Cutaneous leishmaniasis: new developments in diagnosis and treatment evaluation
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Summary

Currently, there is a clear and disturbing increase in the number of cutaneous leishmaniasis (CL) patients worldwide. CL ranges from single to many large skin ulcers to the development of mucosal lesions (muco-cutaneous leishmaniasis), leading to progressive destruction of the nasopharynx and invasion of the respiratory tract. Although CL is not lethal, CL control is very important to prevent serious morbidity. CL control currently depends on early and accurate diagnosis and treatment. However, the diagnosis of leishmaniasis remains problematic. Moreover, many, mostly uncontrolled, treatment schemes are employed with varying success. Up to this date the duration of treatment and definition of cure have been based on pure clinical criteria. Therefore, it is important to determine the number of parasites in a lesion before, during and after treatment as accurate as possible in order to assess the outcome of treatment. This thesis describes the development and evaluation of new tools for the diagnosis of CL, and for the determination of the duration and efficacy of treatment.

The Quantitative Nucleic Acid Sequence-Based Amplification (QT-NASBA) technology was developed and evaluated to detect and quantify *Leishmania* parasites in skin biopsies from CL patients. The assay is based on the detection of a small subunit ribosomal RNA (18S rRNA). The QT-NASBA assay was able to quantify 2 – 11,300,000 parasites per biopsy and test evaluation revealed that the assay had a sensitivity of 97.5% and a specificity of 100% [Chapter 2]. Furthermore, this assay proved to be a valuable instrument for monitoring therapy response of CL and could help in predicting clinical outcome. Positive QT-NASBA results 6 weeks after treatment were significantly associated with treatment failure/delayed healing up to 6 months after treatment [Chapter 3].

Additionally, the QT-NASBA assay was compared with two other molecular assays, the real-time Reverse Transcriptase PCR (qRT-PCR) and real-time PCR (qPCR). The qRT-PCR was developed on the basis of the same gene sequences as QT-NASBA. All three assay were compared for the detection and quantification of *Leishmania* parasites in *in vitro* parasite samples and skin biopsy samples from confirmed CL patients. Additionally, a cost, time efficiency and user-friendliness analysis was done. While the three assays performed
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equally well in skin biopsy samples from CL patients, QT-NASBA and qRT-PCR had a
detection limit of 100 parasites/ml of blood, while qPCR detected 1,000 parasites/ml.
Overall, the qRT-PCR was preferred over QT-NASBA and qPCR, since it was highly
sensitive and reproducible but also had a fast procedure time [Chapter 4].

As molecular tools may not be readily applicable under field conditions a serological
tool for diagnostic purposes was also developed and evaluated. In Suriname and the north
of Brazil > 90% of the patients are infected with *Leishmania (Viannia) guyanensis*, but
serological assays for CL are often based on antigens from other species. In Chapter 8 *L.
(V.) guyanensis* antigen was compared with different antigens in an Enzyme-linked
Immunosorbent Assay (ELISA) in proven *L. (V.) guyanensis* infected CL patients. The use
of *L. (V.) guyanensis* antigen improved the assay significantly in comparison with other
antigens, as *L. (V.) braziliensis*, *L. (Leishmania) amazonensis* and *L. (L.) chagasi*. For this
reason our data are of great importance to improve the efficacy of serological diagnostic
tests for CL.

Another part of the research for this thesis was conducted in Suriname. In this country
Pentamidine Isethionate (PI) is the only available drug for treatment of CL. Recently, local
dermatologists have observed an increase in CL patients not responding adequately to
standard treatment. With QT-NASBA, we measured parasite loads during and after
treatment with PI. We found that treatment compliance was very low; half of CL patients
were treated inadequately. Furthermore, a lower cure rate (76% - 78%) was estimated than
previously observed between 1994 and 2000 (90%). For this reason, a much shorter
treatment protocol was recommended to improve inadequate compliance and efficacy of
treatment [Chapter 5].

In addition, risk factors seem to be increasing in Suriname due to deforestation, gold
mining activities and migration of immigrant workers. While only *L. (V.) guyanensis* is
described as causing species, different clinical manifestations are seen, suggesting that
other *Leishmania* species are responsible for CL disease in Suriname. With PCR-RFLP
(Restriction Fragment Lengths Polymorphism) the majority of the infecting *Leishmania*
species was identified as *L. (V.) guyanensis*, while also *L. (L.) amazonensis* and *L. (V.)
lainsoni* were found [Chapter 7]. The *L. (L.) amazonensis* infected patient did not respond
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to standard treatment with PI, and other medication was donated [Chapter 6]. Furthermore, the annual incidence rate over 2006 was estimated as 5.32 to 6.13 CL patients per 1,000 inhabitants for the forested hinterland and 0.64 to 0.74 patients per 1,000 inhabitants for the whole country.
Summary