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Lyme borreliosis (LB) is caused by spirochetes within the Borrelia burgdorferi sensu lato complex and is the most common tick-transmitted disease in the northern hemisphere. The transmission of the spirochetes to humans in Europe is done by the Ixodes ricinus ticks, which can also transmit the relapsing fever species Borrelia miyamotoi. LB may cause clinical manifestations in the skin, in the central nervous system, in joints, and in the heart. Diagnosis of LB is mainly based on the patient ´s medical history, self-described symptoms, and clinical signs in combination with the detection of Borrelia-specific antibodies (serological methods). In some cases/issues, detection of Borrelia-specific deoxyribonucleic acid (molecular methods) may be used as a complement to serology. All diagnosed LB infections are treated with antibiotics to prevent disease progression, and most patients fully recover without further sequelae. The overall aims of this thesis were to evaluate molecular and serological tools for laboratory diagnosis of LB, with a special focus on Lyme neuroborreliosis (LNB), and to identify potential improvements. The results presented in this thesis showed that the immunoglobulin (Ig) G assays, currently in use in northern Europe for detection of antibodies in serum, had high diagnostic sensitivity (88 %) together with comparable results both between and within assays. For the IgM assays, the diagnostic sensitivity was lower (59 %) with more heterogeneous results. Small variations in diagnostic performance for IgM and IgG were mainly presented for samples within the borderline zone. These results support the theory that separate testing of IgM antibodies in serum has low diagnostic value. However, simultaneous detection in serum and cerebrospinal fluid (CSF) for both IgM and IgG antibodies was essential for the diagnosis of LNB, at least for certain assays. So far (to our knowledge), no systematic evaluation and optimisation of the pre-analytical handling of CSF samples before molecular testing has been performed. By use of the precipitate concentrated by moderate centrifugation, extraction of total nucleic acid followed by reversetranscription to complementary deoxyribonucleic acid, in combination with the absence of polymerase chain reaction (PCR) inhibitors, detection of Borrelia garinii, Borrelia afzelii, Borrelia burgdorferi sensu stricto, and B. miyamotoi was possible. These four species are all known to be pathogenic to humans. The results revealed a high analytical sensitivity and specificity for the optimised pre-analytical conditions. The thesis also presents results showing that the real-time PCR protocols currently used in Scandinavia have high analytical sensitivity, specificity, and concordance. This indicates that the low diagnostic sensitivity for detection of Borrelia in CSF was not a result of poorly designed and evaluated PCR protocols, but was possibly due to the low number of spirochetes in the samples. However, to further evaluate the diagnostic performance for detection of Borrelia in CSF by PCR, clinical samples need to be evaluated based on our new recommendations for the pre-analytical handling of CSF samples. In conclusion, this thesis presents results revealing that both molecular and serological tools for detection of Borrelia have, in general high sensitivity and specificity with results comparable between different protocols and different laboratories. It also presents recommendations for pre-analytical handling of CSF samples before PCR-analysis, and shows the benefits in diagnostic performance by simultaneous detection of IgM and IgG antibodies in serum and CSF for accurate diagnosis of LNB. Even though the techniques mentioned above have high analytical performance, the ability to discriminate an active infection from a previous one is limited and further studies need to be carried out. These studies need to focus on finding diagnostic tools that can help physicians to determine ongoing infection to ensure adequate treatment. It is also desirable to improve the standardisation of the diagnostic tools and to find methods that can discriminate between different Borrelia species. Borrelios är den vanligaste fästingöverförda sjukdomen på norra halvklotet och orsakas av bakterier inom Borrelia burgdorferi sensu lato gruppen. Överföringen av bakterier till människa i Europa sker via Ixodes ricinus fästingar, vilka även överför bakterien Borrelia miyamotoi som ger återfallsfeber. Borreliainfektioner uppvisar kliniska uttryck i huden, i det centrala nervsystemet och i leder. En borrelia-diagnos baseras främst på patientens medicinska historia i kombination med kliniska tecken, egenbeskrivna symptom samt på visning av Borrelia-specifika antikroppar (serologiska metoder). Vid vissa frågeställningar kan påvisning av Borrelia-bakteriens arvsmassa (molekylärbiologiska metoder) användas som komplement till antikroppstester. Alla diagnostiserade borreliainfektioner behandlas med antibiotika för att förhindra utveckling av sjukdomen och merparten av patienterna blir fullt återställda. Det övergripande syftet med avhandlingen var att utvärdera metoder för påvisning av Borrelia-specifika antikroppar samt Borrelia-specifik arvsmassa, men fokus på neuroborrelios, samt identifiera potentiella förbättringar. De metoder som användes för påvisning av immunoglobulin (IgG)-antikroppar (uppträder sent i en infektion) i serum i norra Europa uppvisar hög känslighet (88 %) med jämförbara resultat både mellan och inom en analysmetod. Vid påvisning av IgM-antikroppar (uppträder tidigt i en infektion) i serum uppvisas lägre känslighet (59 %) och mer olikartade resultat. Småvariationer i den diagnostiska förmågan att påvisa IgM och IgG-antikroppar beror till stor del på att flera prover erhållit gränsvärden dvs sett värde som inte kan anses som positivt men inte heller som negativt. Resultaten från denna studie indikerar att påvisning av IgM-antikroppar i serum har lågt värde vid diagnostik av Borrelia. Dock bör parallell analys av både de IgM och IgG-antikroppar i serum och ryggmärgsvätska utföras vid påvisning av neuroborrelios. I dagsläget (till vår kännedom) har ingen systematisk utvärdering och optimering av det preanalytiska tillvägagångssättet vid påvisning av Borrelia-specifik arvsmassa i ryggmärgsvätska genomförts. Genom att använda pelleten (bottensatsen som erhålls genom mättlig centrifugering), framrening av total nukleinsyra i kombination med frånvaro av material som kan påverka PCR-reaktionen på ett negativt sätt (inhibitorer), kan påvisning av Borreliaarterna Borrelia garinii, Borrelia afzelii, Borrelia burgdorferi sensu stricto och B. miyamotoi ske. Dessa Borrelia-arter är alla patogena för människa. De realtids-PCR protokoll som i dagsläget används i Skandinavien har hög analytisk känslighet, tillförlitlighet och överensstämmelse. Detta tyder på att den låga känslighet som uppvisas vid påvisning av Borrelia-specifik arvsmassa i ryggmärgsvätska inte beror på dåligt utvärderade och designade PCR-protokoll, utan är troligtvis orsakad av låg bakteriemängd i proverna. För vidare utvärdering av den diagnostiska förmågan att påvisa Borrelia-specifik arvsmassa i ryggmärgsvätska med PCR, bör kliniska prover samlas in och analyseras utifrån de nya rekommendationerna för pre-analytiskt tillvägagångssätt vid analys av ryggmärgsprover. Sammanfattningsvis visar resultaten i denna avhandling på generellt hög känslighet och tillförlitlighet samt överensstämmelse mellan olika protokoll/test vid påvisningar av Borreliaspecifika antikroppar och Borrelia-specifik arvsmassa. I avhandlingen presenteras även rekommendationer för pre-analytiskt tillvägagångssätt vid omhändertagande och transport av ryggmärgsvätska till laboratoriet. Resultaten visar även på nyttan i att analysera ryggmärgsvätska och serum parallellt för både IgM och IgG-antikroppar för att erhålla rätt diagnos vid frågeställningen neuroborrelios. Ovan nämnda metoder har trots god prestanda svårt att i alla lägen särskilja en aktiv infektion från en tidigare genomgången, varpå vidare studier krävs. Framtida studier bör fokusera på att finna diagnostiska verktyg som hjälper läkarna att urskilja en pågående infektion så att patienten erhåller passande behandling. Det är också mycket viktigt att arbeta vidare mot en standardisering av de diagnostiska metoderna samt finna metoder som har möjlighet att särskilja mellan olika Borrelia-arter.

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