Commentary

The paradox of tegumentary leishmaniasis diagnosis: How the need for accurate and modern techniques is being addressed in low income areas

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Tegumentary leishmaniasis (TL) is a neglected disease that is experiencing an alarming increase in incidence, especially in underdeveloped regions of the world [1]. The causal parasite, *Leishmania,* is transmitted by vectors and can cause cutaneous and mucous lesions [2]. The clinical presentation of the disease differs according to characteristics related to the parasite and the host response.

The diagnosis of TL needs to be specific because the existing treatment has considerable toxicity [3]. However, specific complementary tests that aim to directly identify the parasite, such as cultures or smears, have relatively low sensitivities [2]. Furthermore, the immunological tests that can achieve acceptable sensitivities, such as the Montenegro skin test and serology, tend to be limited by low specificities [2]. Molecular tests that are more accurate require advanced laboratory facilities [4,5]. The implementation of these methods is difficult in the remote areas in which TL is more frequent.

Recent studies, including systematic reviews of the literature, have called attention to the fact that TL is a disease for which no gold standard diagnostic technique exists and that most studies on this topic are performed in extremely controlled environments [6]. It is important to note that new studies aiming to develop diagnostic tools for TL need to determine whether the tests have strong external validity. New diagnostic tools for leishmaniasis should ideally be accurate and easily deliverable to end-users. Other practical characteristics, such as affordability, user-friendliness, reliability and equipment-free, should also be present in ideal conditions.

Vink MMT et al. [7] compared two techniques for the diagnosis of cutaneous leishmaniasis (CL) at the Leishmaniasis Clinic of the National Malaria and Leishmaniasis Control Programme (NMLCP) in Kabul, Afghanistan. The research, designed as a cross-sectional/cohort accuracy study, evaluated the CL Detect™ Rapid Test for Cutaneous Leishmaniasis (InBios International, Inc., Seattle, USA) (CL Detect) and

the Loopamp™ Leishmania Detection Kit (Eiken Chemical Co., Tokyo, Japan) (Loopamp) against a composite reference standard formed by microscopic evaluation of skin smears and real-time polymerase chain reaction (PCR) from samples collected using a dental broach. After recruitment, 257 cases and 17 controls were included.

The CL Detect is a qualitative immunochromatographic assay for the rapid detection of the peroxidoxin antigen of *Leishmania.* Samples are collected from ulcerative leishmaniasis lesions with a dental broach. The sample is added to a lysis buffer and then tested with a testing strip. The authors reported an in-site sensitivity of 65.4% and a specificity of 100%.

Loopamp uses extracted DNA samples and is based on the amplification of genetic material using primers targeting the 18S rRNA gene and kDNA minicircles that are specific to the *Leishmania* genus [8]. An in-site sensitivity of 87.6% and a specificity of 70.6% were reported. The authors also sent DNA samples for testing with the same Loopamp kit to the Academic Medical Centre in Amsterdam (AMC), and the sensitivity and specificity values were 92.2% and 94.1%, respectively.

The authors also showed that, in addition to an inter-site variability in accuracy between NMLCP and AMC, the sensitivity and specificity can also vary according to clinical characteristics such as disease duration and type of lesion. Variation in accuracy was also observed in an in-series use of both index techniques evaluating the Loopamp performance in CL Detect-negative patients. Paradoxical results in the specificity of the in-series evaluation were observed when comparing the NMLCP and AMC centres.

The article presents data with good external validation and shows that there is some unpredictability in the accuracy of these tests, which can even be caused by a change in the processing site. They also showed that the partnership of local authorities with reference laboratories in countries with endemic leishmaniasis can be a solution for difficult parasite identification. It is also clear that this partnership is useful as long as the proposed complementary test has a simple and cost-effective sampling technique.

Accuracy studies that comply with state-of-the-art methodological recommendations tend to achieve lower sensitivity and specificity

results when compared to studies performed in very controlled environments [9]. Studies that include healthy controls, lack a clear case definition, and do not use blinded evaluators for testing can report biased results that can reach 100% specificity and sensitivity.

It is possible to conclude that it is imperative to continue the search for sensitive techniques that can directly identify *Leishmania*. The necessity of applying modern techniques in regions with challenging socioeconomic conditions poses problems that need to be addressed. Investing in simple sampling techniques and forming partnerships between centres in endemic regions and reference laboratories are possible methods of improving the control of neglected infectious diseases.

Authors' contributions

Patrícia Shu Kurizky: Literature search, writing, data interpretation;
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Ciro Martins Gomes: Literature search, writing, data interpretation.

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