Research Article

Treatment failure of pentavalent antimonial therapy for human visceral leishmaniasis: a meta-analysis

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Background

Visceral leishmaniasis is found primarily in developing countries and is a neglected tropical disease that causes approximately 300,000 new cases and 20,000 deaths annually and is often fatal if untreated. Monotherapy with pentavalent antimonials (SSG or MA) is a second-line treatment option in many endemic areas. This meta-analysis provides regional and national analyses of the proportions of antimonial treatment failures at the end-of-treatment and at follow-up according to the drug used.

Methods

This meta-analysis was conducted in accordance with the PRISMA guidelines. Studies were identified by systematic searches of five electronic databases. Studies in English that reported the outcomes of 10 or more patients treated according to the World Health Organization (WHO) pentavalent antimonial regimen (20 mg/kg/day for 28-30 days) were included. Random-effects meta-analyses and funnel plots were performed, and bias was assessed using tools from the Cochrane collaboration.

Results

33 articles were included in the final per protocol analyses, 16 from the Indian subcontinent (India and Nepal), 15 from East Africa and one from each of Iran and Turkey. Data were analysed by drug type regionally, nationally and temporally. Six-month follow-up data from 13 studies (n=797) on the Indian subcontinent gave a sodium stibogluconate (SSG) failure rate of 11.34% (95% confidence interval (CI)=7.05-16.48%). Nationally, the SSG failure rate across 11 studies from northern India greatly exceeded that identified from two Nepalese studies. Six-month follow-up data from three studies (n=638) from East Africa gave an SSG failure rate of 6.25% (95% CI=4.51-8.25%). End-of-SSG-treatment failure rates across the Indian subcontinent (n=1,135), northern India (n=990) and Nepal (n=145) were 39.82% (95% CI=31.67-48.27%), 45.21% (95% CI=39.31-51.17%) and 12.17% (95% CI=7.40-17.93%) respectively. A lower end-of-SSG-treatment failure rate was recorded in East Africa (n=53,335), 6.60% (95% CI=2.94-11.58%). No studies contained six-month follow-up data regarding meglumine antimoniate (MA), and only four studies (n=257) contained end-of-MA-treatment data with a failure rate of 5.01% (95% CI=2.70-7.98%).

Conclusions

Sb(V) remains an important second-line therapy in many areas. Knowledge of failure rates can inform policy decisions at all levels. The findings of this study are most applicable to SSG therapy, confirm the known patterns of resistance and reflect current policies.

Visceral leishmaniasis (VL; also known as kala-azar) is a neglected tropical disease that is typically fatal if left untreated. Globally there are an estimated 300,000 cases and over 20,000 deaths each year, with over 616 million people thought to live endemic areas at risk of VL infection.¹

Two species of *Leishmania* are known to cause VL. The protozoa are transmitted by sand flies, and in East Africa,

the Yemen and the Indian subcontinent VL is caused by anthroponotic transmission of *L. donovani*, while zoonotic transmission of *L. infantum* causes cases in South America, Southwest Asia and in the Mediterranean climates of Europe and North Africa. Both forms have also been reported in China. It is typically the poorest in these regions that are

most at risk, and climate change may increase the at-risk population.²

A diagnosis can be confirmed by culture-based, serological or molecular assays,³ and treatment options vary by disease type and region. Pentavalent antimonial (Sb(V)) monotherapy was the mainstay of treatment for many years, however its side effect profile and increases in the rate of treatment failure led to its replacement as a first-line treatment option.^{4,5} There are two forms of Sb(V): sodium stibogluconate (SSG) and meglumine antimoniate (MA), also known as Pentostam and Glucantime respectively. Historically, cases in East Africa and the Indian subcontinent were mainly treated with SSG, while cases in South America, Europe and North Africa were mainly treated with MA.

The WHO currently recommends Sb(V) monotherapy at 20mg/kg/day intramuscularly or intravenously for 30 days as a second-line treatment option for *L. donovani* cases in East Africa and the Yemen. The same dosage for 28 days is also recommended as a second-line therapy for *L. infantum* cases in Europe, the Middle East, Central Asia and South America. High rates of treatment failure in the Indian subcontinent mean that Sb(V) is no longer deemed efficacious in certain regions (eg, Bihar, India) and 30 days of Sb(V) monotherapy is now the fifth-line option in areas where Sb(V) are known to remain effective.⁵

Successful treatment is indicated by clinical and/or parasitological cure. Complete parasite clearance is thought to be rarely achieved, and it is considered that the majority of successfully treated immunocompetent patients develop effective life-long cellular immunity.^{6,7} However, a proportion of immunocompetent patients relapse, typically within six months of treatment.⁸ Many studies therefore publish the rates of initial (end-of-treatment) and final (six-month follow-up) cure.^{9–11} The rate of relapse rises significantly when patients are co-infected with human immunodeficiency virus type 1 (HIV-1).^{8,12} Many of the reports of apparent resistance to Sb(V) come from clinical interventions where, in the absence of a molecular marker, treatment failure is used as a proxy for resistance.^{13–15}

Although no longer a first-line option, Sb(V) monotherapy remains an important second-line option across East Africa, Europe, the Middle East, Central Asia and Latin America. The objective of this meta-analysis was therefore to provide an up-to-date summary and synthesis of the rates of Sb(V) initial and final treatment failure in human VL. Thus far, no meta-analysis of the treatment failure rate of the WHO Sb(V) regimen has been performed. This analysis is important as it fills this knowledge gap with the statistical power provided by a meta-analysis and an up-to-date appraisal of treatment failure rates will help inform policy makers in determining the order in which VL therapies are offered to patients in different parts of the world. Therefore, systematic searches of the literature were conducted and studies of any design which reported the outcomes of 10 or more primary VL patients treated with Sb(V) according to the recommended WHO regimen were identified for inclusion.

METHODS

LITERATURE SEARCH

Systematic searches were conducted using five electronic databases: EMBASE (Ovid; 1980–2018), MEDLINE In-Process & Other Non-Indexed Citations (Ovid) and MEDLINE (Ovid; 1946–2018), Global Health (Ovid; 1973–2018), Web of Science and BIOSIS Citation Index (1926–2018). The Web of Science Core Collection Citation Indexes searched were: Science Citation Index Expanded (1900–2018), Conference Proceedings Citation Index- Science (1990–2018), Book Citation Index – Science (2005–2018) and the Emerging Sources Citation Index (2015–2018). Two complementary search strategies with no restrictions on language, study date or study design were devised. Strategy one used terms related to all forms of leishmaniasis, combined with terms for Sb(V) and terms for treatment failure. Strategy two used the same terms for *Leishmania* and Sb(V), combined with terms associated with clinical studies in general and studies more specifically of Sb(V) therapy for leishmaniasis. Full details of each search strategy are provided in Appendix S1 in the **Online Supplementary Document**. The results of both search strategies were updated during final preparation of the manuscript. Additional manuscripts were identified from review articles and manual searching.^{16–19} Deduplication was performed using Endnote (version X8.0.1), Zotero (version 5.0.47) and Systematic Review Accelerator [<http://crebp-sra.com>].²⁰ This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, and a PRISMA checklist completed (Appendix S2 in the **Online Supplementary Document**). A study protocol was not published prior to this study.

SELECTION OF STUDIES

The titles and abstracts of the articles were independently screened against primary inclusion criteria by two authors (CMJ, JDJ). Articles that reported the treatment of 10 or more human primary VL patients with Sb(V) were selected, with discrepancies solved by consensus (CMJ, JDJ). Studies that did not contain primary patient data, reported *in vitro* findings, were not available in English, in which Sb(V) was used as part of a combination therapy or the patient population was exclusively HIV-1 positive or otherwise immunocompromised were excluded. No selection criteria were applied to the publication date, geographical area, patient demographics or study design. A full-text analysis of the remaining articles was then conducted independently by two authors, with discrepancies solved by consensus (CMJ, JDJ). Selected studies reported the outcomes of patients treated with either SSG or MA according to the recommended WHO dosing regimen (20 mg/kg/day parenterally for 28–30 days) and contained data for 10 or more patients about the initial (end-of-treatment) and/or final (six-month follow-up) cure that permitted calculation of the proportion of treatment failures. Given that the efficacy of Sb(V) against VL in patients co-infected with HIV-1 has been shown to be reduced,^{21,22} only studies in which fewer than 5% of patients were HIV-1 positive or all HIV-1 patients could be statisti-

cally excluded were selected. Study populations for which HIV-1 prevalence was not reported were included.

DATA EXTRACTION

Data from the remaining studies was independently extracted into separate spreadsheets by two authors, with discrepancies solved by consensus (CMJ, JDJ). Extracted data included study type, study years, study nation(s), form of Sb(V) used, the mean age of the patients (or median, if mean not reported) and the size of the total study population. Also extracted were the number of initial and final treatment successes and failures, number of patients that defaulted (ceased treatment of their own volition) or were withdrawn (on medical advice) during treatment, the number lost or that developed PKDL during follow-up and the number of deaths.

KEY DEFINITIONS

Initial treatment success was defined as clinical and/or parasitological cure after 28–30 days of Sb(V) therapy according to WHO guidelines.⁵ Where clinical and parasitological cure data were provided, parasitological test-of-cure was taken to be more precise. Final cure was defined as initial cure with no relapse or PKDL at six months post-treatment.

The proportion of initial failures was calculated by dividing the number of failures during the study period through the total number of patients treated, where treated is the sum of patients that were initially cured or initially failed treatment. Patients that initially failed treatment and were re-treated with Sb(V) were discounted from follow-up analyses, such that the sum of final cures, failures, PKDL patients and those lost to follow-up was equal to the number of initial cures where such data was available.

The proportion of final failures was calculated by dividing the number of final failures through the sum of the number of patients that were cured or had failed treatment at follow-up. Patients lost to follow-up were discounted.

The assumption was made that withdrawals or defaults were secondary to the toxicity of the drug and that no evaluation of treatment efficacy could be made for these patients. The mortality rate of VL was assumed to be greater than Sb(V) and therefore deaths during treatment or the follow-up period were defined and presented as initial or final treatment failures respectively.

STATISTICS

Random-effects meta-analyses of proportions were performed using a per protocol approach. 95% confidence intervals (CIs) were calculated for individual studies and the principal summary measure of the pooled proportions of treatment failures. Study heterogeneity was assessed by the I^2 statistic, reported with 95% CIs, and interpreted as low ($\leq 25\%$), moderate (25–75%) and high ($> 75\%$).²³ Statistical analyses were performed using MedCalc for Windows, version 18.0 (MedCalc Software, Ostend, Belgium).

RISK OF BIAS

The risk of bias was assessed at the individual study level. Randomised controlled trials (RCTs) were assessed using the Cochrane risk of bias tool for randomised controlled trials.²⁴ The risk of bias in non-randomised studies of interventions (ROBINS-I) assessment tool was used to assess bias for retrospective and cohort studies. Publication bias was assessed using funnel plots.^{25,26}

RESULTS

STUDY SELECTION AND CHARACTERISTICS

[Figure 1](#) illustrates the five databases that were systematically searched using two distinct search strategies. These complementary search strategies were chosen to ensure that cohort and retrospective studies that investigated drug resistance were obtained together with clinical trials. A further 24 references were obtained from reviews and manual searches, and a total of 1,713 deduplicated references were screened against broad inclusion criteria, of which 1,532 were excluded. The full-texts of the remaining 181 articles were subsequently screened against refined inclusion criteria, of which 148 were excluded because: of a dose regimen that did not match the WHO regimen ($n=40$); the full-text was inaccessible ($n=28$, see Appendix S3 of the **Online Supplementary Document**); cutaneous, not VL, was studied ($n=25$); fewer than 10 patients were studied ($n=21$); the patient population in whole or part did not have primary VL ($n=11$); the data were unclear or not relevant to this study ($n=9$); the article was not published in English ($n=7$); the studies used *in vitro* ($n=2$) or animal ($n=1$) approaches; the articles did not contain primary data ($n=2$); greater than 5% of the study population was HIV-1 positive ($n=1$); they were found to be duplicates ($n=1$).

Of the 33 final articles,^{9,10,13–15,27–53} 31 contained data regarding the initial treatment outcome ([Table 1](#)) and 16 regarding the final outcome ([Table 2](#)); 14/31 contained data for both end-of-treatment and six-month follow-up. Of the 54,826 VL patients for which data regarding initial treatment outcome was available, 45,862 (83.7%) were successfully treated, 8,865 (16.2%) failed treatment and 99 (0.2%) defaulted or were withdrawn and did not complete treatment. Of the patients that completed treatment, 93.7% (51,257) were drawn from one large study.⁴² Of the 1,493 VL patients for which data regarding final treatment outcome was available, 1,308 (87.6%) were successfully treated, 125 (8.4%) failed treatment, 59 (4.0%) were lost to follow-up and one (0.07%) developed PKDL.

The mean age of the 3,781 patients for which mean age data were available/calculable was 17.9 years old - excluding two studies which did not report age data and three studies which reported median age data. The median number of patients per study was 61, with a median study period of 2.0 years. The studies were conducted in India (13; $n=1,068$), Sudan (6; $n=52,711$), Ethiopia (3; $n=386$), Kenya (3; $n=142$), Nepal (3; $n=45$), Uganda (1; $n=161$), Iran (1; $n=61$) and Turkey (1; $n=15$), with two studies conducted across Sudan, Ethiopia and Kenya ($n=691$). 18/33 were RCTs, 9/33 were of a cohort design and 6/33 were retrospective analyses.

Table 1. Studies that contained data regarding initial (end-of-treatment) outcome

Citation	Study nation(s)	Study type	Study year(s)	Drug	Mean age (years)	Study population	Treated population	Treatment successes	Treatment failures	Defaults or withdrawals	% treatment failures (95% CI)
52	Iran	Retro	1997-2010	MA	2.0	61	60	57	4	0	6.6 (1.8-16.0)
27	Kenya	RCT	1980-1981	SSG	14.5	26	26	25	1	0	3.8 (0.1-19.6)
14	India	Cohort	2005*	SSG	N/A	182	156	89	67	26	43.0 (35.1-51.1)
15	Ethiopia	Cohort	2011-2012	SSG	8.7	116	116	113	3	0	2.6 (0.5-7.4)
10	Ethiopia	Retro	2008-2009	MA	14.3	24	24	24	0	0	0.0 (0.0-14.2)
11	Sudan, Ethiopia, Kenya	RCT	2004-2008	SSG	16.7	135	132	123	9	3	6.8 (3.2-12.5)
33	India	RCT	1993-1995	SSG	26.1	30	30	22	8	0	26.7 (12.3-45.9)
34	Nepal	RCT	1994-1997	SSG	27.0	27	27	25	2	0	7.4 (0.9-24.3)
35	Sudan	RCT	1989-1995	SSG	18.0	825	825	810	15	0	1.8 (1.0-3.0)
36	India	Cohort	1995-1998	SSG	24.2	24	24	9	15	0	62.5 (40.6-81.2)
40	Kenya	RCT	1997-1998	SSG	9.0	102	98	93	5	4	5.1 (1.7-11.5)
48	Sudan	Retro	2004-2005	SSG	21.0	23	22	22	0	1	0.0 (0.0-15.4)
49	Uganda	Retro	2002-2003	MA	12.5	161	159	152	7	2	4.4 (1.8-8.9)
43	Nepal	Cohort	1999-2001	SSG	23.8	120	118	103	15	2	12.7 (7.3-20.1)
42	Sudan	Cohort	1989-2003	SSG	N/A	51,257	51,257	42,974	8,283	0	16.2 (15.8-16.5)
46	Ethiopia	RCT	2006*	SSG	28.8	246	240	214	26	6	10.8 (7.2-15.5)
29	Sudan	RCT	1992	SSG	15.2	61	61	57	4	0	6.6 (1.8-16.0)
30	Kenya	RCT	1989-1991	SSG	14.7	14	14	14	0	0	0.0 (0.0-23.2)
31	India	RCT	1992-1993	SSG	27.0	15	15	11	4	0	26.7 (7.8-55.1)
32	India	RCT	1995-1996	SSG	20.0	52	50	20	30	2	60.0 (45.2-73.6)
37	India	Cohort	1994-1997	SSG	22.5	320	306	189	117	14	38.2 (32.8-43.9)
47	Turkey	Retro	2000-2003	MA	3.9	15	13	12	1	2	7.7 (0.2-36.0)
53	India	Cohort	1994-1996	SSG	25.2	80	78	48	30	2	38.5 (27.7-50.2)
13	India	RCT	1996	SSG	27.9	50	49	27	22	1	44.9 (30.7-59.8)
38	India	RCT	1996	SSG	22.4	30	30	22	8	0	26.7 (12.3-45.9)
41	India	RCT	2001-2002	SSG	23.0	32	32	11	21	0	65.6 (46.8-81.4)
44	India	Cohort	2000-2001	SSG	35.0†	60	51	30	21	9	41.2 (27.6-55.8)

Citation	Study nation(s)	Study type	Study year(s)	Drug	Mean age (years)	Study population	Treated population	Treatment successes	Treatment failures	Defaults or withdrawals	% treatment failures (95% CI)
45	India	Cohort	2000-2002	SSG	28.0	123	104	47	57	19	54.8 (44.7-64.6)
9	India	RCT	2004-2005	SSG	34.0†	70	65	30	35	5	53.8 (41.0-66.3)
39	Sudan	RCT	1996-1998	SSG	12.3	516	515	464	51	1	9.9 (7.5-12.8)
28	Sudan	RCT	1989-1990	SSG	15.0	29	29	25	4	0	13.8 (3.9-31.7)

CI – confidence interval; MA – meglumine antimoniate; N/A – not available; Retro – retrospective; RCT – randomised controlled trial; SSG – sodium stibogluconate.

*Study period unavailable, publication date listed instead.

†Mean age data were unavailable and median age is presented instead.

Table 2. Studies that contained data regarding final (six-month follow-up) outcome

Citation	Study nation(s)	Study type	Study year(s)	Drug	Mean age (years)	Final cures	Final failures	LTFU	PKDL	% treatment failures (95% CI)
14	India	Cohort	2005*	SSG	N/A	65	24	0	0	27.0 (18.1-37.4)
33	India	RCT	1993-1995	SSG	26.1	19	3	0	0	13.6 (2.9-34.9)
40	Kenya	RCT	1997-1998	SSG	9.0	52	6	35	0	10.3 (3.9-21.2)
51	Sudan, Ethiopia, Kenya	RCT	2004-2010	SSG	15.3	524	32	0	0	5.8 (4.0-8.0)
43	Nepal	Cohort	1999-2001	SSG	23.8	98	1	4	0	1.01 (0.0-5.5)
50	Nepal	Cohort	2001-2003	SSG	23.9†	153	16	12	0	9.5 (5.5-14.9)
31	India	RCT	1992-1993	SSG	27.0	9	2	0	0	18.2 (2.3-51.8)
32	India	RCT	1995-1996	SSG	20.0	18	2	0	0	10.0 (1.2-31.7)
37	India	Cohort	1994-1997	SSG	22.5	168	15	6	0	8.2 (4.7-13.2)
53	India	Cohort	1994-1996	SSG	25.2	46	2	0	0	4.2 (0.5-14.3)
13	India	RCT	1996	SSG	22.4	26	1	0	0	3.7 (0.1-19.0)
38	India	RCT	1996	SSG	27.9	21	1	0	0	4.5 (0.1-22.8)
44	India	Cohort	2000-2001	SSG	35.0†	24	6	0	0	20.0 (7.7-38.6)
45	India	Cohort	2000-2002	SSG	28.0	39	8	0	0	17.0 (7.7-30.8)
9	India	RCT	2004-2005	SSG	34.0†	25	5	0	0	16.7 (5.6-34.7)
28	Sudan	RCT	1989-1990	SSG	15.0	21	1	2	1	4.2 (0.1-21.1)

CI – confidence interval, LTFU – lost to follow-up, N/A – not available, PKDL – post-kala-azar dermal leishmaniasis, RCT – randomised controlled trial, SSG – sodium stibogluconate

*Study period unavailable, publication date listed instead.

†Mean age data were unavailable and median age is presented instead.

Figure 1. Flow diagram of study selection

Flow diagram of study selection.

All studies were assessed for risk of bias using tools from The Cochrane Collaboration.^{24,54} All but one of the RCTs were assessed to be of an Agency for Healthcare Research and Quality standard of fair or good (Table 3). Zijlstra and colleagues 1993 was assessed as poor due to inadequate reporting of their randomisation process and a failure to explicitly mention whether patients with a history of VL treatment were excluded from the study, however it was not excluded as there was no reason to consider this as a substantial source of bias for the purposes of this study. None of the cohort or retrospective studies were assessed as having greater than a moderate risk of bias, consistent with studies of a sound nature for non-randomised trials (Table 4).

META-ANALYSES OF THE EFFICACY OF SB(V) TREATMENT

For all meta-analyses the studies were grouped according to drug type and geographical region.

THE EFFICACY OF SB(V) TREATMENT AT SIX-MONTH FOLLOW-UP

16 studies contained six-month follow-up data for patients treated with Sb(V), SSG was used in all studies; no data for MA was available.

SIX-MONTH EFFICACY OF SSG IN THE INDIAN SUBCONTINENT

Six-month follow-up data were available for a pooled population of 797 patients across the 13 studies conducted in the Indian subcontinent, 2/13 of which contained data from Nepal and 11/13 from the neighbouring Indian states of Ut-

Table 3. Risk of bias assessment for randomised controlled trials*

Citation	Year(s)	Nation(s)	Random'n	Allocation	Selective reporting	Other	Participant/ personnel blinding	Blinding outcome	Incomplete outcome data	AHRQ standard	Clinical ± Micro' TOC
27	1980-81	Kenya	H	U	L	L	L	L	L	Fair	C, P
11	2004-08	Sudan, Ethiopia, Kenya	L	L	L	L	L	L	L	Good	C, P
33	1993-95	India	L	L	L	L	L	L	L	Good	C, P
34	1994-97	Nepal	U	U	L	L	L	L	L	Fair	C
35	1989-95	Sudan	U	U	L	L	L	L	L	Fair	C, P†
40	1997-98	Kenya	H	L	L	L	L	L	L	Fair	C, P
51	2004-10	Sudan, Ethiopia, Kenya	L	L	L	L	L	L	L	Good	P
46	2006‡	Ethiopia	L	L	L	H§	L	L	L	Fair	C, P
29	1992	Sudan	H	L	L	L	L	L	L	Fair	C, P
30	1989-91	Kenya	L	U	L	L	L	L	L	Fair	C, P
31	1992-93	India	L	U	L	L	L	L	L	Fair	C, P
32	1995-96	India	L	U	L	L	L	L	L	Fair	C, P
13	1996	India	L	L	L	L	L	L	L	Good	C, P
38	1996	India	L	L	L	L	L	L	L	Good	C, P
41	2001-02	India	U	U	L	L	L	L	L	Fair	C, P
9	2004-05	India	U	U	L	L	L	L	L	Fair	C, P
39	1996-98	Sudan	H	U	L	L	L	L	L	Fair	C, P
28	1989-90	Sudan	U	U	L	H§	L	L	L	Poor	C, P

AHRQ – Agency for Healthcare Research and Quality, H – high, L – low, Micro' – microbiological, Random'n – randomisation, U – unclear

*The Cochrane Risk of Bias Tool for Randomised Controlled Trials was used. The test-of-cure (TOC) assay(s) are listed as clinical (C) and/or parasitological (P).

†Parasitological test of cure was not explicitly stated.

‡Study period unavailable, publication date listed instead.

§It was not explicitly stated in the study whether patients with previous anti-leishmanial therapy were excluded.

Table 4. Risk of bias assessment for non-randomised studies*

Citation	Study type	Year(s)	Nation(s)	Confounding	Selection	Classification	Deviation	Missing data	Measurement	Reported	ROBINS-I overall risk	Clinical ± Micro/TOC
52	Retro	1997-10	Iran	Low	Mod	Mod	Low	Low	Mod	Mod	Mod	Unclear
14	Cohort	2005†	India	Low	Mod	Low	Low	Low	Low	Low	Mod	C, P
15	Cohort	2011-12	Ethiopia	Mod	Mod	Low	Low	Low	Mod	Low	Mod	C or P
10	Retro	2008-09	Ethiopia	Low	Mod	Mod	Low	Low	Low	Low	Mod	C, P
36	Cohort	1995-98	India	Mod	Mod	Low	Low	Low	Low	Low	Mod	C, P
48	Retro	2004-05	Sudan	Mod	Mod	Mod	Low	Low	Low	Low	Mod	C
49	Retro	2002-03	Uganda	Low	Mod	Mod	Low	Low	Low	Low	Mod	C, P
43	Cohort	1999-01	Nepal	Low	Mod	Low	Low	Low	Low	Low	Mod	C, P
50	Cohort	2001-03	Nepal	Low	Mod	Low	Low	Low	Low	Low		C, P
42	Retro	1989-03	Sudan	Low	Mod	Low	Low	Low	Mod	Mod	Mod	Unclear
37	Cohort	1994-97	India	Low	Mod	Low	Low	Low	Low	Low	Mod	P
47	Retro	2000-03	Turkey	Low	Mod	Mod	Low	Low	Mod	Low	Mod	Unclear
53	Cohort	1994-96	India	Low	Mod	Low	Low	Low	Low	Low	Mod	C, P
44	Cohort	2000-01	India	Low	Mod	Low	Low	Low	Low	Low	Mod	P
45	Cohort	2000-02	India	Low	Mod	Low	Low	Low	Low	Low	Mod	C, P

Micro – microbiological

*The ROBINS-I tool was used, and scores of low, moderate (mod) or high given. The test-of-cure (TOC) assay(s) are listed as clinical (C) and/or parasitological (P).

†Study period unavailable, publication date listed instead.

Figure 2. The six-month efficacy of SSG in the Indian subcontinent

Panel A. Forest plot of the proportion of treatment failures (n=797). **Panel B.** Funnel plot. **Panel C.** Temporal analysis of the proportion of treatment failures.

tar Pradesh and/or Bihar. We therefore elected to analyse the data at both regional and national levels.

Figure 2, Panel A, shows that, across the region, the proportion patients found to have failed SSG treatment was 11.34% (95% CI=7.05-16.48%). The inconsistency between study findings was moderate, $I^2=74.44\%$ (95% CI=55.86-85.20%). All forest plots are presented in the same way, the boxes show effect estimates for each study, weighted according to a random-effects model; the horizontal lines indicate 95% CIs; the centre of the diamond shows the pooled treatment failure rate and the horizontal tips represent 95% CIs. A funnel plot - on which the vertical line represents the summary estimate derived by fixed-effects meta-analysis and the diagonals represent 95% CIs around the summary effect - was found to be symmetrical (Figure 2, Panel B). When failure proportions were plotted by publication year (Figure 2, Panel C), the most recent studies were observed to have the highest rates of treatment failure, but the overall trend was negligible ($R^2=0.0574$). The highest rate of treatment failure was 26.97% (95% CI=18.10-37.42%), recorded in a 2005 study of 89 patients in Bihar.¹⁴ The lowest rate of treatment failure was 1.01% (95% CI=0.03-5.50%) recorded in a 2003 study of 99 patients in Nepal.⁴³

At a national level, 2/13 studies were conducted in Nepal. Meta-analysis of these data revealed a treatment failure rate of 4.83% (95% CI=0.09-16.13%); $I^2=89.64\%$ (95% CI=61.62-97.2%) across 268 patients. Sub-analysis of the

529 patients drawn from the 11 studies conducted in northern India revealed a higher rate of treatment failure of 13.34% (95% CI=8.62-18.90%) and reduced inconsistency between study findings, $I^2=62.16\%$ (95% CI=27.21-80.33%). Temporal analysis of the Indian data did not reveal a significant increase in the rate of treatment failure over time ($R^2=0.2299$).

SIX-MONTH EFFICACY OF SSG IN EAST AFRICA

Three East African studies (Kenya n=1, Sudan n=1, and a mixed study across Sudan, Ethiopia and Kenya n=1) contained six-month follow-up data for a pooled population of 638 patients. Meta-analysis showed that the pooled proportion of treatment failure was 6.25% (95% CI=4.51-8.25%), with too few studies to accurately appraise inter-study inconsistency ($I^2=0.00\%$, 95% CI=0.00-96.47%). No funnel plot was generated for these data as the influence of chance increases with fewer than 10 studies.⁵⁵ The highest rate of treatment failure (10.35%, 95% CI=3.90-21.17%) was recorded in a study that contained data from 58 Kenyan patients.⁴⁰ Insufficient data was available for national sub-analyses.

THE EFFICACY OF SB(V) TREATMENT AT THE END OF TREATMENT

31 studies contained data regarding patient outcomes at the end-of-treatment (initial cure). In the majority of stud-

Figure 3. The end-of-treatment efficacy of SSG in the Indian subcontinent

Panel A. Forest plot of the proportion of treatment failures (n=1,135). **Panel B.** Funnel plot. **Panel C.** Temporal analysis of the proportion of treatment failures.

ies, 27/31, patients were treated with SSG, although in 4/31 studies MA was used.

END-OF-TREATMENT EFFICACY OF SSG IN THE INDIAN SUBCONTINENT

15 studies from the Indian subcontinent contained end-of-treatment outcome data, two from Nepal and 13 from the Bihar and/or Uttar Pradesh regions of northern India, representing 1,135 patients. Figure 3, Panel A, shows that 39.82% (95% CI=31.67-48.27%) failed SSG treatment ($I^2=86.86\%$, 95% CI=79.93-91.39%). A funnel plot of standard error against proportion of failures wasn't notably asymmetrical (Figure 3, Panel B). Figure 3, Panel C, shows that there was no obvious increase in the rate of treatment failure over time ($R^2=0.0923$). The highest rate of treatment failure was 65.63% (95% CI, 46.81-81.43%), recorded in a 2003 study of 32 patients in Bihar.⁴¹ The lowest rate of treatment failure was 7.41% (95% CI, 0.91-24.29%) recorded in a 1998 study of 27 patients in Nepal.³⁴

Nationally, 2/15 studies contained data from Nepal, meta-analysis of these data gave a treatment failure rate of 12.17% (95% CI=7.40-17.93%); $I^2=0.00\%$ (95% CI=0.00-0.00) across 145 patients. Sub-analysis of the remaining 990 patients drawn from the 13 studies conducted in northern India revealed a high rate of treatment failure of 45.21% (95% CI=39.31-51.17%) and reduced inter-study inconsistency, $I^2=67.19\%$ (95%CI=41.32-81.66%). Temporal analysis

of the studies from northern India revealed a greater magnitude of increase in the rate of treatment failure over time ($R^2=0.1406$).

Only 14 of the 33 studies identified contained data about both initial and follow-up failure rates, 11 of these studies were from India. Given the large difference between the initial and follow-up failure rates identified thus far, we sought to confirm this pattern by repeating the analysis using only the data from the 11 Indian studies that covered both time-points (n=528). This provided an initial treatment failure rate of 42.91% (95% CI=37.27-48.63%); $I^2=62.4\%$ (95% CI=27.73-80.44%) and a follow-up failure rate of 13.38% (95% CI=8.65-18.96%); $I^2=62.25\%$ (95% CI=27.40-80.37%).

END-OF-TREATMENT EFFICACY OF SSG IN EAST AFRICA

A total of 12 studies from East Africa (Kenya n=3, Ethiopia n=2, Sudan n=6, and a mixed study across Sudan, Ethiopia and Kenya n=1) contained end-of-SSG-treatment data, representing 53,335 patients. 96.1% of patients were drawn from one large retrospective study. While meta-analyses are weighted, to investigate the influence of this sample size all analyses were conducted with and without this large study population. When all 12 studies were included (Figure 4, panel A), the pooled proportion of treatment failure was 6.60% (95% CI=2.94-11.58%; $I^2=96.73\%$, 95% CI, 95.53-97.61%).

Figure 4. The end-of-treatment efficacy of SSG in East Africa

Panel A. Forest plot of the proportion of treatment failures (n=53,335). **Panel B.** Funnel plot. **Panel C.** Temporal analysis of the proportion of treatment failures.

A funnel plot of all 12 studies appeared asymmetrical (Figure 4, panel B). The vertical line in a funnel plot represents the pooled estimate calculated using a fixed-effects model. In this case there was a marked discrepancy between the fixed- (15.63%, 95% CI=15.33-15.95%) and random- (6.60%, 95%CI=2.94-11.58%) effects estimates. This difference reflects the comparative influence of the weighting given by the fixed or random effects models to the smaller studies or the large 2003 study and was responsible for the appearance of the funnel plot. Repetition, excluding the large 2003 study provided a pooled population of 2,078 and gave more closely aligned fixed- and random-effects estimates of 5.19% (95% CI=4.28-6.23%) and 5.88% (95% CI=3.17-9.36%) respectively $I^2=84.63$ (95% CI=74.12-90.87%); the funnel plot was found to be symmetrical, suggesting no publication bias. Temporal analysis (Figure 4, Panel C) did not reveal any change in failure rate over time.

Sub-analyses for Kenya, Ethiopia and Sudan revealed treatment failure rates of 5.01%, 6.56% and 7.57% respectively (Table 5). Exclusion of Ritmeijer and 2003 from the Sudanese analysis gave a failure rate of 5.96%.

END-OF-TREATMENT EFFICACY OF MA

The four studies in which MA was used took place in Iran, Ethiopia, Uganda and Turkey and represented 257 patients. Meta-analysis of these data gave a treatment failure rate

of 5.01% (95% CI=2.70-7.98%), and too few studies to accurately appraise inter-study inconsistency ($I^2=0.00\%$, 95% CI=0.00-85.86%). Insufficient data was available for national analyses and the small number of studies precluded generation of a funnel plot.

THE INFLUENCE OF HIV-1 STATUS

HIV-1 and VL both typically affect patients in lower socioeconomic demographics. Of the 33 studies, the majority (19/33) didn't report the HIV prevalence of their study population, while 14/33 excluded HIV+ patients (n=11) or reported a prevalence below 5% (n=3). To provide a better estimate of the failure rate of Sb(V), while acknowledging that HIV-1 can increase the rate of treatment failure,^{21,22} we sought to establish a threshold of acceptable HIV-1 prevalence for inclusion. The HIV-1 prevalence data for adults aged 15-49 from the study countries ranged from 5.9% (Uganda) to <0.1% (Iran).⁵⁶ However, the HIV-1 prevalence among earlier study populations may be higher, reflecting the progressive roll out of ART. We sought to exclude studies that examined solely HIV-1+ patients or groups in which HIV-1 might be over-represented. Instead, we wanted to include populations in which the HIV-1 prevalence would reflect the wider national population. Given the range of HIV prevalence values identified for included studies, we therefore selected an arbitrary 5% threshold. Note that this led

Table 5. Sub-analyses of treatment failure rates in East African nations

Nation	Studies	Pooled population	Proportion of failures (%)	95% CI (%)	I ² (95% CI)
Ethiopia	2	356	6.56	0.96-16.64	88.28 (55.16-96.94)
Kenya	3	138	5.01	2.04-9.21	0.00 (0.00-88.98)
Sudan	6	52,709	7.57	2.14-15.95	98.20 (97.35-98.77)
Sudan*	5	1,452	5.96	1.66-12.68	91.86 (84.00-95.86)

CI – confidence interval

*Represents repetition of the analyses excluding data from.⁴²

to the exclusion of one Ethiopian study which examined the efficacy of Sb(V) among HIV co-infected VL patients.⁵⁷

Using SSG treatment data from East Africa and India, the locations in which most studies were performed, we compared failure rates from studies that reported an HIV-1 prevalence of <5% to those that did not report prevalence data (Table 6). The failure rate of the population with an unknown HIV-1 prevalence was not significantly different to that of the population with <5% HIV-1 for any of the Indian data. Among the African data, there was a significant ($P < 0.0001$) difference between the end-of-treatment failure rates reported in studies with <5% (5.09%) or an unknown (9.18%) HIV-1 prevalence. The significant difference remained after exclusion of Ritmeijer and Davidson 2003, (7.5% failure rate; $n=690$; $P=0.028$). No significant difference was reported at six-month follow-up.

DISCUSSION

Sb(V) are no longer recommended as a first-line therapy for VL, however Sb(V) monotherapy remains an important later treatment option in most regions if required.⁵ An appreciation of the Sb(V) treatment failure rate is therefore useful in guiding regional, national and clinical policy decisions.

In the absence of reliable molecular or genetic markers of parasite resistance, clinical treatment failure is often taken as a proxy for drug resistance.^{13–15,58} However, treatment failure ultimately represents the outcome of a complex interplay of host (eg, immunological, genetic and pharmacokinetic), parasite and drug factors.^{59,60} The clinical use of treatment failure as a proxy for drug resistance may therefore overestimate the prevalence of parasite resistance, but in the absence of molecular markers remains a valuable predictor of resistance.

The highest rate of SSG treatment failure was identified in northern India, where the rate of initial treatment failure (45.21%) was greater than that recorded at six-month follow-up (13.34%). Compared to the resistance rates recorded for other antileishmanial therapeutics in India,⁶¹ these data imply that Sb(V) monotherapy should remain a last-line treatment option. The discrepancy between the initial and six-month follow-up figures shows that the initial failure rate was greater than the relapse rate among patients that were initially treated successfully. The high rates of Sb(V) treatment failure recorded in northern India, particularly Bihar, are well-known.¹⁴ Several factors are thought to have contributed, including the misuse of Sb(V) in the region which may have driven parasite resistance. For example, it has been estimated that in 2011 only 26% of VL patients

were treated with Sb(V) according to guidelines.⁶² It has also been suggested that high levels of arsenic in the drinking water in some parts of northern India may have selected for parasites with reduced Sb(V) sensitivity.⁶³ Notably, temporal analysis did not show a significant increase in the rate of resistance over time. Given the high rate of treatment failure, it is unsurprising that Sb(V) is no longer recommended in the Indian subcontinent, except in regions where it remains effective.⁵ Among the latter is Nepal, which was found to have much lower failure rates, suggesting that the drivers of treatment failure are, wholly or partially, not present in Nepal.

The proportion of SSG treatment failures in East Africa was substantially lower than in India, perhaps reflecting a lower degree of historical Sb(V) misuse or arsenic contamination. The data from this study therefore suggest that Sb(V) monotherapy remains a valid treatment option in East Africa when the first-line therapy is unavailable or ineffective. There were 12 East African studies from which end-of-treatment data could be drawn, compared to 15 for the Indian subcontinent. In contrast, while 13 studies from the Indian subcontinent contained six-month follow-up data, only three East African studies did so. This may reflect greater difficulty in tracing patients, many of whom may have been nomadic.⁴²

This study identified four articles ($n=257$) in which MA was used instead of SSG, and the failure rate of these studies was found to be 5.01%. Strict study selection criteria were applied to ensure comparability. However, as *L. infantum* often infects children many studies have used lower doses and/or shorter courses^{64,65} and these studies were therefore excluded from this analysis. Nevertheless, shorter courses of up to 21 days have been shown to be efficacious with low levels of treatment failure.^{52,66–68} Moreover, this likely explains why no studies from South America were identified, despite ongoing research there.

This analysis may somewhat overestimate the proportion of treatment failures for two reasons. First, HIV-1 infection is known to increase the rate of treatment failure.^{8,12} To increase the number or eligible studies, those with a cohort with greater than 5% HIV-1 prevalence were excluded while studies that did not report prevalence data were included. Analysis of the two largest SSG treatment datasets showed no influence of including studies with unreported HIV-1 prevalence on the Indian data. However, there was a significant influence upon the end-of-treatment data from East Africa. The higher failure rate among those without known HIV-1 status likely reflects the sum of the background failure rate and underlying HIV-1 prevalence.

Table 6. The influence of HIV-1 status upon treatment failure in India and East Africa

	Studies	n	No or <5% HIV		Studies	n	Unknown HIV prevalence		P value
			% TF (95% CI)	I ² (95% CI)			% TF (95% CI)	I ² (95% CI)	
India:									
EOT (initial)	4	265	49.24 (38.21-60.31)	68.92 (10.15-89.25)	9	725	43.3 (36.25-50.49)	66.06 (31.14-83.28)	0.5707
6MFU (final)	3	125	13.33 (4.76-25.35)	67.62 (0.00-90.63)	8	404	13.35 (7.57-20.45)	65.43 (26.47-83.75)	0.99
East Africa:									
EOT (initial)	6	1,388	5.09 (1.94-9.63)	85.97 (71.55-93.08)	6	51,947	9.18 (5.05-14.37)	87.99 (76.34-93.91)	< 0.0001
6MFU (final)	1	556	5.76	N/A	2	80	9.58 (4.20-16.85)	0.00 (0.00-0.00)	0.187

6MFU – six-month follow-up, CI – confidence interval, EOT – end of treatment, TF – treatment failure

Future studies examining the efficacy of anti-leishmanial chemotherapeutics must account for HIV-1 prevalence. Second, this study assumed that the death rate from VL exceeded the treatment. However, there were studies in which patients died from drug toxicity or other causes, eg,^{44,45,49} and inclusion of these as treatment failures will lead to a slight overestimation.

The results of this study may also be limited by the moderate to high heterogeneity associated with the results. This is likely due to variations that couldn't adequately be accounted for by sub-analysis because of insufficient volume or reporting of data to enable this - eg, national or patient variables such as ethnicity, age or unreported comorbidities. Further heterogeneity may arise from methodological diversity, including the use of parasitological or clinical test-of-cure. It's important that future clinical evaluations of antileishmanial treatment efficacy report as much data about the participants as possible to enable future meta-analyses to explore and account for heterogeneity.

Taken together, this study supports current policy, confirming high rates of Sb(V) treatment failure in parts of the Indian subcontinent while comparatively lower rates exist in East Africa. Given that Sb(V) is no longer a first-choice treatment, future research is likely to focus on other chemotherapeutics, the efficacies of which will need to be carefully monitored. Such monitoring should continue to make use of meta-analyses to offer more highly powered studies where possible.

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Conceived and designed the experiments: JDJ, SCW; Performed the experiments: MJ, JDJ; Analysed the data: CMJ, JDJ; Wrote the manuscript: JDJ; Reviewed the manuscript: CMJ, SCW, JDJ.

COMPETING INTERESTS

The authors completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available upon request from the corresponding author), and declare no conflicts of interest.

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