Optimisation of PCR Sampling Techniques for Assessment of Parasitological Response in Patients with Chronic Chagas Disease

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INTRODUCTION

An increasing body of data has pointed to a strong biological rationale for the use of PCR as a surrogate marker of therapeutic response in CD. However, in chronic Chagas Disease (CCD), parasite burden is low and consequently, even most sensitive standardized PCR techniques have achieved around 70% of diagnostic sensitivity (Schijman et al, PLOS NTD, 2011; Ramirez et al, J.Mol.Diag 2015).

A DNDi/MSF-sponsored trial evaluated sampling procedures for PCR testing to assess parasitological response in CCD benznidazole (BZN)-treated patients in Bolivia (NCT01678599)

OBJECTIVES

Primary Objectives

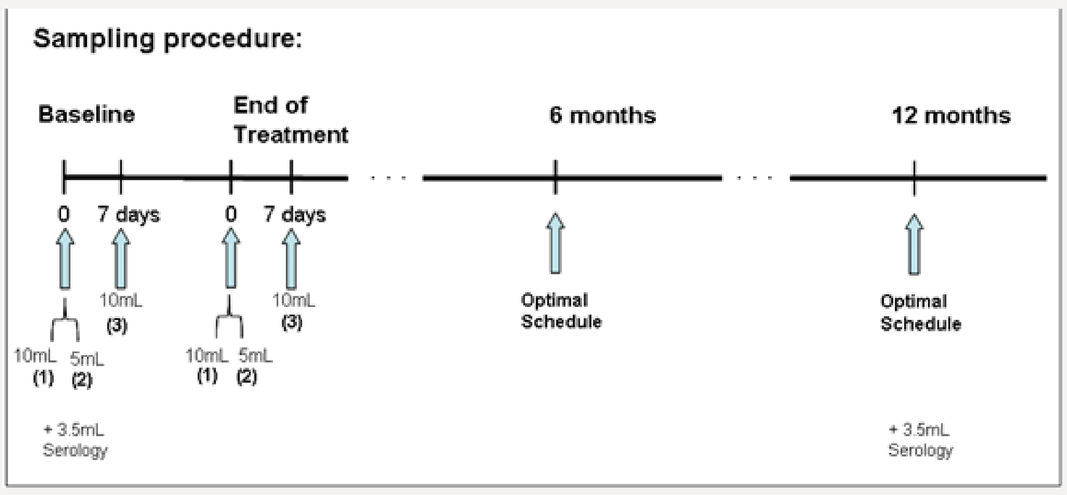
- To estimate gain in sensitivity of several multiple-sample strategies of PCR compared to the current standard (single sample of 10 ml) to detect chronic stage of CD at baseline.

Secondary Objectives

- To identify optimal sampling schedule at EOT (Day 60± 7 days) and primarily to verify whether the optimal strategy and the ranking of sensitivity at baseline are confirmed at EOT.
- To evaluate the parasitological response after treatment with BZ by determining the proportion of patients PCR (+) at baseline (prior to treatment) who convert to PCR negative at EOT (Day 60 ± 5 days) using different sampling schedules and if possible: the most feasible sampling schedule (the current one), the most sensitive one, and the optimal one (small loss of sensitivity for a large gain in feasibility and availability of data).
- To describe the changes in parasitological load after treatment, as measured by quantitative PCR at baseline and at the end of treatment, 6 months and 12 months.
- To estimate the relative reduction of parasitemia (parasite load) at the end of treatment, 6 and 12 months [(parasite count at baseline – parasite count at EOT or 6 or 12 m.) parasite count at baseline].
- To compare negative PCR at 12 months and negative serology at 12 months knowing that the proportion should be lower with the PCR method.

Study Design

Open label, longitudinal, prospective, non-randomized, one arm, multicenter, methodological study. The study consists in the follow-up of a cohort of patients treated with Benznidazole.



Sampling strategies

- The **Current strategy** (**CS**) consists in drawing one blood sample (**S1**: sample 1).
- The **Reinforcement Strategy** (**RS**) consists in adding blood sampling(s) to the current single-sample approach (volume of 10 ml). Unless additional samplings do not allow the detection of additional PCR-positive cases (which is unlikely) such strategy are inevitably more sensitive than the current one.

- The **Substitution Strategy** (**SS**) consists in replacing the current approach (sample 1 = 10 ml) by one 5 ml sample (sample 2) or by two samples (sample 2 and sample 3 = 10 ml).

RESULTS

- 16 communities involved in Narciso Campero district – Bolivia, between 1 to 27 patients were recruited per site.
- 220 patients enrolled (positive serology), and had at least 2 PCR results at baseline.
  - 194 patients had their 3 PCR results at baseline
  - 176 patients were treated for at least 30 days
  - 163 patients received full treatment (55-60 days)

Table 1: PCR Results at Baseline

Percentage of positive PCR is NOT dependent upon the sample

- **Sample 1 and 2 provided similar results (McNemar test: p = 0.2858).**
- **Sample 1 and 3 provided similar results (McNemar test: p = 0.8759)**

Baseline	Sample 1	Sample 2	Sample 3
Positive PCR	168	175	148
Negative PCR	52	45	47
Missing PCR	0	0	25
Sample size	220	220	195
% positive PCR	76.36%	79.55%	75.90%

Combination of results	**Sample 1+2 2 PCR done**	**Sample 1+2+3 3 PCR done**	**Sample 1+2+3 At least 2 PCR**
True Positives	**193**	**180**	**202**
False Negatives	**27**	**15**	**18**
Missing PCR	**0**	**25**	**0**
Sample size	**220**	**195**	**220**
% true positives	**87.73%**	**92.31%**	**91.82%**

Table 2: PCR Results at EOT

- Percent of positive PCR depends upon the sample (p = 0.0003 Sample 1 vs 2 McNemar test)

EOT	**Sample 1**	**Sample 2**	**Sample 3**
Positive PCR	8	29	12
Negative PCR	137	113	106
Missing PCR	75	78	102
Sample size	145	142	116
% positive	5.52%	20.42%	10.34%

- The pragmatic gain in sensitivity was 15% (n = 220) and significant (95%CI = 10.56% - 20.42%).

- A larger volume of blood (10 ml instead of 5 ml) did not increase sensitivity. The three samples could be taken one after the other some minutes apart, with no need for 7 days interval.
- Sustained parasitological response to benznidazole treatment was 64% at M12 (based on 3 PCR at EOT and 12 months, n = 111) .
- Depending on the definition of success rate there is significant variability with several intermediary estimations.

CONCLUSIONS

- Multiple, serial samples lead to a significant gain in PCR sensitivity.
- A proposed optimal strategy for PCR sampling in CD patients would involve a total of three 5 ml samples taken minutes apart, at each of the timepoints of planned evaluation.

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