Treatment failure and drug resistance in natural *Leishmania* populations: A multi-disciplinary research consortium

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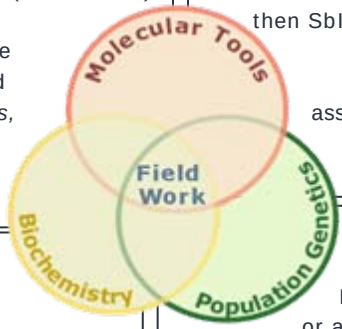
- Control of leishmaniasis is challenged by increasing antimonial (SbV) treatment failure. This can be due to the host, the drug and/or the parasite. Parasite resistance is a well-established fact and several mechanisms were reported in **artificially** induced resistant strains. It is unknown if they are valid in naturally resistant strains.
- We aimed to understand the phenomenon of treatment failure in a global and integrated way and particularly to tackle the genetic bases of parasite resistance in **natural** populations.
- We achieved our objectives through a unique multi-disciplinary study, combining:

Clinical work. 282 Patients with VL (Nepal), 746 with CL (Peru & Bolivia). Treatment failure: 11,3 % in Nepal, 23,9 % in Peru. 562 fully documented strains (110 from treatment failure); identification of clinical risk factors for treatment failure.

Characterisation of isolates.
SbV Susceptibility assays (99 isolates) and species typing
a) 47 resistant, 52 sensitive
b) SbV resistance identified in *L.donovani, L.braziliensis, L.guyanensis*, *L.lainsoni*
c) No cross-Resistance wih miltefosine

Putative SbV resistance markers.
Resistance as a stepwise process (SbV then SbIII)?; upregulation of genes encoding oxidative stress protective proteins; Q-PCR assay for promastigotes, good predictive value for treatment outcome



Immunology.
Cytokine measurement *in situ* (CL), LST
High level of IL-13 is linked to poor response to treatment. LST induration size smaller in treatment failure

Parasite Population Genetics.
Effect of transmission (zoo- or anthroponotic) on spreading resistance ? Result *L. donovani*: Polyclonal structure of the resistant parasite populations; independent events leading to emergence of drug resistance ?

General data processing. Low correlation between treatment outcome and *in vitro* drug susceptibility: need to upgrade *in vitro* assays!!!

Prospects:
- Validation of markers on our collection of strains and clinical samples
- Proteomics and complementation genomics
- Definition of reference strains, dissemination of standardised methods
- Recommendations for further use of antimonials

LeishNatDrug Consortium ©:
Funding: EC-INCO-Dev, DGOS, OFES

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Impact

Recommendations (written in february 2006) should be adapted with care, by endemic area.

In Nepal, it is crucial that suspected VL patients are referred early to the district hospitals and centers for confirmation of the diagnosis and early initiation of therapy. It is also important to ensure that the patients are monitored closely so that they complete the therapy. A mechanism to follow the patient in the nearest health facility at the end of therapy and at 6 months follow up should be made to record the clinical outcome of therapy. All kala-azar cases treated at non government health facilities including private facilities should be reported to the district public health office. In view of the increasing failure rates of SSG in kala-azar patients from the districts bordering the Bihar high resistance it would be preferable to treat patients with advanced disease from these areas with Amphotericin B in order to decrease fatalities. We recommend a further assessment of the efficacy of antimonials in districts bordering the focus of high antimonial resistance in Bihar. If a low efficacy is confirmed in these districts, a change of first line therapy should be considered (***Note:*** *since the writing of these recommendations, Miltefosine has been recommended to be the 1st line in the elimination campaign. However SSG continues to be used in Nepal and supplied by the MOH as they plan to phase it out gradually*). Based on our finding that drug resistance is likely to emerge several times through independent events, we also recommend the implementation of regional networks for monitoring of drug efficacy and emergence of drug resistance. These could use the markers and technologies we have developed after their further validation.

In Peru, considering the species-specific differences in treatment outcome, organisations involved in leishmaniasis control should consider treatment strategies adapted to the parasites circulating in the region. This might have a direct economic implication. New therapeutic schemes are necessary and particularly adapted to children; combination of treatment should be considered, involving immuno-modulatory drugs. Diagnostic procedures should be improved and standardised, whilst follow-up strategies should be implemented for the early detection of treatment failure. Several clinical features have been identified as significant predictors of treatment failure in CL patients. Further work to develop clinical scores predicting treatment failures should be conducted.