Institute that any positive result in the Q-RIBb test is significant. The validation of the Q-RIBb test claimed by the Bowen Institute was not scientifically sound because the comparative method (Mattman culture medium) was not an acceptable external testing method and had been shown to be invalid in peer-reviewed literature. As well as the links to the discredited Mattman culture medium as the validation of the Q-RIBb test, the Institute also uses the commercially obtained *B. burgdorferi* fluorescent antibody inappropriately. The antibody was used in a more concentrated (undiluted) form than recommended by the manufacturer and the Bowen Institute then interpreted even the faintest of staining as a positive result. This interpretation would not be accepted by reputable scientists in the field of immunofluorescence microscopy. The Institute also reports that all samples tested to date have given positive results, which gives a strong indication that they are reporting weak, non-specific antibody binding. Further details of the Inspector's report are on file.

Review of the unorthodox diagnostic tests

There is no evidence of validation or other evidence to support the use of these tests. Conclusions are drawn from individual patient's experience and unsubstantiated claims which, when published, are in non-peer-reviewed journals. The blood microscopy and culture methods use equipment and methods that the practitioners are not trained or qualified to use and are not performed in an accredited laboratory.

There is no evidence of proper quality control or external quality assurance although the results are passed on to other medical practitioners and patients as a validated diagnostic work-up.

1. Microscopy of whole blood

The objects purported to be borreliae in the whole blood films are not considered to be borreliae but to represent artefacts of the method used. If they were spirochaetes the number demonstrated by light microscopy in such a small sample would indicate a substantial spirochaetaemia which could readily be confirmed or refuted by electron microscopy, immunofluorescence or PCR. Some of the structures appear to be contaminating debris, as would be expected in samples collected by inexperienced individuals (patients, carers etc) in non-sterile conditions. Other strands appear to be fibrin produced by the clotting mechanism that would occur in whole blood held for several hours in this way, collagen fibrils or cell membrane fragments shed from degenerating red and white blood cells. Both fibrin and cell membrane fragments are well recognised artefacts in microscopy of blood cells. All have been known to be confused with spirochaetes (particularly leptospires) on microscopy.

Details of how the blood samples for these tests are collected and transported are scanty but what information is known suggests that the methodology from specimen collection to microscopic examination would not eliminate or minimise the presence of such artefacts and the risk of their misidentification as borrelia.

Furthermore, the biological basis for the test is fundamentally wrong. *B. burgdorferi sensu lato* is an extracellular bacterium in the bloodstream. It is not an intracellular bacterium that could be seen 'emerging from infected blood cells'. Moreover, any spirochaetes in a thick blood smear, covered by a cover slip, would not remain viable for long. Blood is not an 'ideal medium' for borreliae, the organisms are fragile *in vitro*, requiring special media and careful temperature regulation. Spirochaetemia occurs in the early stages of infection and is intermittent and short lived, with a low number of organisms. In untreated or inadequately treated infection spirochaetes may become sequestered in niches deep organs and tissues, causing persistent infection and disease hence the difficulty in treating with antibiotics after the acute period of infection, and their ability to cause chronic conditions of nerves, heart, joints, etc.' *In vitro* work has shown intracellular localisation of a few *B. burgdorferi* within human endothelial cells, macrophages and fibroblasts. However, *B. burgdorferi* can adhere to the surface of many types of mammalian cells and is a potent inducer of pro-inflammatory cytokines, including TNF and interleukin 1b from peripheral blood mononulcar cells.

Diagnostic tests that require microscopy or culture of samples from patients should be supervised and the results interpreted by consultant medical microbiologists or clinical scientists of equivalent standing. Tests should also be done in an accredited laboratory with quality assurance standards.

2. Fluorescent antibody tests – Bowen Institute

The recommendations from CDC as set out in Dr Johnson's communication and the MMWR that this test should not be used for the diagnosis of Lyme borreliosis are supported by Lyme borreliosis experts in the UK and Europe. The Institute's claim for

validation rests upon culture confirmation by Professor I. Mattman using a medium (MPM) reported by Phillips et al. Other reputable workers have shown that this medium fails to grow *B. burgdorferi*. The fact that all 306 blood samples were positive in the Q-RIBb test and by MPM culture and no sample gave negative results indicates that any results in this tests are spurious and give no indication of Lyme borreliosis status. Patients should not be diagnosed and treated on the basis of these test results.

3. Culture for *Micrococcus luteus* and *Staphylococcus cohnii*

This diagnostic test has no evidence base and the methods used show a lack of knowledge and understanding of the basic requirements for performing blood cultures. Because blood should be normally sterile, any growth in a blood culture is potentially significant and the most important confounding factor in performing blood cultures is the risk of introducing contaminants from the skin of the patient or the person taking the blood sample. The most common contaminants are coagulase-negative staphylococci of which *S. cohnii* is one of the species commonly found on human skin. *M. luteus* is also common on the skin and in the general environment. It is one of the most common contaminants of laboratory cultures.

Few details are provided about how the blood for culture is collected and transported and what methods are used for examination in the laboratory, except that it includes ultrafiltration. Any such procedure increases the chance of contamination in addition to the likelihood of contamination if samples are collected and handled by inexperienced and untrained people. In view of the ease with which blood cultures taken in hospital wards can yield the skin organisms as contaminants it would not be surprising to detect these bacteria in specimens collected under less than the strictest aseptic procedures.

There are no Standard Operating Procedures for these tests and the laboratory work is not performed in a laboratory accredited for clinical diagnostic work or by registered healthcare scientists trained and qualified in clinical microbiology.

Conclusions

None of the unorthodox diagnostic tests purported to support the diagnosis of Lyme borreliosis or other infections in chronic fatigue syndrome are validated and should not form the basis of any medical diagnosis or treatment prescription or recommendation.

Clinical Practice:
The consequences of diagnosis and advice based on unorthodox and unvalidated tests

The application of diagnostic approaches based on unvalidated and inappropriate tests as described above can result in the diagnosis of patients as suffering from chronic Lyme borreliosis when the internationally accepted criteria do not show any evidence of *B. burgdorferi* infection. This has two significant consequences in terms of clinical governance and standards of medical practice.

1. Failure to investigate, diagnose and treat other identifiable medical conditions that might be the cause of the patient's signs and symptoms.

   It is also significant that the information provided to patients can be misleading and inaccurate..

2. The treatments prescribed or recommended on the basis of the tests include combinations of long term cocktails of antibiotics with various complementary medicine items.

   All of the antibiotics are known to have some specific side effects, especially when given in prolonged courses. In addition, they have a damaging effect on the normal commensal flora and there is a risk of inducing antibiotic associated diarrhoea. The patients would certainly be expected to develop a resistant bacterial flora.