TITLE

What to expect and when: Benznidazole toxicity in chronic Chagas Disease treatment

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RUNNING TITLE: Benznidazole toxicity in chronic Chagas Disease

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SYNOPSIS

Background: Benznidazole is one of the two most effective antiparasitic drugs for Chagas Disease treatment. However, the knowledge about its toxicity profile is mostly based on post-marketing observational studies.

Objectives: Our study combines data from two prospective clinical trials designed to control safety for the drug newly produced by ELEA Laboratories (Abarax®).

Methods: Eligible participants were selected by consecutive sampling strategy in CINEBENZ and BIOMARCHA study between 2013 and 2016 (EUDRACT 2011-002900-34, 2012-002645-38; and clinicaltrials.gov NCT01755403, NCT01755377, respectively). Enrolled subjects received treatment with benznidazole 5mg/kg/day orally in two divided doses for eight weeks and were followed-up fortnightly.

Results: We observed 305 adverse reactions among 85 out of 99 participants (85.9%). Each patient had a median of three adverse reactions, 89.5% were mild and the median duration was 12 days. Most adverse reactions appeared in the first month of treatment except arthritis and peripheral neuropathy. 26 patients did not complete treatment: two were withdrawn, one for ectopic pregnancy and one for epilepsy relapse due to cysticercosis; two were lost to follow-up and 22 due to adverse reactions, two of them severe. We observed some unexpected adverse reactions that have not been described previously such as psychiatric symptoms, erectile dysfunction, menstrual cycle alterations or lung infiltration.

Conclusions: There is a very high frequency of adverse reactions to benznidazole. Most adverse reactions are mild but the treatment burden is significant and unexpected reactions are not rare. Severe reactions are uncommon but they can be life threatening. Further studies are necessary to optimise treatment.

INTRODUCTION

Chagas Disease is a chronic anthropo-zoonotic disease caused by the protozoan *Trypanosoma cruzi* and it is most frequently transmitted through a vectorial route. However, it is also a major public health problem in endemic and non-endemic countries given its transmissibility from mother to child or through blood products and organ transplants. Outbreaks with oral transmission have been also described.[1-3] It is estimated that 6-7 million people could be infected worldwide and 20-30% of them could develop a potentially life-threating cardiac condition.[1]

The treatment in acute cases is very effective whereas in chronic disease the effectiveness has been highly debated.[4-6] There is a general understanding that parasitic persistence increases the risk of cardiac lesions in chronically infected patients and therefore parasite eradication may be necessary in the early stages of the disease.[7] On the other hand, the advanced cardiac stage is irreversible and treatment of this stage is futile. The BENEFIT trial supported this concept considering that it did not show significant morbidity or mortality reduction with antiparasitic treatment in advanced cardiac stage.[5,8-9] However, this study did not provide answers about the long-term effectiveness of the antiparasitic approach in early stages of the chronic disease. In the latest years the evidence from observational studies has supported treating most of the chronic patients with the available antiparasitic drugs.[10-11]

At the moment, the most effective drugs to treat Chagas disease are benznidazole and nifurtimox. Both drugs have shown poor tolerance, significant adverse reactions (ARs) and high treatment suspension rates. They were registered and

used in clinical practice since their launch in the 1970s with little understanding of their mechanism of action and toxicity. Early comprehensive preclinical toxicological investigation were conducted for nifurtimox whereas no equivalent information has been available for benznidazole.[12] Given that no pre-marketing safety studies were performed for, most of the available knowledge on human safety has been built up based on post-marketing retrospective observational studies. Very few studies have been designed to offer a systematic post-marketing surveillance, which could explain the variability in side effect reports between different groups in addition to race or genetics differences.

Expert's forums have discussed about the possibility of different safety profiles depending on the drug manufacturer. This question was raised mostly when Elea laboratories took responsibility of producing after a dramatic period of 2 years of lack of benznidazole stock worldwide between 2011 and 2013.[13] There were some concerns that the drug produced by the Brazilian state-owned laboratory LAFEPE and Elea Laboratories from Argentina may have a different toxicity profile, even if some retrospective studies did not observe any significant differences in treatment withdrawals.[14]

The aim of this study is to offer a systematic evaluation of safety in a prospective study about the drug manufactured by Elea Laboratories in chronically infected adults.

PATIENTS AND METHODS

Study design

From 2013 to 2016 we conducted two prospective, open-label and single centre clinical trials in the International Health Department at Hospital Clinic of Barcelona. CINEBENZ was designed to study the population pharmacokinetics of benznidazole and BIOMARCHA monitored cardiac biomarkers before and after treatment (EUDRACT 2011-002900-34 and 2012-002645-38, and clinicaltrials.gov NCT01755403 and NCT01755377 registration numbers respectively). The protocols were approved by the Ethics Committee of the Hospital Clinic of Barcelona and AEMPS (the Spanish Agency of Medicines and Medical Devices), they were conducted in accordance with the Declaration of Helsinki and national and institutional standards. Before inclusion in the study, all patients provided written informed consent. Both studies had the same treatment and follow-up protocol during treatment and the ARs of both studies were analysed together.

Eligible subjects met the inclusion criteria of being at least 18 years old, diagnosed by two different positive *Trypanosoma cruzi* serologic tests, not to have received treatment before and not to have advanced cardiac disease. Exclusion criteria were hypersensitivity to benznidazole, difficulties to follow-up, immunodeficiency, hepatic or renal impairment, cardiac disease of other origin, pregnancy or lactation. Enrolled subject received treatment with benznidazole 5 mg/kg/day orally with or without food in two divided doses for eight weeks, with a maximum of 400mg/day (Abarax®, Elea Laboratory, Argentina).

We selected all subjects attending health facilities meeting the inclusion and

exclusion criteria with consecutive sampling strategy. Patients were followed-up fortnightly with clinical review and blood test. The clinical review consisted of an open interview plus an exhaustive structured questioning, a physical examination and vital sign measurements. Patients had a contact telephone number available 24h and they could be seen ad-hoc in the clinic if needed. As per site protocol at the time, no food restriction was applied but commitment to avoid alcohol during treatment was required.

Data management and Statistical analysis

Data collection and management were performed using OpenClinica open source software, version 2.0.3.1. (Copyright© OpenClinica LLC and collaborators, Waltham, MA, USA, www.OpenClinica.com). Data were described using frequencies for discrete variables and median (IQR) for continuous variables. ARs incidence rates and confidence intervals based on Poisson distribution were estimated. Multilevel Poisson regression models were employed to assess fortnightly incidence rate ratio (IRR) of ARs.[15] To assess the existence of a time trend of the IRR of ARs, the same models were performed using day instead of fortnight. Following clinical criteria, all models were adjusted by sex and age. The significance level was set at 0.05. The analysis was carried out using Stata (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

RESULTS

Out of 100 eligible patients, 99 received treatment with BNZ and one patient declined to participate. Baseline demographic and clinical characteristics can be seen in Table 1. In the cohort, 97 patients (98.0%) were Bolivian, 73.7% were women and the median age was 36 (IQR 9). Regarding to Chagas Disease form, six had a mild heart disease and the rest did not have any evidence of organic disease. Out of 99 participants, 26 patients (26.3%) did not complete treatment: 22 due to ARs; two were lost to follow-up; two had withdrawals criteria with severe consequences, one for pregnancy and one for epilepsy relapse. (Table 2)

Eighty-five patients out of 99 (85.9%) had 305 ARs. Among patients with ARs each patient had a median of 3 ARs (IQR 1). Following WHO Toxicity Grade scale, the majority of ARs were mild (n=273, 89.5%), 30 were moderate (9.8%) and two were severe (0.7%). The median time to onset to the first AR was nine days (IQR 12) and the majority of the ARs (81.8%) appeared in the first month of treatment. (Figure 1) The median duration of all ARs was 12 days (IQR 9.5). 136 (44.6%) of the ARs required some intervention or medication whereas the rest resolved with no medical treatment. Among the 85 patients who had ARs, the majority had dermatological (74%) or neuropsychiatric symptoms (60%), followed by digestive (40%), general (32%), musculoskeletal (21%) and reproductive system disorders (5%), and only one patient had pulmonary symptoms. (Table 3)

The ARs leading to suspend treatment were: rash (n=7), arthritis (n=3), peripheral neuropathy (n=3), anxiety (n=3), fever (n=2), general malaise (n=1), angioedema and pulmonary symptoms (n=1), drug rash with eosinophilia and systemic

symptoms (DRESS) syndrome (n=1) and treatment intolerance in context of consumption of high dose of alcohol (n=1). Among them, four patients decided to stop treatment before medical advice and three other patients suspended treatment because they were unable to return to the clinic for follow-up. By site protocol, we suspended treatment to patients reporting fever, peripheral neuropathy (including ageusia) and arthritis. The rest of patients who did not complete treatment had unexpected or severe ARs. (Table 2)

Among patients who did not complete treatment the median duration of treatment was 25.5 days (IQR 27) (Figure 2) and the median cumulative dose of benznidazole at the end of treatment 7450.0mg (IQR 9400.0). Among patients who completed treatment the median duration of treatment was 57 days (IQR 6) and the median cumulative dose at the end of treatment 18350.0mg (IQR 5950.0).

Ten unexpected ARs were recognised during treatment. Four cases of psychiatric symptoms were observed, one severe, two moderate and one mild. The cases involved sleeping disorders, anxiety and panic attacks and in the most severe cases tremor and persecutory delusions. One case had history of anxiety and one patient had personal difficulties during treatment. No other explanation was found in two other cases. There were two cases of amenorrhea during treatment. One woman missed only one cycle whereas the other missed three cycles. Pregnancy test were negative, there was no history of irregular menstrual periods and no other reason was found. Two men reported erectile dysfunction during treatment after 8 and 36 days of treatment. The dose until onset was 2200mg and 11850mg and symptoms lasted between 100 and 148 days. Only one patient recognised personal issues at

the time and no vascular or hormonal disorders were found. Finally, one patient presented with bronchospasm, basal lung infiltrations in the chest X-ray and basal tree in bud nodules in the CT scan with no other explanation after the study. (Table 2)

We had blood test data of 98 out of 99 patients during treatment and 61 patients (62.2%) had blood test alterations. The majority of blood test disorders were mild and none of them leaded to treatment suspension by itself. Two cases with moderate increase of transaminases could not be contacted to stop treatment and by the next appointment liver tests were back to normal. All the severe blood results were related to the DRESS syndrome and treatment was suspended for clinical reasons before blood test were acknowledged. (Table 4) The median time to onset to present with alterations in liver function tests was 28.5 days (IQR 35) whereas white blood cell disturbances appeared earlier with a median time to onset of 14 days (IQR 14).

The overall AR incidence rate was 9.2 ARs (95%CI 8.2-10.3) per ten person-fortnights. The incidence rate for mild ARs per ten person-fortnights was 8.2 (95%CI 7.3-9.3), 0.9 for moderate ARs (95%CI 0.6-1.3) and 0.1 for severe ARs (95%CI 0-0.2). Dermatological and neuropsychiatric reactions had the highest incidence rate among different AR types. (Table 5)

A Multilevel Poisson Regression analysis adjusted by age and sex showed strong evidence that the overall incidence rate was much higher in the first fortnight with a decreasing trend in the following fortnights. This pattern was similar in all

different categories of ARs except for musculoskeletal ARs where there was no evidence of different incidence rates among the four fortnights (p=0.3005). (Table 5)

There are two types of ARs that tend to appear later during treatment: arthritis and peripheral neuropathy. We observed 11 cases of possible peripheral neuropathy. Four of them were subtle and non-typical: bitter taste (n=1), temporary burning feeling in hands and feet (n=1) and intermittent dysesthesia in hands (n=2). The time to onset in these cases varied between 5-32 days and no treatment suspension was medically advised. The other seven cases had established peripheral neuropathy, two with ageusia and five with peripheral numbness or neuropathic pain. The median time to onset in these cases was 46 days (IQR 13) and the median dose to onset 15350mg (IQR 4550). All cases on treatment were advised to discontinue treatment. The median symptoms duration was 31 days (IQR 48). One patient had an electromyoneurography where small fibre polyneuropathy was observed and symptoms lasted 321 days. Arthritis was observed in six cases: two monoarthritis, two polyarthritis, one migratory and one additive case. Large joints such as knees or shoulders were affected in all cases. The patients with polyarthritis and migratory arthritis also presented with wrist, hip and ankle involvement and the migratory arthritis case was the only one where metacarpophalangeal joints were affected. All cases of arthritis appeared after at least 31 days of treatment (median 33, IQR 23), with the median of cumulative dose of 9300mg (IQR 5750) and all cases were women.

Finally, there was some evidence that women may have higher incidence of ARs compared to men when adjusted by age (p=0.0164). Overall, taking into account all ARs, women had 53% higher incidence of ARs than men (95%CI 1.08-2.17).

DISCUSSION

The results of this study are in agreement with most previous observational studies showing poor tolerance to benznidazole.[14,16-20] With the most widely used dosage of 5mg/kg/day for 60 days there is a high frequency of ARs with an important disease burden for patients. Only 14 % of patients were free of ARs whereas the majority had several consecutive or simultaneous ARs and suffered for each of them the median of 12 days. Most ARs were mild but significant ARs appeared up to 10% of the cases. Skin reactions, neuropsychiatric ARs (including headache) and digestive symptoms were consecutively the most frequent ARs involving 82% of all ARs.

The most severe ARs presented in this study have been already described in literature. Eight cases of DRESS syndrome, two of them overlapped with Stevens-Johnson syndrome and toxic epidermal necrolysis; another case of Stevens-Johnson syndrome and two cases of acute generalised exanthematous pustulosis have been reported.[14,18-19,21-22] Thus, life-threatening skin ARs, even uncommon, are not rare and underlines the need of close benznidazole treatment monitoring. In our cohort angioedema appeared in 3% of exposed with the only severe case being the patient with DRESS syndrome. Molina et al observed angioedema in 1.1% of their patients whereas Miller *et al.* described it up to 20% of their cohort.[14,16] Patients in this latter study were mostly from Northern and Central America and different ethnicity or *Trypanosoma* genotype may explain the difference in ARs prevalence.[19]

279 Eighty per cent out of the patients with ARs had their first AR in the first 30 days of
280 treatment, which supports the theory that most ARs are secondary to
281 hypersensitivity reactions.[19] However, it is consistent in all studies that some
282 cumulative dose or treatment duration is necessary for arthritis and peripheral
283 neuropathy to appear. In the previous arthritis series published by our group
284 arthritis symptoms appeared at least after 40 days of treatment and a cumulative
285 dose of 7500mg.[23]. In the current study all cases of arthritis appeared after at least
286 31 days of treatment and a cumulative dose of 7250mg, and typical peripheral
287 neuropathy after at least 22 days of treatment and a cumulative dose of 5850mg.
288 These results support the theory that arthritis and peripheral neuropathy may be
289 secondary to toxicity produced by BNZ and therefore 30 days of treatment would
290 prevent most of the cases.[24] However, data from this study do not support the
291 threshold of 18g described in some other studies as a threshold for toxicity driven
292 ARs.[25] Our results do not agree either with previous study results were bone
293 marrow depression appeared late in treatment as white blood cell disturbances
294 were observed with a median time to onset of 14 days.[24]
295
296 We have observed some unexpected ARs that have not been described in previous
297 studies. Even if sleeping disorders, either insomnia or sleepiness, have been
298 already reported in some cohorts, it is the first time in our knowledge that
299 psychiatric symptoms such as anxiety, panic attacks, emotional lability or
300 persecutory delusions have been described. [14,16,18] These results may suggest some
301 central nervous system effect by BNZ. Some patients also presented with erectile
302 dysfunction or delay in menstrual cycle with no other good explanation for these
303 symptoms. One patient had bronchospasm and bibasal lung infiltration that

disappeared after treatment suspension and could not be explained with her medical history. All ARs had no permanent consequences except one case of erythema dyschromicum perstans in both legs diagnosed by histopathology six months after having a moderate skin rash reaction. Finally, we suspended treatment in a patient with an ectopic pregnancy during treatment. She had other risk factors such as a long history of infertility and exposition to two days of Mequitazine. Mequitazine has not demonstrated any teratogen effect in animal studies but information in humans is insufficient to ensure safety in pregnancy. On the other hand, some studies in pregnant rats observed that BNZ could cross the placental barrier and reach the foetuses.[12] The association between the ARs and BNZ could not be ruled out in all those cases. Future pharmacovigilance data will be needed to conclude if there is any real association.

Treatment suspension rate was 26% in our study with free and flexible health care access. However, six patients presented difficulties to have a safe social environment to endure with the treatment: three patients had difficulties to attend to clinical review due to work-related limitations and three other patients could not cope and felt ashamed because of stigmatising skin reactions in an already vulnerable population. These results manifest how social support has an impact in treatment's effectiveness and migrants are exposed to health care access limitations attributable to the lack of social support.[26-27]

Our study may have a generalisability limitation given almost all the patients were migrants from Bolivia. Race and genetics seem to be significant in drug ARs and even some HLA alleles have been proposed to be associated to BNZ related

329 ARs.[19,28] Moreover, their migrant status may determine their social support and
330 health access during treatment compared to different locations.
331
332 It is estimated that over one million people are affected with cardiac Chagas
333 disease worldwide and based on the latest studies mortality in 5 years despite
334 treatment could reach 17-18%.[8,29] These results underline the urgent need of long-
335 term studies with strong clinical outcomes to understand treatment's efficacy as a
336 secondary prevention or therapeutic treatment. It is also critical to optimise
337 treatment with BNZ considering it will still be part of the treatment in the near
338 foreseeable future. Combined drug therapy or different dosages need to be
339 evaluated to achieve the maximum efficacy with the minimum of toxicity.[30] At the
340 moment we have reached a point where there is no effective treatment for the
341 advanced Chagas disease and the antiparasitic drug toxicity is intolerably high as a
342 potential secondary preventive treatment in chronically infected patients.
343

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TRANSPARENCY DECLARATION

Authors declare no conflict of interest.

Author contribution: Study design: EA, DS, JG. Patient selection, data collection: EA, EP. Clinical assessment and patient care: EA, NS, ARM, ACC, JG. Data management: EA, EP, ACC. Data analysis: EA, AC, SS. Data interpretation: EA, JG. Writing of the document: EA, EP, ARM, AC, SS, MJP, JG.

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TABLES

Table 1: Baseline demographic and clinical characteristics of patients treated with benznidazole (N=99)

Variable	N (%)
Age[a]	36 (9.0)
Sex	
Female	73 (73.7)
Height (cm)[a]	157.0 (12)
Weight (kg)[a]	66.0 (20.0)
Country of origin	
Bolivia	97 (98.0)
Paraguay	1 (1.0)
El Salvador	1 (1.0)
Non smokers	91 (91.9)
Alcohol intake (Grams per day)	
0	65 (65.7)
0.1-4.9	18 (18.2)
5-14.9	7 (7.1)
>15	4 (4.0)
Other medication[b]	27 (27.3)
Chagas disease form	
Indeterminate	93 (93.9)
Cardiac (mild)	6 (6.1)

[a]Median and IQR

[b]Other medication: 12 hormonal contraception, five proton pump inhibitors, three non-steroidal anti-inflammatory drugs, two Paracetamol, two antihypertensive drugs, two statins and one oral antidiabetic medication

Table 2: Clinical characteristics and outcome of patients who did not complete treatment due to unexpected ARs, poorly controlled ARs or severe ARs

Medical history	Clinical description	Main reason for suspension	Severity of AR	Date onset	Day of suspension	Outcome
Female, 35, Bolivia. H. Pylori treated in the past, eosinophilia with negative parasite studies, long history of infertility	Positive pregnancy test	Withdrawal criteria	Severe	52	52	Treated with BNZ for 52 days and Mequitazine for 2 days due to mild rash until positive pregnancy test. Diagnosed with ectopic tubal pregnancy. Salpingectomy and medical treatment with Metotrexate needed. Complete recovery 60 days after treatment suspension
Female, 35, Bolivia, neurocysticercosis treated 2 years prior research study (no symptoms with no treatment for 2 years)	Right side hemiparesis, initially temporary episodes and finally permanent	Withdrawal criteria	Severe	11	15	Treatment suspension when symptoms were reported. Hospitalisation for 5 days, treated with high doses of Dexametasone and Levetiracetam. Craneal MRI showed vascular oedema around one out of the three known lesions. Full recovery with no sequela in 18 days.
Female, 35, Bolivia, no PMH	Fever, angioedema, generalised pruritic maculo-papular rash, elevated liver enzimes, neutropenia, eosinophilia, thrombocytopenia	DRESS syndrome	Severe	21	22	High doses of steroids and hospitalisation needed for 5 days. Full recovery including blood test normalisation in 55 days
Female, 33, Bolivia, past history of anxiety	Maculo-papular rash, pyrosis, sleepiness and panick attacks	Anxiety	Severe	12	12	Treated with Diazepam, Clorazepate and Alprazolam. Enough recovery to contiune everyday activities in 281 days

Female, 34, Bolivia, no PMH, no known previous history of anxiety	Moderate insomnia after 9 days of treatment and panic attack at day 30 which led to suspend treatment	Anxiety	Moderate	9	30	Treated with Zolpidem and Diazepam. Anxiety under control since treatment suspension, insomnia improved 2-3 weeks after treatment suspension
Male, 44, El Salvador, no PMH	Anxiety, insomnia, distal tremor and later on persecutory delusion	Anxiety	Moderate	1	18	Treated with Lorazepam long-term. Assessed by Psychiatry it was diagnosed with anxiety and possible depression. Unemployment and relationship problems recognised afterwards.
Female, 48, Bolivia, rosacea on treatment with Doxycycline, two previous episodes of pneumonia (17 and 4 years ago)	Low grade fever, angioedema (periorbital and lips), hand and feet oedema, urticaria in trunk and limbs, dry coughing, mild bronchiespasm and bibasal lung infiltration (previously not known), joint pain	Skin and pulmonary reaction	Moderate	19	19	Treated with Prednisone 40mg/day, Mequitazine and Salbutamol. Full symptoms recovery in 12 days. Reviewed by respiratory and autoimmune specialist. Complete resolution of the lung disease with no other explanation. Posterior studies found a possible scleromyositis with no link with lung symptoms
Female, 37, Bolivia, no PMH	Angioedema, generalised pruritic maculo-papular rash	Skin	Moderate	11	11	Treated with IV Metilprednisolona 40mg and IV Dexchlopherinamine 5mg followed by oral Prednisone 30mg and Mequitazine. Full recovery in 5 days

PMH: previous medical history

Table 3: Distribution and description of ARs among patients who had ARs during treatment with (N=85)

AR type	AR per person, N(%)[a]			Frequency, N(%)[b]	Severity, N(%)[a]		Medication needed, N(%)[c]	Time to onset (days), median (IQR)	Dose to onset (mg), median (IQR)	ARs duration (days), median (IQR)
	1	2	>=3		Mild	Moderate-Severe				
General	22(81)	5(19)	0(0)	32(10)	29(91)	3(9)	4(13)	12(22)	3575(5250)	15(23)
Asthenia	16(100)	0(0)	0(0)	16(50)	15(94)	1(6)	0(0)	8(28)	1675(6575)	15(30)
Adenopathy	5(100)	0(0)	0(0)	5(16)	4(80)	1(20)	3(60)	16(11)	4050(3700)	24(27)
Hyporexia	4(100)	0(0)	0(0)	4(13)	4(100)	0(0)	0(0)	36(34)	10700(14225)	34(22)
Fever (high & low grade)	4(100)	0(0)	0(0)	4(13)	3(75)	1(25)	1(25)	16(6)	3575(1375)	4(5)
Others[d]	3(100)	0(0)	0(0)	3 (9)	1(100)	0(0)	0(0)	NA	NA	NA
Digestive	25(74)	7(21)	2(6)	45(15)	45(100)	0(0)	22(49)	15(19)	3750(4850)	12(12)
Sickness & vomiting	18(100)	0(0)	0(0)	18(40)	18(100)	0(0)	4(22)	15(27)	3750(9100)	9(10)
Gastric pain	13(100)	0(0)	0(0)	13(29)	18(100)	0(0)	8(62)	21(16)	4750(4650)	9(11)
GERD & pyrosis	6(86)	1(14)	0(0)	8(18)	8(100)	0(0)	6(75)	12(19)	3475(5475)	16(6)
Dyspepsia	6(100)	0(0)	0(0)	6(13)	6(100)	0(0)	4(67)	16(45)	3775(4800)	26(52)
Skin disorders	23(37)	30(48)	10(16)	118(39)	101(86)	17(14)	102(86)	14(17)	3800(6100)	10(11)
Rash	51(94)	3(6)	0(0)	57(48)	49(86)	8(14)	50(88)	12(15)	3450(5550)	9(10)
Pruritus	46(96)	2(4)	0(0)	50(42)	45(90)	5(10)	44(50)	13(16)	3800(5400)	11(12)
Others[e]	11(100)	0(0)	0(0)	11(9)	7(63)	4(36)	8(73)	NA	NA	NA
Musculoskeletal	18(100)	0(0)	0(0)	18(6)	15(83)	3(17)	8(44)	27(35)	6550(11900)	13(14)
Joint & muscle pain	12(100)	0(0)	0(0)	12(67)	12(100)	0(0)	2(17)	11(26)	3150(9425)	11(15)
Arthritis	6(100)	0(0)	0(0)	6(33)	3(50)	3(50)	6(100)	33(23)	9300(5750)	19(13)
Neuropsychiatric	29(57)	14(27)	8(16)	86(28)	77(90)	9(10)	31(36)	13(24)	3300(7250)	14(26)
Headache	33(83)	5(13)	2(5)	50(58)	47(94)	3(6)	23(46)	11(20)	3075(6550)	1(12)

Sleeping disorders	18(100)	0(0)	0(0)	18(21)	16(89)	2(11)	3(17)	9(28)	2425(9200)	20(32)
Peripheral neurology	11(100)	0(0)	0(0)	11(13)	11(100)	0(0)	1(9)	34(28)	12000(10750)	31(44)
Anxiety	4(100)	0(0)	0(0)	4(5)	1(25)	3(75)	3(75)	14(27)	3450(9350)	157(270)
Others[f]	3(100)	0(0)	0(0)	3()	2(66)	1(33)	1(33)	NA	NA	NA
Reproductive	4(100)	0(0)	0(0)	4(1)	4(100)	0(0)	0(0)	15(24)	5000(8650)	96(63)
Respiratory	0(0)	1(100)	0(0)	2(1)	2(100)	0(0)	2(100)	19(0)	4650(0)	12(0)

[a] Row percentages. [b]Column percentages . [c]Percentage= total frequency/medication needed

NA: non-applicable if different categories are collapsed

[d]Other general ARs: one general malaise (mild), one dry eyes (mild), one dizziness (mild)

[e]Other skin disorders: three cases of angioedema (one mild, two moderates), one DRESS (severe), three skin peeling (mild), two skin plaques or thickening (mild), one chronic hyperpigmentation (mild), one distal swelling in ankles (moderate)

[f]Other neuropsychiatric ARs: two cases of emotional lability (one mild, one moderate), one case of persecutory delusion (mild)

Table 4: Distribution of the 119 significant blood test disorders during treatment with BENZNIDAZOLE (N=98)

Blood test disorder	N (%)	Severity		
		Mild	Moderate	Severe
Liver function test	16 (16)	11 (69)	3 (19)	2 (12)
Haemoglobin	4(4)	4 (100)	0 (0)	0 (0)
WBC	48 (49)	28 (58)	18 (38)	2 (4)
Lymphopenia	37 (38)	19 (51)	16 (43)	2 (5)
Neutropenia	14 (14)	10 (71)	3 (21)	1 (7)

Liver function test (GOT, GPT): mild 60-100 U/l, moderate 101-200 U/l, severe >201 U/l. Haemoglobin: mild 95-105 g/l, moderate 80-94g/l, severe <79 g/l. White Blood Cells (WBC): mild 3-3.9x10^3/µL, moderate 2-2.9x10^3/µL, severe <1.9x10^3/µL. Lymphocytes and neutrophils: mild 1.5-1.9 x10^3/µL, moderate 1-1.4 x10^3/µL, severe <0.9 x10^3/µL.

Table 5: AR incidence by type and incidence rate ratio (IRR) in fortnightly periods

Type of AR	Overall and time period (days)[a]	Incidence rate (95%CI)[b]	IRR (95%CI)[c]	p-value
TOTAL	Overall	9.2(8.2-10.3)		
	1-15	16.8(14.4-19.6)	4.67 (3.07-7.09)	<0.0001
	16-30	8.0(6.2-10.1)	2.19 (1.39-3.44)	
	31-45	4.1(2.8-5.7)	1.16 (0.69-1.94)	
	46-60	4.3(2.8-6.3)	1	
General	Overall	1.0(0.7-1.4)		
	1-15	2.0(1.2-3.0)	7.35 (1.71-31.61)	0.0020
	16-30	0.9(0.4-1.8)	3.35 (0.71-15.78)	
	31-45	0.2(0.0-0.9)	0.92 (0.13-6.51)	
	46-60	0.3(0.0-1.2)	1	
Digestive	Overall	1.4(1.0-1.8)		
	1-15	2.5(1.6-3.7)	4.75 (1.65-13.71)	0.0005
	16-30	1.3(0.6-2.2)	2.34 (0.74-7.35)	
	31-45	0.2(0.0-0.9)	0.46 (0.08-2.51)	
	46-60	0.7(0.2-1.7)	1	
Skin	Overall	3.6(2.9-4.3)		
	1-15	6.5(5.0-8.3)	4.81 (2.46-9.39)	<0.0001
	16-30	3.3(2.2-4.8)	2.42 (1.18-4.97)	
	31-45	1.5(0.8-2.6)	1.10 (0.48-2.55)	
	46-60	1.6(0.8-3.0)	1	
Musculoskeletal	Overall	0.5(0.3-0.9)		
	1-15	0.7(0.3-1.5)	2.69 (0.56-12.97)	0.3005
	16-30	0.2(0.0-0.8)	0.84 (0.12-5.97)	
	31-45	0.7(0.3-1.6)	2.76 (0.56-13.68)	
	46-60	0.3(0.0-1.2)	1	
Neuropsychiatric	Overall	2.6(2.1-3.2)		
	1-15	5.0(3.7-6.7)	4.50 (2.12-9.56)	<0.0001
	16-30	1.9(1.1-3.1)	1.69 (0.73-3.94)	
	31-45	1.2(0.6-2.3)	1.14 (0.45-2.88)	
	46-60	1.3(0.6-2.6)	1	

[a] Number of participants on treatment on each period: 1-15=99, 16-30=92, 31-45=84, 46-60=77. [b]Incidence rate: ten person-fortnights. Exact Poisson 95% CI. [c]Multilevel Poisson Regression adjusted by age and sex, 352 observations.

FIGURES

Figure 1: Kaplan-Meyer survival estimates of the time until first adverse reaction among patients treated with benznidazole (N=99)

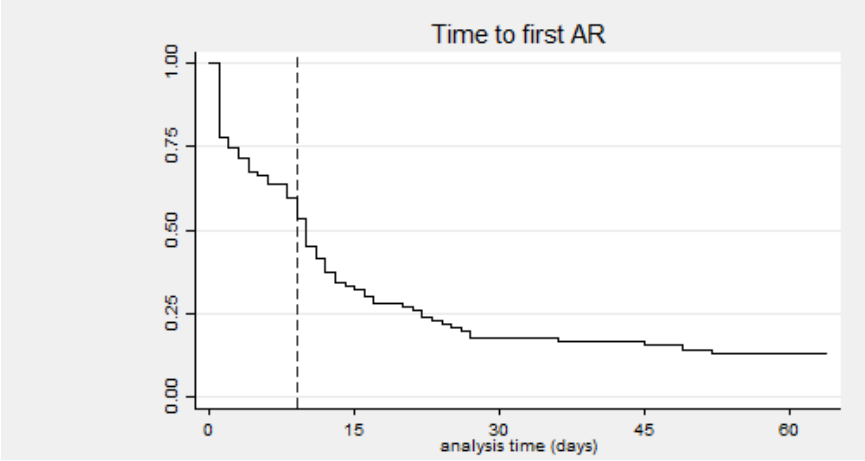

Figure 2: Kaplan-Meier survival graph of the suspension of treatment among patients who did not complete treatment (N=26) compared to those who did (N=73)