KALADRUG-R: Overview clinical sampling

April 8th 2010
ITMA

A. Symptomatic VL cases (see flow diagram 1)

Aim: to collect (i) parasite isolates for characterisation current natural *L. donovani* population regarding drug resistance and its relation to clinical treatment failure and (ii) clinical samples to apply developed laboratory tools for assessment frequency molecular markers parasite resistance and/or treatment failure in symptomatic VL cases.

Patient cohort: cohort of active VL cases admitted in WP3 and WP4

Sampling: all 3 following types of samples need to be taken

Sample type 1*: Bone marrow/spleen aspirates for parasite isolation/culture (ref. cSOP#A & G):*

1. on admission for **all** first-time kala-azar patients
2. on admission of **all** retrospective kala-azar cases
3. at end of treatment of all Kaladrug-R enrolled patients **only** when treatment failure is suspected
4. during follow-up of all Kaladrug-R enrolled patients **only** when relapse is suspected

Sample type 2*: Bone marrow/spleen aspirates for PCR (ref cSOP#B):*

1. on admission for **all** first-time kala-azar patients
2. on admission of **all** retrospective kala-azar cases
3. at end of treatment of **all** Kaladrug-R enrolled patients (tissue aspirates need to be taken anyway for evaluation treatment outcome)
4. during follow-up of all Kaladrug-R enrolled patients **only** when relapse is suspected

Sample type 3*: blood for PCR + two separate drops of plain blood on filter paper (ref cSOP#C):*

5. on admission for **all** first-time kala-azar patients
6. on admission of **all** retrospective kala-azar cases
7. at end of treatment of **all** Kaladrug-R enrolled patients
8. during follow-up of all Kaladrug-R enrolled patients **only** when relapse is suspected

B. Asymptomatic cases

Aim: to collect clinical samples to apply developed laboratory tools for assessment frequency molecular markers parasite resistance and/or treatment failure in asymptomatic VL cases representative for whole endemic region.

Cohort: 500 household contacts (per country) of (i) active KA cases, (ii) recently miltefosine treated KA cases (up to 1 yr ago), and (iii) recently treated KA cases treated with an alternative drug (up to 1 yr ago)

Location of sampling: Area with active transmission, to be carefully chosen/country.

Sampling: a protocol is being developed with the specifics for sampling of asymptomatics.

C. PKDL

aim: PKDL can be considered as a partial unresponsiveness (the skin acts as a sanctuary for *L. donovani* and, after reactivation, lesions appear and are generally restricted to the skin); PKDL is thus a form of treatment failure, and its relation with parasite drug resistance will be investigated, comparatively to VL non-responders/relapse cases. In order to find out what happened with the infecting parasite from acute VL stage to acute PKDL stage, samples will be taken from PKDL patients and compared to samples of VL patients. Ideally, paired samples should be obtained that is pre-treatment VL and post-treatment PKDL samples to comparatively characterise the parasite isolates or test molecular markers.

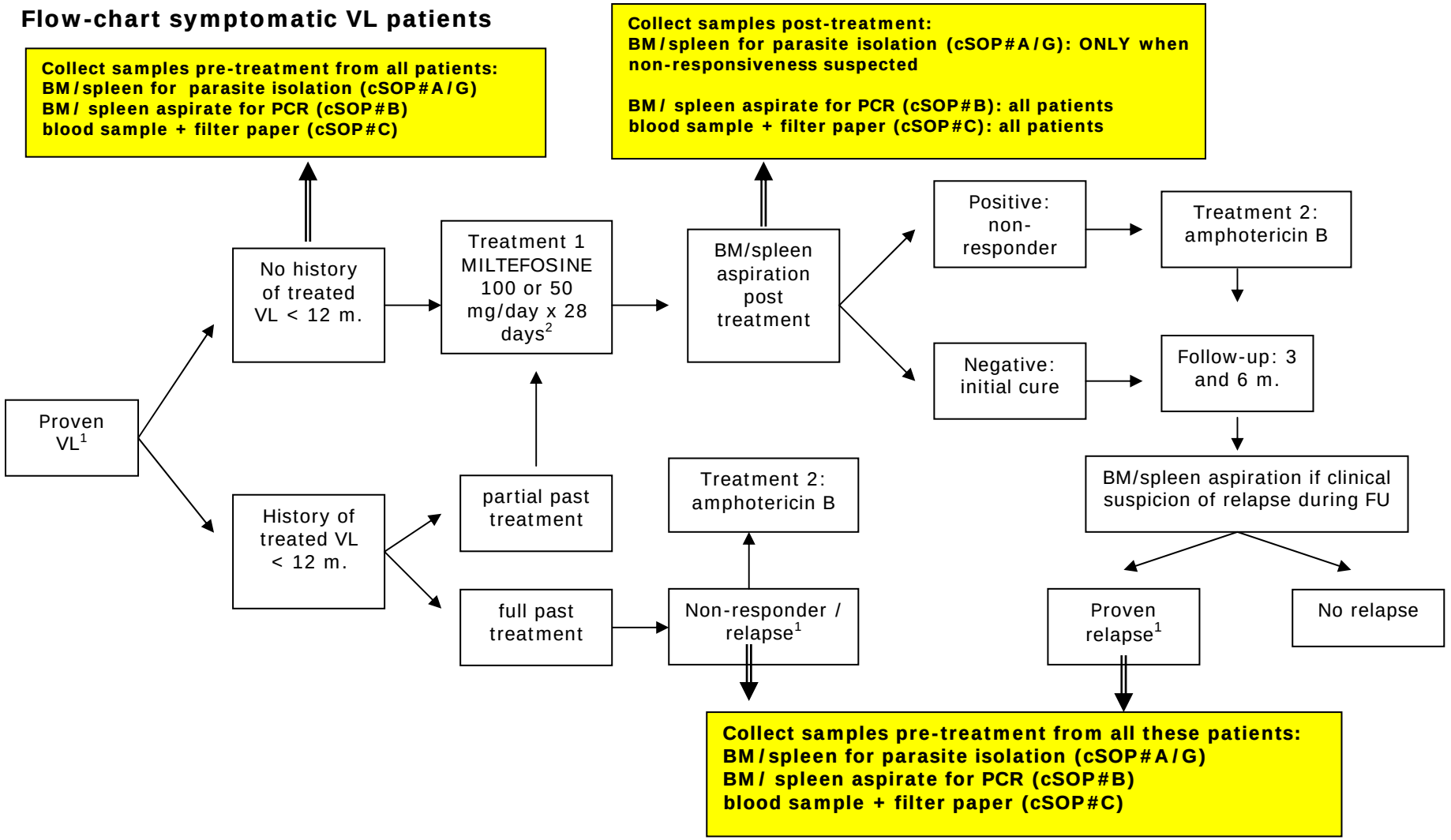
Sample type 1*: Skin punch biopsy for culture and PCR (cSOP#D):*

on admission for all PKDL patients with a past history of kala-azar fully treated with SAG, MIL or amphotericin B.

Sample type 2: *blood for PCR + two separate drops of plain blood on filter paper (ref cSOP#C):*

1. on admission for all PKDL patients with a past history of kala-azar fully treated with SAG, MIL or amphotericin B.
2. at end of treatment of **all** Kaladrug-R enrolled PKDL patients
3. during follow-up of all Kaladrug-R enrolled PKDL patients **only** when relapse is suspected

Flow-chart symptomatic VL patients



[1] proven by positive parasitology
[2] 100 or 50 mg daily for patients weighing ≥ 25 kg or < 25 kg respectively