Adverse events associated with nifurtimox treatment for Chagas disease in children and adults

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ABSTRACT

BACKGROUND: Nifurtimox (NF) is one of the only two drugs currently available for Chagas disease (ChD) treatment. However, there is scarce data on NF safety, and many physicians defer or refuse NF treatment because of concerns about drug tolerance.

METHODS: Retrospective study of adverse drug reactions (ADRs) associated with NF treatment of ChD. Children received NF doses of 10-15 mg/kg/day for 60-90 days, and adults 8-10 mg/kg/day for 30 days.

RESULTS: 215 children (median age: 2.6yrs, range 0-17) and 105 adults (median age: 34yrs, range 18-57) were enrolled. Overall, 127/320 (39.7%) patients developed ADRs, with an incidence of 64/105 in adults, and 63/215 in children (OR = 3.7, 95%CI [2.2;6.3]). We observed 215 ADRs, 131 in adults (median: 2 events/patient (IQR_{25-75}= 1-3) and 84 in children (median: 1 event/patient (IQR_{25-75}= 1-1.5) ($P_{Adjusted}$ < 0.001). ADRs were mainly mild and moderate. Severe ADRs were infrequent (1.2% in children and 0.9% in adults). Nutritional, central nervous and digestive systems were the most frequently affected, without differences between both groups.

Treatment was discontinued in 31/320 (9.7%) patients without differences between groups. However, ADR-related discontinuations occurred more frequently in adults than in children (OR = 5.5, 95%CI = [1.5;24]).

CONCLUSIONS: Our study supports the safety of NF for ChD treatment. Delaying NF treatment due to safety concerns does not seem to be supported by the evidence.

Background

Chagas disease (ChD) is a silent but devastating disease caused by infection with the parasite *Trypanosoma cruzi*. The disease is endemic to the Americas, from the USA to Argentina, with over 7 million people currently infected in Latin America. ChD has expanded to many countries of the world via immigration, most cases reported in Europe, North America, Australia and Japan(1).

Most patients are asymptomatic during acute ChD. The acute phase is followed by a chronic asymptomatic stage that will eventually lead to irreversible heart disease in up to 30% of the infected patients many years later(2). Over 7,000 deaths occur yearly due to complications of Chagas.

The current treatment for ChD is limited to two nitro-heterocyclic drugs, nifurtimox (NF) and benznidazole (BZ), both with similar effectiveness. Despite both drugs having been available since the early 70s, treatment recommendations vary significantly from country to country and the evidence-base for the current treatment regimens is limited. This failure to treat may possibly be explained by many obstacles, including health care providers' low awareness of the diseases and its treatment options, overblown concerns about side effects, low access to healthcare for many patients, lack of an optimal straightforward test of treatment response, widespread drug shortages and irregular supplies, and regulatory barriers .

The most commonly observed NF´ADRs are anorexia and weight loss, irritability, sleepiness, and other nervous system signs and symptoms (4, 5). NF is also associated with rash, pruritus, and drug-associated hepatitis but less frequently than BZN. Depression, peripheral neuropathy, and psychiatric symptoms have also been reported. Similar to BZN, NF-associated ADRs seem much more common and severe in adults (6)and are usually mild in children, including neonates (7, 8) . However, current tolerability data comes mainly from small cohort studies. The pharmacological basis for the differences in the incidence of adverse events remain to be studied (9).

Here, we present results from a large cohort of ChD patients including infants, children and adults treated with NF, describing and comparing safety among adults and pediatric patients.

Results

Population characteristics

Medical records of ChD patients treated at our Institution were reviewed, and 372 patients who were prescribed NF were identified. However, 52 patients were excluded because they did not start treatment (i.e. did not fill in NF prescription). The remaining 320 patients were included in the study. A total of 215 pediatric patients [0-17yrs) and 105 adults were included. Among children were: n=56 (0 - 7mos) n=43 (8mos - 1yr), n=44 (2 - 6yrs), n=37 (7 - 11yrs) and n=35 (12-17yrs). A low rate of loss to follow-up was observed, as only 16/320 (5%) patients abandoned the study (see flowchart diagram in Figure 1).

In general, male and female subjects were well balanced in children but not in adults where 87.6% of subjects were female (most of them were mothers of children assisted in our service). The route of infection was: congenital in 131, undetermined in 154, vectorial in 32 and by blood transfusion in 3 cases (Table 1).

Overall, most patients were asymptomatic 289/320 (90.3%) and only 31/320 (9.7%) were symptomatic. Considering the route of infection, patients infected by the vector route were predominantly symptomatic 12/32 (37.5%) and the most frequent symptom was the ocular chagoma in 11/12 cases. In patients infected by the remaining routes (congenital, undetermined and blood transfusion), symptomatic cases were infrequent 19/288 (6.6%). Symptomatic cases were observed mainly in infants under 2 years 16/19 (84.2%), and the most affected organ was the liver (12/19 cases).

A clinical improvement was observed in all but one symptomatic patient during treatment. Only one 3-month-old infant, coinfected with T.cruzi and HIV by the transplacental route, did not show clinical improvement. , The patient developed encephalitis and myocarditis related to HIV infection, and died during NF treatment due to respiratory complications.

ADRs incidence and relationship

Overall, 127/320 patients (39.7%) developed ADRs, with an incidence in adults of 64/105 (60.9%), and of 63/215 (29.3%) in children (OR = 3.7, CI95% = [2.2 - 6.3]; $P_{Adjusted}$ <0.001).

A total of 215 ADRs in 127 patients were observed. In 64 adults, 131 ADRs were observed with a median of 2 events per patient (IQR_{25-75} = 1-3) and in 63 children,

84 ADRs were observed with a median of 1 event per patient (IQR_{25-75} = 1- 1.5); ($P_{Adjusted}$ <0.001).

NF-related ADRs were more frequent in adults (79.7%) than in children (7.9%) (OR=9.9, CI95% = [3.7 - 33]; $P_{Adjusted}$ <0.001; see Table 2 for further details). No significant differences were observed in the amount of ADRs comparing patients during the acute (vectorial and infants younger than 8 months of age) and the chronic phase of infection (P_Adjusted = 0.4).

The number of ADRs was associated with incomplete treatment: 2.58 events/patient in subjects that discontinued treatment vs 1.55 events/patient in those with completed treatment ($P_{Adjusted}$ <0.001). Moreover, differences remained when considering both cohorts separately (adults: $P_{Adjusted}$ = 0.028; children: $P_{Adjusted}$ = 0.016).

The profile of the 215 ADRs is shown in Table 3. The systems most commonly affected were nutritional 75/215 (34.9%), Central Nervous System (CNS) 61/215 (28,4%) and digestive 38/215 (17.7%) without differences between adults and children. Few adverse skin effects (20/215, 9.3%) were observed in both groups and hematological ADRs (7/215 events) were observed only in children (OR = Inf, CI95% = [2.3 - Inf]; $P_{Adjusted}$=0.005).

Time of onset of ADRs was recorded for 130/215 ADRs in 64/127 patients (50.4%). Overall, 93.8% of ADRs appeared within 30 days of treatment. ADRs median onset time (IQR_{25-75}) was: 5 (2.5 - 10.2) days for digestive, 5.5 (1.5 - 11) days for CNS, 8 (0.75 - 26) days for nutritional, and 13 (10 - 20) days for skin.

ADRs had an earlier onset in adults, who presented a median onset time of 6.5 days (IQR_{25-75} = 1.5 - 9), compared to children, who presented a median onset time of 12 days (IQR_{25-75} = 9.7 - 21; $P_{Adjusted}$ <0.001).

Severity

ADRs severity is described in Table 2. Most ADRs were mild (74.9%) and moderate (16.3%) and resolved without sequelae. Severe ADRs were infrequent: 2/215 (0.9%). Severe ADRs occurred in 1 adult and in 1 child (see Table 2). The adult was a woman in her 30´s, who developed a headache with a defined relationship to NF. ADR resolved without consequences, but NF treatment was discontinued. The child was in the 8mos-2yrs age range, who presented severe leukopenia, but also resolved without consequences and he was able to complete treatment.

The severity of ADRs was associated with treatment discontinuation in adults, with 67% of discontinuations in the 15 subjects that presented moderate/severe ADRs compared to 6.1% in the 49 subjects that presented mild ADRs (OR = 27.8, CI95% = [5.1 - 212]; $P_{Adjusted}$ <0.001). In children no association was observed (OR = 2.4, CI95% = [0.04 - 35]; $P_{Adjusted}$ = 0.4).

Serious events were observed in 2 patients. One patient showed a serious event unrelated to NF. The patient died due to complications related to HIV infection and it was described previously. The other patient, a female in her 30´s (not the one mentioned in the previous paragraph), presented tremors, dysarthria and panic attacks which required hospitalization. This patient made a full recovery, but treatment was discontinued.

Treatment completion

Overall treatment was completed in 289/320 patients (90.3%) without differences between children (92.6%) and adults (85.7%; Table 4). Treatment discontinuation took place in 31/320 (9.7%) patients, but only 14/320 (4.4%) were related to ADRs (Table 5). ADRs-related discontinuations occurred more frequently in adults (9.5%) than in children (1.9%; OR = 5.5, CI95% = [1.5 - 24]; $P_{Adjusted}$ = 0.008). Notably, the main cause of treatment discontinuation in children was related to moderate skin ADRs (3/4 children, see tables 4 and 5).

A total of 20/320 (6.2%) subjects temporarily interrupted NF (see table 4), without differences among adults (9.5%) and children (4.7%, OR = 2.1, CI95% = [0.77 - 5.9]; $P_{Adjusted}$ = 0.14). Temporary interruption causes were in 7 adults and 5 children due to ADRs and the remaining 8 patients by personal decision. The median temporary interruption length was 7 days (IQR_{25-75}: 2- 9 days) with no differences between adults and children.

ADRs leading to temporary treatment interruptions were digestive (5), CNS (3), skin (2), cardiovascular (2), and nutritional (2) among adults, and digestive (2), skin (2) and hematologic (2) among children. Treatment discontinuation occurred in 4 of these 12 patients (2 adults, 2 children). The remaining patients completely recovered after symptomatic treatment and/or transient interruption of NF.

For those 289 patients who completed treatment, mean dose, number of tablets and the length of treatment are described in Table 6.

Pediatric cohort analysis

A sub-analysis by age group of the pediatric cohort was carried out to elucidate whether there was any trend in the number, frequency or type of ADRs.

A high rate of treatment completion (92.6%) was observed without differences within pediatric age groups (table 7).

No significant differences among pediatric subgroups were observed in the rates of temporary interruption or in the rate of ADRs (25-38%). In addition, a high

compliance (greater than 70%) was observed in all groups and notably > 90%) in patients under 2 years old (Table 7).

The most frequently observed pediatric ADRs were nutritional, followed by CNS adverse reactions, without clear differences among age groups. Headache was most frequent in children older than 7 years of age. Digestive and hematological events were mainly observed in children younger than 2 years old (Table 8).

Discussion

We present a large retrospective study of ChD patients (children and adults), treated with NF. We observed a low loss to follow-up, similar to other prospective pediatric and adult ChD studies (7, 8, 10, 11). Our service followed the standard of care guidelines for CD patients. However, as with any retrospective study conducted over a long period of time, possible sources of bias must be considered, due to insufficient detailed information about the incidence or severity of ADRs

ADRs incidence in our study was strongly associated with patient age, since adults had higher incidence of ADRs and related treatment discontinuations than children. Moreover, NF-related ADRs were significantly more frequent in adults. Although pediatric pharmacologic studies on NF are still lacking, it is possible that observed differences in incidence of ADRs among the pediatric and adult sub-populations could be due to age-related differences in drug metabolism. NF is metabolized in the liver, and, similar to many other drugs (12–14), it would be expected to undergo faster liver clearance in children compared to adults, leading in shorter half-lives and steady-state plasma concentrations. Pharmacological NF studies are currently underway to clarify this issue (www.clinicaltrials.gov NCT01927224, NCT02625974).

NF related ADRs had a lower incidence in our study compared to previous reports in children (6, 8, 10) and in adults (4, 6, 11) Moreover, the rate of ADRs per patient in our adult cohort was 4-fold lower than the reported in previous studies (11). These differences could be explained by a larger sample size of our cohort that would yield a more accurate estimate of the incidence of ADRs and a shorter time of treatment (30 days) prescribed in our patients. However, some alternative explanations are possible, such as socio-economic, ethnical differences among the studied populations, detection bias (our data were retrospectively collected for this analysis), or other yet unknown issues.

A high incidence of ADRs in patients with acute oral acute *T.cruzi* infection was reported (6). In our study we did not observe a higher incidence of ADRs in

patients during the acute phase of infection. This difference could be related to the different route of infection, mainly congenital, and that the majority of our cases were asymptomatic or with mild symptoms of infection

Regarding severity, the ADRs observed in our cohort were mostly mild. Severe events were infrequent. and all patients recovered with no sequelae. This is comparable to previous studies in children (8, 10). However, in adult patients, we report a lower incidence of severe and serious adverse events than previous studies(4, 11).

ADRs appeared earlier in adults than in children but most of them occurred within the first month of treatment, suggesting that most NF ADRs are not dependent on cumulative doses.

ADRs profiles were similar between pediatric and adult populations, except for hematological ADRs, which appeared only in children, mainly those under 2 years of age. The most frequent ADRs were nutritional, mainly hyporexia and weight loss, in line with the observations of many other ChD researchers (4, 7, 8, 11)

CNS ADRs are a major concern. Seizures and psychiatric ADRs related to NF have been previously described (15)**.** NF has a high level of fat solubility and is well distributed throughout the tissues, including the CNS. Even though NF is a substrate of breast cancer resistance protein (BCRP), which may be responsible for the active transfer of NF out of the CNS, it is possible that some patients may have BCRP polymorphisms that decrease this transfer, thus exposing them to high level of NF in the CNS for longer periods of time (i.e. exposing them to CNS ADRs) (16, 17). However, this remains a hypothesis to be tested. We observed mostly headaches and CNS irritability as NF ADRs (16). An adult patient presented tremors, dysarthria and panic attacks which required hospitalization, but recovered without consequences after temporary treatment interruption. Evaluation of CNS ADRs such as headaches, was difficult in children and particularly in infants because only older children can accurately express these symptoms. However, associated signs, such as unexplained irritability, food refusal, or vomiting, were not reported by caregivers or observed by our pediatricians who have significant experience in evaluating ChD pediatric patients.

The profile of digestive ADRs was similar to that reported in other NF studies (4, 5, 10, 11, 18). In children, digestive ADRs could be related to the lack of an appropriate pediatric formulation of NF which requires pill fractioning. As pill fragments are not easily (or willingly) swallowed by small children, this sometimes results in vomiting and other problems that may not be specifically related to the active drug. A new pediatric NF formulation in the late stages of clinical development (clinicaltrials.gov NCT02625974), over time, would eventually be

helpful to address the pediatric formulation gap and possibly decrease the incidence of digestive ADRs.

Skin reactions are the main ADRs observed during treatment with the alternative drug BZ (19), but are much less frequently described with NF. Accordingly, we observed few skin manifestations in our cohort, and only 3 children developed skin reactions that led to treatment discontinuation. The differences in ADR profiles between BZ and NF are not clearly explained to date, particularly given that they are both nitro-drugs. Unfortunately, NF metabolism, and metabolite profiles for both drugs remain poorly studied, which hampers any speculation on the pharmacological reasons behind these ADR differences (20, 21).

Pediatric treatment discontinuation rates due to NF ADRs in our study were comparable with those in other studies (8, 10). Even though we observed higher discontinuation rates in adults compared to children, these adult rates (i.e. 14.3%) were lower than those reported in previous studies, which ranged from 19.8% to 43.8% (4, 11). This difference could be due to the fact that most of the adult patients in our study were relatives of previously treated and cured children, to whom we offered treatment as part of our ChD family screening and treatment protocol. This population is highly motivated to persist and complete treatment.

In summary, our results suggest that NF is a safe drug to use in both pediatric and adult ChD patients. Considering the retrospective nature of the study, these results are not conclusive and further prospective studies would be required in order to confirm our results.

Since more primary infections of ChD occur during childhood, early diagnosis and treatment of children is vital to prevent long-term ChD sequelae. In the light of our findings, which strongly suggest, that NF is safe in childhood, we believe that treatment should not be delayed.

Methods

Study design and population

This is a retrospective age-stratified study to assess safety and tolerability of oral NF in subjects with ChD.S All patients were treated and followed-up at the Parasitology and Chagas service, Hospital de Niños “Ricardo Gutiérrez”, Buenos Aires, Argentina from January 1980 to July 2019.

Patients were stratified according to age. Sub-analysis among children was done considering the following age groups: (0 - 7mos), (8mos - 1yr), (2 - 6yrs), (7-11yrs), (12 - 17yrs).

Chagas Disease diagnostic criteria: For infants younger than 8 months: direct observation of *T.cruzi* using parasitological concentration method (microhematocrit test, MH) or xenodiagnosis (XD); for older patients: 2 reactive serological tests: Enzyme Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination (IHA) or Direct agglutination (DA).

Exclusion criteria: Cases where nifurtimox was prescribed but not taken (patients did not come back; n = 52) or cases where medication data was not properly documented. (n = 10).

Study population: For the safety analysis, all patients who started treatment were considered, regardless of whether or not they completed the treatment.

Treatment

NF treatment (120-mg tablets, Bayer) was prescribed in doses of 10-15 mg per kg per day divided in two or three daily doses for 60 to 90 days for infants and children, and 8-10 mg per kg for 30 days for adults, according to national guidelines at the moment of diagnosis. Note that regimens were modified for shorter treatment in the last years. Enrollment of children started in January 1980, and enrollment of adults started in July 2008. Infant NF doses were provided as fractionated tablets prepared by a pharmacist and administered with water or mother's milk. Medication was provided to patients or their guardians in monthly batches, and compliance was assessed by counting remaining tablets at each visit. Treatment was considered complete when patients took the medication for at least 60 days for children and 30 days for adults.

Data Collection

Data were collected from medical records of treated patients and entered into an Access clinical database (ACD) designed for this study. All individual datasets were anonymized.

Demographic data, clinical and biochemical assessments and complementary studies were collected during follow-up. Baseline data values were obtained at the beginning of the treatment. Following the standard of care of our service for CD treated patients, visits were carried out at 7, 30 days and at the end of treatment,

every 3 months during the first-year post-treatment and every 6 -12 months thereafter.

ADRs were evaluated through laboratory tests, clinical interviews and physical examinations, and classified according to World Health Organization (WHO) definitions (22, 23). Causality assessment was performed using the WHO criteria for causality assessment

Information on treatment duration and dosage, temporary interruptions and concomitant medications was systematically collected from medical records and documented in the clinical Database.

Statistical Analysis

Continuous variables were expressed with mean and median, as applicable, with the corresponding standard deviation or interquartile range. Categorical variables were expressed in percentages. To test for significance, as appropriate, T test or Wilcoxon unpaired rank test (W.T.) for continuous variables and Fisher exact tests (F.E.T.) for categorical ones. P-values were adjusted by false discovery rate (Benjamini-Hochberg procedure). Adjusted p-values with $P_{Adjusted} < 0.05$ were considered statistically significant. The statistical package R was used (24).

Ethics statement

Study protocol was approved by the research & teaching committee and the bioethics committee of the Buenos Aires Children's Hospital "Dr Ricardo Gutierrez". The protocol was registered at ClinicalTrials.gov (NCT#04274101).

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Potential conflicts of interest

JA is a consultant of Bayer. All other authors report no potential conflicts.

FIGURES

Fig. 1. Study flowchart. Medical records included in this study are described in the figure.

TABLES

Table 1. Demographic data.

	Children (%)	Adults (%)	Total Patients (%)
Gender			
F	109 (50.7)	92 (87.6)	201 (62.8)
M	106 (49.3)	13 (12.4)	119 (37.2)
Age (months)			
Median [Q1, Q3]	31.0 [6.00, 108] mos.	34 [29, 38] yrs.	-
Mean (SD)	59.5 (63.1) mos.	34.5 (7.37) yrs.	-
Min-Max	1.00-215 mos.	19-57 yrs.	-
Route of Infection			
Vector	22 (10.2)	10 (9.5)	32 (10.0)
Congenital	120 (55.8)	11 (10.5%)	131 (40.9)
Blood transfusion	1 (0.5)	2 (1.9)	3 (0.9)
Undetermined	72 (33.5)	82 (78.1)	154 (48.1)
Clinical Examination at diagnosis			
Asymptomatic	185(86.0)	104(99.0)	289(90.3)
Symptomatic	30(14.0)	1(1.0)	31(9.7)
Total	**215(67)**	**105(33)**	**320(100)**

F: Female, M: Male

362 Table 2. Adverse events classified by severity and their relationship to treatment

	Children		Adults	
	Patients (%) n = 63	Number of ADRs (%) n = 84	Patients (%) n = 64	Number of ADRs (%) n = 131
Severity				
Mild	47 (74.6)	60 (71.4)	54 (84.4)	101 (77.1)
Moderate	8 (12.7)	8 (9.5)	14 (21.9)	27 (20.6)
Severe	1 (1.6)	1 (1.2)	1 (1.6)	1 (0.8)
No-Data	11 (17.5)	15 (17.9)	1 (1.6)	2 (1.5)
Relationship				
Not Related	5 (7.9)	10 (11.9)	2 (3.1)	2 (1.5)
Unlikely	1 (1.6)	1 (1.2)	1 (1.6)	1 (0.8)
Probable	47 (74.6)	53 (63.1)	20 (31.2)	31 (23.7)
Certain	5 (7.9)	5 (6)	51 (79.7)	95 (72.5)
No-Data	11 (17.5)	15 (17.9)	1 (1.6)	2 (1.5)
Total	63 (100.0)	84 (100.0)	64 (100.0)	131 (100.0)

363 Relationship classification was recorded according to OMS criteria. For each age group, two columns are
364 shown. The first one, *Patients with ADRs,* shows the number of patients presenting at least one ADR and its
365 corresponding percentage. The second column, *Number of ADRs*, depicted the observed number of ADRs and
366 its corresponding percentage. Notice that patients could present more than one ADR belong to different
367 categories (i.e Mild - Moderate).

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370 Table 3. ADR occurrence and patient incidence by organ system.

	Children		Adults	
	Patients (%) n = 63	**Number of ADRs (%) n = 84**	**Patients (%) n = 64**	**Number of ADRs (%) n = 131**
Body as a whole	**1 (1.6)**	**1 (1.2)**	**4 (6.2)**	**4 (3.1)**
Fever	1 (1.6)	1 **(1.2)**	2 (3.1)	2 (1.5)
Asthenia	-	-	2 (3.1)	2 (1.5)
Cardiovascular	-	-	**2 (3.1)**	**2 (1.5)**
Syncope	-	-	1 (1.6)	1 (0.8)
Tachycardia	-	-	1 (1.6)	1 (0.8)
Digestive	**11 (17.5)**	**12 (14.3)**	**21 (32.8)**	**26 (19.8)**
Vomiting	8 (12.7)	8 (9.5)	4 (6.2)	4 (3.1)
Nausea	2 (3.2)	2 (2.4)	8 (12.5)	8 (6.1)
Dyspepsia	1 (1.6)	1 (1.2)	8 (12.5)	8 (6.1)
Abdominal pain	1 (1.6)	1 (1.2)	4 (6.2)	4 (3.1)
Others	-	-	2 (3.1)	2 (1.5)
Hematological	**6 (9.5)**	**7 (8.3)**	-	-
Eosinophilia	3 (4.8)	3 (3.6)	-	-
Leukopenia	3 (4.8)	3 (3.6)	-	-
Plaquetopenia	1 (1.6)	1 (1.2)	-	-
Nutritional	**30 (47.6)**	**31 (36.9)**	**40 (62.5)**	**44 (33.6)**

Weight loss	13 (20.6)	13 (15.5)	33 (51.6)	33 (25.2)
Hyporexia	18 (28.6)	18 (21.4)	11 (17.2)	11 (8.4)
Musculoskeletal	**1 (1.6)**	**1 (1.2)**	**2 (3.1)**	**2 (1.5)**
Myalgias	-	-	2 (3.1)	2 (1.5)
Chest pain	1 (1.6)	1 (1.2)	-	-
CNS	**19 (30.2)**	**22 (26.2)**	**28 (43.8)**	**39 (29.8)**
Headache	6 (9.5)	6 (7.1)	20 (31.2)	20 (15.3)
Irritability	15 (23.8)	15 (17.9)	7 (10.9)	7 (5.3)
Dizziness	-	-	5 (7.8)	5 (3.8)
Others	1 (1.6)	1 (1.2)	5 (7.8)	7 (5.3)
Respiratory	**1 (1.6)**	**3 (3.6)**	-	-
Acute bronchitis	1 (1.6)	1 (1.2)	-	-
Rhinorrhea	1 (1.6)	1 (1.2)	-	-
Influenza Syndrome	1 (1.6)	1 (1.2)	-	-
Skin	**6 (9.5)**	**7 (8.3)**	**10 (15.6)**	**13 (9.9)**
Rash	6 (9.5)	7 (8.3)	6 (9.4)	6 (4.6)
Urticaria	-	-	4 (6.2)	4 (3.1)
Others	-	-	3 (4.7)	3 (2.3)
Psychiatric	-	-	**1 (1.6)**	**1 (0.8)**
Depression	-	-	1 (1.6)	1 **(0.8)**
Total	**63 (100.0)**	**84 (100.0)**	**64 (100.0)**	**131 (100.0)**

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372 Detailed description of the 215 ADRs occurring in the 127 patients segregated by organ system.
373 For each age group, two columns are shown. The first column, *Patients with ADRs,* shows the
374 number of patients presenting at least one ADR and its corresponding percentage. The second
375 column, *Number of ADRs*, depicted the observed number of ADRs and its corresponding
376 percentage. Low frequency symptoms were grouped into a general category, namely, "*others". digestive: (epigastralgia; pyrrhosis). CNS:(depression;* nightmare*; dysarthria; insomnia; loss of*
377
378 *memory; panic attacks; tinnitus). Skin: (facial edema; pruritus).*

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381 Table 4. Treatment discontinuation and interruption.

	Children (%)	Adults (%)	Total Patients (%)
Complete treatment			
Yes	199 (92.6)	90 (85.7)	289 (90.3)
No	16 (7.4)	15 (14.3)	31 (9.7)
Discontinuation cause			
Patient Decision	6 (2.8)	1 (1.0)	7 (2.2)
Adverse Effect	4 (1.9)	10 (9.5)	14 (4.4)
Death	1 (0.5)	-	1 (0.3)
Lost of follow-up	5 (2.3)	4 (3.8)	9 (2.8)
Treatment Complete	199 (92.6)	90 (85.7)	289 (90.3)
Temporary Interruption			
Yes	10 (4.7)	10 (9.5%)	20 (6.2)
No	205 (95.3)	95 (90.5)	300 (93.8)
Total	215 (67.0)	105 (33.0)	320(100.0)

382 Detailed description of reasons of treatment discontinuation and interruption for all patients included
383 in this study (n = 320 patients).
384

385 Table 5. ADRs causing treatment discontinuation.

	Age range (yrs.)	**Gender**	**Symptoms**	**Treatment Length** (days)	**Second Treatment**
Pediatrics	0-1	F	Irritability	15	NF, Completed
	7-17	M	Rash	15	-
	7-17	F	Rash	27	-
	2-6	M	Abdominal pain, Rash, Fever, Eosinophilia	21	-
Adults	30-39	F	Headache, Irritability, Nausea	3	BZ, discontinued because of ADR
	40-49	F	Hiporexia	23	BZ, Completed
	50-59	F	Hiporexia	11	BZ, Completed
	30-39	F	Syncope, Dizziness, Headache, Dyspepsia, Nausea	12	BZ, Completed
	40-49	F	Headache, Abdominal pain	15	-
	20-29	F	Abdominal pain, Vomiting, Myalgias	11	-
	30-39	F	weight loss, Irritability, tremors, dysarthria, Panic attack	18	BZ, discontinued because of ADR
	20-29	F	Rash, Itching, Headache, Dyspepsia	12	BZ, Completed
	30-39	F	Headache. Dizziness	8	BZ, Completed
	30-39	F	Psychomotor agitation	10	BZ, discontinued because of ADR

386 Detailed ADR description for those patients who discontinued treatment due to ADRs. NF: nifurtimox, BZ:
387 benznidazole. All patients had a good response to symptomatic treatment.

388 Table 6: Treatment description.

	Children	Adults	Total Patients
Dose (mg/kg body weight)			
Median [Q1, Q3]	11.0 [10.0, 12.0]	9.00 [8.20, 9.70]	10.0 [9.19, 12.0]
Missing (%)	5 (2.5)	-	5 (1.7)
Number of doses			
Median [Q1, Q3]	2.00 [2.00, 3.00]	2.00 [2.00, 3.00]	2.00 [2.00, 3.00]
Missing (%)	6 (3.0)	-	6 (2.1)
Days of treatment			
Median [Q1, Q3]	62.0 [60.5, 73.0]	30.0 [29.0, 32.0]	61.0 [33.0, 69.0]
Concomitant medication			
Yes (%)	3 (1.5)	-	3 (1.0)
No (%)	196 (98.5)	90 (100)	286 (99.0)
Compliance			
Yes (%)	166 (83.4)	75 (83.3)	241 (83.4)
No (%)	33 (16.6)	15 (16.7)	48 (16.6)
Temporary Interruption			
Yes (%)	7 (3.5)	8 (8.9)	15 (5.2)
No (%)	192 (96.5)	82 (91.1)	274 (94.8)
Total	199 (92.6)	90 (100)	289 (90.3)

389 Treatment dosification, length and concomitant medication for all patients that completed NF treatment
390 (n=289).

391

392 Table 7: Treatment details for the pediatric cohort.

	(0-7mos) Patients (%) n = 56	(8mos - 1yr) Patients (%) n = 43	(2 - 6yrs) Patients (%) n = 44	(7 - 11yrs) Patients (%) n = 37	(12 - 17yrs) Patients (%) n = 35	Total Patients (%) n = 215
Complete Treatment						
Yes	53 (94.6)	41 (95.3)	41 (93.2)	35 (94.6)	29 (82.9)	**199 (92.6)**
No	3 (5.4)	2 (4.7)	3 (6.8)	2 (5.4)	6 (17.1)	**16 (7.4)**
Treatment discontinuation						
Patient decision	-	1 (2.3)	2 (4.5)	1 (2.7)	2 (5.7)	**6 (2.8)**
Adverse event	-	1 (2.3)	1 (2.3)	0 (0)	2 (5.7)	**4 (1.9)**
Death	1 (1.8)	-	-	-	-	**1 (0.5)**
Loss of follow-up	2 (3.6)	-	-	1 (2.7)	2 (5.7)	**5 (2.3)**
Temporary Interruption						
Yes	2 (3.6)	1 (2.3)	2 (4.5)	1 (2.7)	4 (11.4)	**10 (4.7)**
No	54 (96.4)	42 (97.7)	42 (95.5)	36 (97.3)	31 (88.6)	**205 (95.3)**
Compliance						
Yes	48 (90.6)	37 (90.2)	29 (70.7)	27 (73.0)	25 (71.4)	166 (83.4)

No	5 (9.4)	4 (9.8	12 (29.3)	12 (27.0)	10(28.6)	33 (16.6)
Adverse Events						
Yes	14 (25.0)	14 (32.6)	17 (38.6)	11 (29.7)	7 (20.0)	63 (29.3)
No	42 (75.0)	29 (67.4)	27 (61.4)	26 (70.3)	28 (80.0)	152 (70.7)
Number of Events per patient.						
Median [Q1, Q3]	1.0 [1.0, 1.00]	1.0 [1.0, 1.0]	1.0 [1.0, 2.0]	1.0 [1.00, 1.0]	2.0 [1.0, 2.0]	1.0 [1.0, 1.5]

393 Treatment completion, compliance, interruption and discontinuation causes for different age groups
394 in the pediatric cohort. Also, the number and rates of adverse events are shown

395
396 Table 8: ADR occurrence and patient incidence by organ system in the pediatric cohort.

	(0-7mos)		(7mos-1yr)		(2 – 6yrs)		(7-11yrs)		(12-17yrs)	
	Patients with ADRs (%) n = 14	Number of ADRs (%) n = 17	Patients with ADRs (%) n = 14	Number of ADRs (%) n = 16	Patients with ADRs (%) n=17	Number of ADRs (%) n = 24	Patients with ADRs (%) n = 11	Number of ADRs (%) n = 13	Patients with ADRs (%) n = 7	Number of ADRs (%) n = 14
Body as a whole	-	-	-	-	**1 (5.9)**	**1 (4.2)**	-	-	-	-
Fever	-	-	-	-	1 (5.9)	1 (4.2)	-	-	-	-
Digestive	**4 (28.6)**	**4 (23.5)**	**2 (14.3)**	**2 (12.5)**	**2 (11.8)**	**2 (8.3)**	**-**	**-**	**3 (42.9)**	**4(28.6)**
Vomiting	4 (28.6)	4 (23.5)	2 (14.3)	2 (12.5)	1 (5.9)	1 (4.2)	-	-	1 (14.3)	1(7.1)
Nausea	-	-	-	-	-	-	-	-	2 (28.6)	2(14.3)
Dyspepsia	-	-	-	-	-	-	-	-	1 (14.3)	1(7.1)
Abdominal pain	-	-	-	-	1 (5.9)	1 (4.2)	-	-	-	-
Hematological	**2 (14.3)**	**2 (11.8)**	**2 (14.3)**	**3 (18.7)**	**1 (5.9)**	**1 (4.2)**	**1 (9.1)**	**1(7.7)**	-	-
Eosinophilia	-	-	1 (7.1)	1 (6.2)	1 (5.9)	1 (4.2)	1 (9.1)	1(7.7)	-	-
Leukopenia	2 (14.3)	2(11.8)	1 (7.1)	1 (6.2)	-	-	-	-	-	-
Plaquetopenia	-	-	1 (7.1)	1 (6.2)	-	-	-	-	-	-
Nutritional	**6 (42.9)**	**6 (35.3)**	**8 (57.1)**	**8 (50)**	**8 (47.1)**	**8 (33.3)**	**5 (45.5)**	**5(38.5)**	**3 (42.9)**	**4(28.6)**

Weight loss	1 (7.1)	1 (5.9)	4 (28.6)	4 (25)	3 (17.6)	3 (12.5)	3 (27.3)	3(23.1)	2 (28.6)	2(14.3)
Hiporexia	5 (35.7)	5 (29.4)	4 (28.6)	4 (25)	5 (29.4)	5 (20.8)	2 (18.2)	2(15.4)	2 (28.6)	2(14.3)
Musculoskeletal	-	-	-	-	-	-	**1 (14.3)**	**1(7.1)**	-	-
Chest pain	-	-	-	-	-	-	1 (14.3)	1(7.1)	-	-
CNS	**3 (21.4)**	**3 (17.6)**	**3 (21.4)**	**3 (18.7)**	**8 (47.1)**	**10 (41.7)**	**5 (45.5)**	**6(46.2)**	-	-
Headache	-	-	-	-	2 (11.8)	2 (8.3)	4 (36.4)	4(30.8)	-	-
Irritability	3 (21.4)	3 (17.6)	3 (21.4)	3 (18.7)	7 (41.2)	7 (29.2)	2 (18.2)	2(15.4)	-	-
Nightmare	-	-	-	-	1 (5.9)	1 (4.2)	-	-	-	-
Respiratory	-	-	-	-	-	-	**1 (14.3)**	**3(21.4)**	-	-
Acute bronchitis	-	-	-	-	-	-	1 (5.6)	1 (3.7)	-	-
Rhinorrhea	-	-	-	-	-	-	1 (5.6)	1 (3.7)	-	-
Influenza Syndrome	-	-	-	-	-	-	1 (14.3)	1(7.1)	-	-
Skin	**2 (14.3)**	**2 (11.8)**	**-**	**-**	**2 (11.8)**	**2 (8.3)**	**1 (9.1)**	**1(7.7)**	**1 (14.3)**	**2(14.3)**
Rash	2 (14.3)	2 (11.8)	-	-	2 (11.8)	2 (8.3)	1 (9.1)	1(7.7)	1 (14.3)	2(14.3)
Total	**14(100)**	**17 (100)**	**14(100)**	**16(100)**	**17(100)**	**24(100)**	**11(100)**	**13 (100)**	**7 (100)**	**14 (100)**

Detailed description of the 84 ADRs occurring in the 64 pediatric patients segregated by organ system. For each age group, the first column, *Patients with ADRs,* displays the number of patients presenting at least one ADR and its corresponding percentage. The second column, *Number of ADRs*, depicted the observed number of ADRs and its corresponding percentage.

Bibliography

1. Schmunis GA, Yadon ZE. 2010. Chagas disease: a Latin American health problem becoming a world health problem. Acta Trop 115:14–21.

2. Altcheh JM, Freilij H. 2019. Chagas Disease: A Clinical Approach. Springer Nature.

3. Pereiro AC. 2019. Guidelines for the diagnosis and treatment of Chagas disease. Lancet 393:1486–1487.

4. Jackson Y, Alirol E, Getaz L, Wolff H, Combescure C, Chappuis F. 2010. Tolerance and safety of nifurtimox in patients with chronic chagas disease. Clin Infect Dis 51:e69–75.

5. Olivera MJ, Cucunubá ZM, Álvarez CA, Nicholls RS. 2015. Safety Profile of Nifurtimox and Treatment Interruption for Chronic Chagas Disease in Colombian Adults. The American Journal of Tropical Medicine and Hygiene.

6. Alarcón de Noya B, Ruiz-Guevara R, Noya O, Castro J, Ossenkopp J, Díaz-Bello Z, Colmenares C, Suárez JA, Noya-Alarcón O, Naranjo L, Gutiérrez H, Quinci G, Torres J. 2017. Long-term comparative pharmacovigilance of orally transmitted Chagas disease: first report. Expert Rev Anti Infect Ther 15:319–325.

7. Freilij H, Altcheh J. 1995. Congenital Chagas' Disease: Diagnostic and Clinical Aspects. Clinical Infectious Diseases.

8. Moya PR, Paolasso RD, Blanco S, Lapasset M, Sanmartino C, Basso B, Moretti E, Cura D. 1985. [Treatment of Chagas' disease with nifurtimox during the first months of life]. Medicina 45:553–558.

9. Garcia-Bournissen F, Altcheh J, Giglio N, Mastrantonio G, Della Védova CO, Koren G. 2009. Pediatric Clinical Pharmacology Studies in Chagas Disease. Pediatric Drugs.

10. Bianchi F, Cucunubá Z, Guhl F, González NL, Freilij H, Nicholls RS, Ramírez JD, Montilla M, Flórez AC, Rosas F, Saavedra V, Silva N. 2015. Follow-up of an Asymptomatic Chagas Disease Population of Children after Treatment with Nifurtimox (Lampit) in a Sylvatic Endemic Transmission Area of Colombia. PLOS Neglected Tropical Diseases.

11. Forsyth CJ, Hernandez S, Olmedo W, Abuhamidah A, Traina MI, Sanchez DR, Soverow J, Meymandi SK. 2016. Safety Profile of Nifurtimox for Treatment of Chagas Disease in the United States. Clin Infect Dis 63:1056–1062.

12. Hines RN. 2007. Ontogeny of human hepatic cytochromes P450. Journal of Biochemical and Molecular Toxicology.

13. Hines RN. 2008. The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacology & Therapeutics.

14. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Steven Leeder J, Kauffman RE. 2003. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. New England Journal of Medicine.

15. Wegner DH, Rohwedder RW. 1972. The effect of nifurtimox in acute Chagas' infection. Arzneimittelforschung 22:1624–1635.

16. Watson CP, Dogruel M, Mihoreanu L, Begley DJ, Weksler BB, Couraud PO, Romero IA, Thomas SA. 2012. The transport of nifurtimox, an anti-trypanosomal drug, in an in vitro model of the human blood–brain barrier: Evidence for involvement of breast cancer resistance protein. Brain Research.

17. Garcia-Bournissen F, Altcheh J, Panchaud A, Ito S. 2010. Is use of nifurtimox for the treatment of Chagas disease compatible with breast feeding? A population pharmacokinetics analysis. Arch Dis Child 95:224–228.

18. Valencia NC, Mancilla M, Ramos D, Zulantay I, Molina M, Torres A, Corral G, Apt W. 2012. Tratamiento de la enfermedad de Chagas crónica en Chile: efectos adversos de nifurtimox. Revista Ibero-latinoamericana de parasitología 71:97–108.

19. Altcheh J, Moscatelli G, Moroni S, Garcia-Bournissen F, Freilij H. 2011. Adverse events after the use of benznidazole in infants and children with Chagas disease. Pediatrics 127:e212–8.

20. Hall BS, Bot C, Wilkinson SR. 2011. Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J Biol Chem 286:13088–13095.

21. Hall BS, Wilkinson SR. 2012. Activation of benznidazole by trypanosomal type I nitroreductases results in glyoxal formation. Antimicrob Agents Chemother 56:115–123.

22. Edwards IR, Ralph Edwards I, Aronson JK. 2000. Adverse drug reactions: definitions, diagnosis, and management. The Lancet.

23. Edwards IR, Ralph Edwards I, Biriell C. 1994. Harmonisation in Pharmacovigilance. Drug Safety.

24. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.