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Haematological dynamics following treatment of visceral leishmaniasis: a protocol for systematic review and individual participant data (IPD) meta-analysis

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Complete List of Authors:	<p>Munir, Abdalla; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Dahal, Prabin; University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Kumar, Rishikesh; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Singh-Phulgenda, Sauman; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Siddiqui, Niyamat; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Naylor, Caitlin; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Wilson, James; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Buck, Gemma; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Rahi, Manju; Indian Council of Medical Research (ICMR)</p> <p>Alves, Fabiana; Drugs for Neglected Disease Initiative</p> <p>Malaviya, Paritosh; Banaras Hindu University, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences</p> <p>Sundar, Shyam; Banaras Hindu University, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences</p> <p>Ritmeijer, Koert; Médecins Sans Frontières</p> <p>Stepniowska, Kasia; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Pandey, Krishna; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Guérin, Philippe J.; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine</p> <p>Musa, Ahmed; University of Khartoum, Institute of Endemic Diseases</p>

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Keywords:	Anaemia < HAEMATOLOGY, EPIDEMIOLOGY, INFECTIOUS DISEASES, Tropical medicine < INFECTIOUS DISEASES

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Manuscripts

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3 1 Haematological dynamics following treatment of visceral leishmaniasis: a
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5 2 protocol for systematic review and individual participant data (IPD) meta-
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8 3 analysis
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10 4 Abdalla Munir^{1,2,3}, Prabin Dahal^{1,2§}, Rishikesh Kumar⁴, Sauman Singh-Phulgenda^{1,2}, Niyamat
11
12 5 Ali Siddiqui⁴, Caitlin Naylor^{1,2}, James Wilson^{1,2}, Gemma Buck^{1,2}, Manju Rahi⁵, Fabiana Alves⁶,
13
14 6 Paritosh Malaviya⁷, Shyam Sundar⁷, Koert Ritmeijer⁸, Kasia Stepniewska^{1,2}, Krishna Pandey⁴,
15
16 7 Phillipe J Guerin^{1,2§}, Ahmed Musa^{3§}
17
18
19
20
21 8

22 9 ¹Infectious Diseases Data Observatory (IDDO), Oxford, UK

23
24 10 ²Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine,
25
26 11 University of Oxford, Oxford, UK

27
28 12 ³Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan

29
30 13 ⁴Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, India

31
32 14 ⁵Indian Council of Medical Research (ICMR), New Delhi, India

33
34 15 ⁶Drugs for Neglected Diseases initiative, Geneva, Switzerland

35
36 16 ⁷Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical
37
38 17 Sciences, Banaras Hindu University, Varanasi, India

39
40 18 ⁸Médecins Sans Frontières, Amsterdam, Netherlands
41
42
43
44

45 21 [§]Correspondence: prabin.dahal@iddo.org, musaam2003@yahoo.co.uk
46
47
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53
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55
56
57
58
59
60

Authors' email:

22
23 AM abdalla.munir@iddo.org
24 PD prabin.dahal@iddo.org
25 RK rishikeshkumar@live.in
26 SSP sauman.singh@iddo.org
27 NAS niyamatalisiddiqui@yahoo.com
28 CN caitlin.naylor@iddo.org
29 JW james.wilson@iddo.org
30 GB gemma.buck@iddo.org
31 MR drmanjurahi@gmail.com
32 PM paritosh_malaviya@yahoo.com
33 FA falves@dndi.org
34 SS drshyamsundar@hotmail.com
35 KR koert.ritmeijer@amsterdam.msf.org
36 KS kasia.stepniewska@iddo.org
37 PJG philippe.guerin@iddo.org
38 KP drkrishnapandey@yahoo.com

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42 **Abstract**

43
44 **Introduction:** Visceral leishmaniasis (VL) is a parasitic disease with an estimated 50,000 to
45 90,000 new cases occurring annually. Anaemia is the most common haematological
46 manifestation of visceral leishmaniasis (VL). However, the evolution of different
47 haematological characteristics following treatment remains poorly understood. An
48 individual participant data meta-analysis (IPD-MA) is planned to characterise the
49 haematological dynamics in VL patients.

50
51 **Methods and analysis:** The Infectious Diseases Data Observatory (IDDO) VL data platform is
52 a global repository of IPD from therapeutic studies identified through a systematic search of
53 published literature (PROSPERO registration: CRD42021284622). The platform currently
54 holds datasets from clinical trials standardised to a common data format. Contacting
55 authors and principal investigators of the studies indexed in the IDDO VL data platform
56 meeting the eligibility criteria for inclusion in this IPD-MA were invited to be part of the
57 collaborative IPD-MA. Mixed effects multivariable regression models will be constructed to
58 identify determinants of haematological parameters by taking clustering within study-sites
59 into account.

60
61 **Ethics and dissemination:** The results of this analysis will be disseminated at conferences,
62 IDDO website and any peer-reviewed publication arising will be made open source. Findings
63 of this research will be critically important for the control programmes at regional/global
64 levels, policy makers and groups developing new VL treatment.

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3 66 **PROSPERO registration:** The systematic review component of the IPD-MA has the following
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6 67 PROSPERO registration: CRD42021284622.
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11 69 **Keywords**
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13 70 Visceral Leishmaniasis; Kala-azar; Statistical Analysis Plan, meta-analysis, individual
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15 71 participant data; anaemia, haemoglobin; platelets, white blood cells, red blood cells
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20 73 **Word count: 2841 (main text); abstract (220)**
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3 77 ***Strengths and limitations of this study***
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- 6 78 • Visceral Leishmaniasis clinical trials are relatively small, which limits the ability to
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9 79 identify predictors associated with it any clinical outcomes in any single study. An
10
11 80 individual participant data (IPD-MA) will increase the statistical power to detect the
12
13 81 predictors and moderators of associated with evolution of haemoglobin.
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16 82
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18 83 • The identification of studies eligible for inclusion in the IPD-MA has been made through
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21 84 a comprehensive literature search of all published studies since 1980 with pre-defined
22
23 85 inclusion-exclusion criteria.
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28 87 • This IPD-MA will utilise the VL repository of individual participant data hosted by
29
30
31 88 Infectious Diseases Data Observatory (IDDO VL data platform). A major strength of this
32
33 89 study is that data on the IDDO VL data platform is harmonised to a common standard
34
35 90 based on an extensive consultation with the VL research community.
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40 92 • Retrieval of data from trials published prior to 2000 can be a major challenge.
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93 Introduction

94 Anaemia is the most common haematological manifestation of Visceral Leishmaniasis
95 (VL)[1]. In patients with VL, anaemia can arise due to one or more associated factors:
96 sequestration and haemolysis of red blood cells in the spleen associated with hypersplenism,
97 bone marrow suppression caused by nutritional deficiencies such as iron, vitamin B12 and
98 folate deficiencies, or due to clotting dysfunction leading to blood loss [1–5]. At the time of
99 clinical diagnosis, haemoglobin levels are often around 7–10 g/dL but can be as low as 4 g/dL
100 [1]. The severity of anaemia depends on the duration of clinical illness and can be exacerbated
101 by comorbidities and iron deficiency [6,7].

102 After treatment with an antileishmanial drug, haematological improvement generally
103 occurs within a few weeks with significant recovery expected within 4–6 weeks [3]. The
104 trajectory of haematological recovery may be affected by patients' age or initial parasite load
105 [8], but the influence of drug regimen and other patient characteristics remains poorly
106 understood. To characterise the haematological changes in VL after treatment, and
107 understand the role of host, parasite and drug related characteristics, we propose to
108 undertake an individual participant data meta-analysis (IPD-MA) of therapeutic studies.

109 Objectives

110 The objectives of this IPD-MA are:

- 111 1. To identify the determinants of haemoglobin concentration at enrolment, at initial
112 cure assessment and at definitive cure assessment
- 113 2. To characterise the haemoglobin dynamics following treatment
- 114 3. To identify the determinants of anaemia or severe anaemia at enrolment, at initial
115 cure assessment and at definitive cure assessment

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3 116 4. To describe white blood cells and platelets dynamics following treatment (data
4
5
6 117 permitting)
7
8 118 5. To identify predictors of blood transfusion during treatment or follow-up (if
9
10 119 available)

120 **Methods and analysis: Patients, interventions and outcomes**

121 **Elements of the research aim (PICOT)**

122 *Population:* Any patient enrolled in an interventional study with a confirmed or suspected
123 diagnosis of VL defined by serological and/or parasitological testing.

124 *Interventions:* Any antileishmanial therapy

125 *Comparator:* Not restricted by the use of a comparator drug

126 *Outcome:* At least one of the following outcomes reported: anaemia (or haemoglobin
127 measurements) at enrolment, anaemia and other haematological measurements at any
128 post-baseline time-points

129 *Time:* Studies published on or after 1980

131 **Criteria for study eligibility**

132 Studies in the IDDO VL data platform [9] will be deemed eligible for the purpose of this
133 analysis if they meet the following criteria:

- 134 • Prospective clinical efficacy studies on patients with confirmed or suspected VL
135 either using microscopy/serology/molecular method (i.e. clinical diagnosis followed
136 by a confirmatory method)

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3 137 • Information is available on treatment regimen including drug, dose, and duration
4
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6 138 • Data on anaemia (or either haemoglobin or haematocrit measurements) measured
7
8 139 at enrolment
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11 140 ***Desirable criteria***
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- 13
14 141 • Methodology used for haematological quantification, e.g. name of devices
15
16 142 • Information regarding if blood transfusion were given before or after treatment
17
18 143 initiation
19
20
21 144 • Information of co-administration of iron supplement
22
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26
27 146 ***Criteria for participant eligibility***
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29
30 147 The following minimum information are required for participants from each of the identified
31
32 148 studies for inclusion in the IPD-MA analysis:
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36 149 • Details of antileishmanial treatment(s) administered
37
38 150 • Baseline information on age and gender
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41 151 • Outcome is recorded
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47 153 **Outcomes**
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50 154 ***Outcomes and definitions:***
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53 155 **Primary outcome:** The primary endpoint is the haemoglobin concentration measured at any
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55 156 time-period during the treatment and follow-up phase
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57

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59 157 **Secondary outcomes:** The following endpoints are identified as secondary
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3 158 • Anaemia and severe anaemia at baseline, at the time of initial cure assessment and
4
5
6 159 at the time of definitive cure assessment
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8 160 • White blood cells (WBC) at baseline and at any time during the treatment and
9
10
11 161 follow-up
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13 162 • Platelets count at baseline and at any time during the treatment and follow-up
14

15 163 • Requirement of blood transfusion during treatment or follow-up (if available)
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18
19 164 Anaemia will be defined following the WHO guidelines [10]. The timing of initial cure
20
21 165 assessment would typically take place within 28 days of treatment completion but this
22
23 166 varies slightly across studies; assessments undertaken between 15 to 60 days will be
24
25
26 167 considered as time of initial cure assessment. Similarly, the timing of definitive cure
27
28 168 assessment will vary according to study design and hence assessments made between 5 to 7
29
30
31 169 months will be considered as 6 months.
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37 171 **Statistical methods**

38 39 40 172 **Identification of relevant studies**

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43 173 We searched all the articles indexed in the open-access Infectious Diseases Data
44
45 174 Observatory (IDDO) visceral leishmaniasis clinical trials library [11]. The IDDO VL clinical
46
47
48 175 trials library is based on a living systematic review and the database is continually updated
49
50 176 every six months in accordance with the Preferred Reporting Items for Systematic-Reviews
51
52 177 and Meta-Analyses (PRISMA) guidelines [12]. The trial library indexes publications identified
53
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55 178 from the following databases: PubMed, Embase, Scopus, Web of Science, Cochrane,
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58 179 clinicaltrials.gov, WHO ICTRP, Global Index Medicus, IMEMR, IMSEAR, and LILACS. For this
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3 180 current review, the search includes all clinical trials published between 1st of Jan 1980 and
4
5
6 181 2nd of May 2021. Details of the search strategy adopted is described elsewhere [12]. Studies
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8 182 indexed in the IDDO VL library will be eligible for inclusion in this review if they meet the
9
10 183 inclusion and exclusion criteria outlined above. This review is not limited by language.

13 14 184 **Collating IPD**

15
16 185 Principal investigators and the corresponding authors of the eligible studies
17
18 186 identified from IDDO VL LSR were invited to share IPD. At least two emails will be sent out in
19
20
21 187 case of non-response. Researchers agreeing to the terms and conditions of the submission
22
23 188 were invited to upload anonymised IPD to the IDDO repository through a secure web portal
24
25
26 189 [9]. Data in the IDDO VL platform are fully pseudonymised to protect personal information
27
28 190 and patient privacy.

30 31 191 **Data management**

32
33 192 Raw data from individual studies shared with IDDO are currently being standardised
34
35
36 193 using the Clinical Data Interchange Standards Consortium (CDISC) compliant curation
37
38 194 standards [13]. Investigators will be further contacted for validation or clarification, if
39
40
41 195 required, and individual study protocols will be requested. On standardisation, the data will
42
43 196 be stored in a relational database of several tables containing information on drug regimen,
44
45
46 197 parasitological, clinical, and haematological assessments and therapeutic outcomes.

198 **Statistical methods for analysis of primary and secondary outcomes**

199 **Descriptive summary of the studies included**

200 Summary of included studies will be presented with respect to study location, years
201 of study, study population, duration of follow-up, drug regimen, methodology for diagnosis,
202 supervision of drug administration or treatment adherence (if available), methods and
203 devices used for haematology measurements.

204 **Summary of the participants included**

205 Summary of baseline characteristics of the participants included in the analysis will
206 be presented for each study, by region and overall. The following will be presented: age;
207 weight, parasite grade on enrolment, presence of fever (body temperature > 37.5 degrees
208 Celsius), haemoglobin (or haematocrit), anaemia or severe anaemia as defined using the
209 WHO definitions [10], spleen size, treatment, total mg/kg dose, and supervision of drug
210 administration. The number of available patients will be summarised for all variables,
211 proportions will be used for categorical or binary variables, and mean and standard
212 deviation (or median and interquartile range) will be used for continuous variables.

214 **Analysis of the primary endpoint**

215 Separate linear mixed effect regression models will be undertaken to identify
216 predictors of haemoglobin concentration at baseline, at the time of initial cure assessment
217 and at the time of assessment of definitive cure in a one-stage IPD meta-analysis. The
218 regression models constructed at the time of initial and final cure assessments will adjust for
219 the baseline measurements of haemoglobin as covariates along with the drug regimen.

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3 220 If repeated haemoglobin measurements are available for more than 3 time points, the
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6 221 longitudinal haemoglobin profile will be characterised using a linear mixed effects regression
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8 222 model by considering time as a continuous variable. Fractional polynomials will be used to
9
10 223 explore any non-linear relationship in the evolution of haemoglobin concentration. If there
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12
13 224 are few numbers of time-points, then time will be considered as a discrete variable (three
14
15 225 time-points: baseline, day 30, and day 180) in the regression model.
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18 226 The difference between haemoglobin concentration at baseline and at the time of
19
20 227 evaluation of definitive cure status (usually at day 180) will be used to quantify the absolute
21
22
23 228 mean disease attributed haematological fall. The difference between haemoglobin
24
25
26 229 concentration at baseline and at the end of the active treatment phase (day 30) will be used
27
28 230 to characterise if there is any drug attributed haematological fall.
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31 231 Further description of candidate predictors and multivariable modelling is described
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34 232 in next.
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37 233 **Candidate predictors and core set**

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40 234 The following variables will be considered for inclusion in the analysis of primary and
41
42 235 secondary endpoints.
43
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45 236 The following host variables are considered: age, gender, body weight, nutritional status,
46
47 237 comorbidity status (such as HIV), and duration of illness prior to study enrolment.
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50 238 Nutritional status in children under 5 years of age will be assessed using standardised age,
51
52 239 weight, height and gender specific growth reference standards according to the WHO 2006
53
54 240 recommendations using igrowup Stata package [14] (or equivalent library will in R be used).
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56

57 241 Anthropometric indicators include weight-for-age (WAZ), height-for-age (HAZ), and weight-
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3 242 for-height (WHZ). The nutritional status of a child will be given as a Z-score and classified as
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6 243 stunted, underweight or wasted as defined in the WHO guidelines [14].
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9 244 The following parasite related baseline factors will be considered: parasite load and
10
11 245 information regarding the nature of infection (primary vs. previously treated cases). Any
12
13 246 cases described as previously untreated (or “fresh”) cases for leishmaniasis will be
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16 247 considered as primary VL.
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19 248 The following drug related variables will be considered for inclusion in analysis: drug
20
21 249 regimen, mg/kg total dose (or target dose), concomitant infections.
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25 250 The following study or arm level variables will be considered in the analysis of primary and
26
27 251 secondary endpoints: geographical region, country, study site, and calendar year of the
28
29 252 study conduct.
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33 253 The following covariates will be examined in the regression model and considered as core
34
35 254 predictor set: age, sex, baseline parasite density, HIV co-infection, and geographical region,
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37 255 baseline haematological measurements. These variables along with the drug regimen will
38
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40 256 form the minimal adjustment set for assessment of other risk factors and will be kept in the
41
42 257 regression model regardless of statistical significance [15].
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45 258 **Considerations for multivariable model construction**

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49 259 Multivariable model construction will follow the recommendations of Heinze et al (2017)
50
51 260 [16]. Nested models will be compared by assessing the change in log-likelihood estimates
52
53 261 and Akaike’s information criterion (AIC) will be used for comparing competing non-nested
54
55
56 262 models. The functional form of the continuous variables will be determined using
57
58 263 multivariable fractional polynomials [17] or restricted cubic splines [15]. Stability
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3 264 investigations will be undertaken to account for uncertainty introduced in multivariable
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6 265 modelling through bootstrap resampling [16].
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8 9 266 **Handling missing data**

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12 267 To assess the impact of missing data, sensitivity analysis will be performed to see if
13
14 268 the main conclusions are affected or not by the exclusion of patients with missing data using
15
16 269 multiple imputation [18]. The imputation model will include all the variables in the target
17
18 270 analysis and additional auxiliary variables. The target analysis will be carried out in each of
19
20 271 the completed (observed plus imputed values) datasets and the estimates will be combined
21
22 272 across the imputed datasets using Rubin's combination rules [18].
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27 273 **Sensitivity analyses**

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29 274 Two sensitivity analyses will be carried using resampling techniques to assess model
30
31 275 stability. Bootstrap resampling will be used to assess the robustness of the derived
32
33 276 estimates and its variance using the recommendations in Heinze et al (2017) [16]. In the
34
35 277 second analysis, model will be refitted with one study excluded at a time, and a coefficient
36
37 278 of variation around the parameter estimates will be calculated. This would identify any
38
39 279 influential studies, that is, studies with unusual results (due to variations in methodology,
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41 280 patient population, and so on) that affect the overall pooled analysis findings.
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281 **Analysis of secondary endpoints**

282 **Anaemia and severe anaemia at baseline, at test of initial cure and at end of the study** 283 **follow-up**

284 A mixed effects logistic regression model will be constructed to identify the
285 predictors associated with anaemia (or severe anaemia) at baseline using a one-stage IPD-
286 MA. Random effects for the study sites will be used to adjust for study-site effect [19].
287 Potential non-linear relationships between continuous predictors and the outcome will be
288 investigated using multivariable fractional polynomials [20]. Similar analysis will be
289 undertaken for anaemia (or severe anaemia) at the time of initial cure, and at the end of the
290 study follow-up. Multivariable model construction will be undertaken as outlined for the
291 primary endpoint.

292 **WBC and Platelets (if data are available)**

293 The distribution of WBC and platelets will be summarised at baseline, at the time of
294 initial cure assessment and at the time of the definitive cure assessment. If data are
295 available, predictors associated with WBC (or platelet counts) will be carried out using a
296 mixed effects linear regression (appropriate transformation will be used if distribution is
297 skewed). Statistical modelling will be undertaken as for the primary endpoint.

298 **Blood transfusion during the study follow-up (if data are available)**

299 Predictors associated with requirement of blood transfusion at any stage during the
300 study period will be identified (if data are available) using a mixed effects logistic regression.
301 Multivariable regression modelling will be undertaken as described for the other endpoints.

302

303 **Subgroup analyses**

304 The following two sub-group analyses are planned. Patients living with HIV who are treated
305 for VL typically have worse outcomes and higher mortality risk than those who are not living
306 with HIV [21,22]. A separate sub-group analysis will be carried out among patients with
307 defined VL-HIV coinfections (data permitting). There is a known regional variation in
308 treatment response [23]; a separate analysis could be undertaken for each of the
309 geographical regions.

310 **Risk of bias assessment in included studies**

311 To examine the risk of bias in IPD-MA, the first four domains of the quality In
312 prognosis studies (QUIPS) tool and the first three domains of the prediction model risk of
313 bias assessment tool (PROBAST) will be considered as recommended in Riley et al (2021)
314 [15]. The relevant domains from the QUIPS checklist are study participation, study attrition,
315 prognostic factor measurement, and outcome measurement, and the relevant domains
316 from PROBAST checklist are participant selection, prognostic factors, and outcomes. Two
317 reviewers will independently assess the risk of bias in the studies included in the analysis.

318 Risk of bias results will be incorporated into analyses by conducting subgroup
319 analyses among studies with overall low risk of bias or by conducting formal interaction
320 analyses with risk of bias responses [24].

321 **Assessment of risk of potential bias in missing studies**

322 Despite best possible efforts, it is anticipated that raw data from all the identified
323 studies will not be available. The characteristics of patient population and study meta-data
324 from the missing studies will be summarised to explore if the missing studies are

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3 325 systematically different from the studies that are included in the meta-analysis. A two-stage
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5 326 IPD may be conducted if sufficient details (or any covariate adjustment) are reported in the
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8 327 original studies.
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10 11 328 **Further development of statistical analysis plan** 12

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14 329 The main analysis is planned as described above. Modification or additional analyses
15
16 330 may be required as the data collection progresses. An updated statistical analysis plan will
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19 331 be available on the IDDO study group website [25].
20

21 22 332 **Software** 23

24
25 333 All the analysis will be carried out using R software or Stata 17 software [26,27]. Use
26
27
28 334 of any other data analysis tools will not change the statistical analysis plan.
29

30 31 335 **Ethics and dissemination** 32

33
34 336 This IPD-MA meets the criteria for waiver of ethical review as defined by the Oxford
35
36 337 Tropical Research Ethics Committee (OxTREC) granted to IDDO, as the research consists of
37
38
39 338 secondary analysis of existing anonymised data. Ethical approval was granted to each study
40
41 339 included in this pooled analysis by their respective ethics committees. This IPD-MA will
42
43 340 address research questions similar to that of included studies. Findings of this IPD-MA will
44
45 341 be reported in open-access, peer-reviewed journals following the PRISMA-IPD guidelines
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48 342 [28].
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50 51 52 343 **Patient and public involvement** 53

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55 344 The research questions considered in this IPD-MA is based on a research agenda
56
57 345 developed by the global VL research community [29]. The design and development of this
58
59
60 346 IPD-MA were done by the study authors only and no patient was involved at any stage.

347 Discussion

348 Anaemia is the most common haematological manifestation of visceral leishmaniasis
349 (VL). Despite this, the impact of treatment on different haematological parameters remains
350 to be fully understood. The aim of this IPD meta-analysis is to explore the dynamics of
351 different haematological parameters during the treatment and the convalescence phase of
352 the disease. IPD-MA is being used increasingly to explore factors affecting treatment
353 outcomes which otherwise would not be possible through standard aggregate data meta-
354 analysis [30].

355 This pooled analysis will provide critical information regarding the trajectory of
356 haematological recovery among VL patients. The assessment of the host, parasite and drug
357 determinants that influence the haematological response can provide evidence-based
358 guidance for optimal case management and monitoring drug safety. The IDDO VL LSR, which
359 is a comprehensive library of all published studies since 1980 has been used for
360 identification of the studies eligible for this IPD-MA. A major strength of this study is that
361 data from several studies will be harmonised to a common standard based on an extensive
362 consultation with the VL research community [13], thus allowing us to address some of the
363 methodological sources of heterogeneity. A major challenge is that a substantial proportion
364 of the studies in the IDDO library were conducted prior to the year 2000; the retrieval of
365 data from historical trials is a major challenge.

366 This IPD-MA will characterise the haematological profile of VL patients at enrolment
367 and at the time-points of initial and definitive cure assessments. Findings of this research
368 will generate important information regarding the evolution of different haematological
369 characteristics.

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3 370 **Authors' contributions**
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6 371 All authors listed were responsible for the study conceptualisation. AM, PD, PJG, AM, KS
7
8 372 wrote the first draft of the manuscript. All authors were involved in reading, revising it
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11 373 critically, editing and approving the final manuscript.
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14 374
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19

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21
22
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25

26 378 **Conflict of Interest**
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28

29 379 None
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32 380 **Data Availability**
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35 381 N/A as this is an analysis protocol.
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Haematological dynamics following treatment of visceral leishmaniasis: a protocol for systematic review and individual participant data (IPD) meta-analysis

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Complete List of Authors:	<p>Munir, Abdalla; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Dahal, Prabin; University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Kumar, Rishikesh; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Singh-Phulgenda, Sauman; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Siddiqui, Niyamat; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Naylor, Caitlin; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Wilson, James; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Buck, Gemma; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Rahi, Manju; Indian Council of Medical Research (ICMR)</p> <p>Alves, Fabiana; Drugs for Neglected Disease Initiative</p> <p>Malaviya, Paritosh; Banaras Hindu University, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences</p> <p>Sundar, Shyam; Banaras Hindu University, Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences</p> <p>Ritmeijer, Koert; Médecins Sans Frontières</p> <p>Stepniewska, Kasia; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Pandey, Krishna; Rajendra Memorial Research Institute of Medical Sciences (RMRIMS)</p> <p>Guérin, Philippe J.; Infectious Diseases Data Observatory (IDDO); University of Oxford, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine</p> <p>Musa, Ahmed; University of Khartoum, Institute of Endemic Diseases</p>

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Secondary Subject Heading:	Public health
Keywords:	Anaemia < HAEMATOLOGY, EPIDEMIOLOGY, INFECTIOUS DISEASES, Tropical medicine < INFECTIOUS DISEASES



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3 1 Haematological dynamics following treatment of visceral leishmaniasis: a
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6 2 protocol for systematic review and individual participant data (IPD) meta-
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10 4 Abdalla Munir^{1,2,3}, Prabin Dahal^{1,2§}, Rishikesh Kumar⁴, Sauman Singh-Phulgenda^{1,2}, Niyamat
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12 5 Ali Siddiqui⁴, Caitlin Naylor^{1,2}, James Wilson^{1,2}, Gemma Buck^{1,2}, Manju Rahi⁵, Fabiana Alves⁶,
13
14 6 Paritosh Malaviya⁷, Shyam Sundar⁷, Koert Ritmeijer⁸, Kasia Stepniewska^{1,2}, Krishna Pandey⁴,
15
16 7 Phillipe J Guerin^{1,2§}, Ahmed Musa^{3§}
17
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21 8

22 9 ¹Infectious Diseases Data Observatory (IDDO), Oxford, UK

23
24 10 ²Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine,
25
26 11 University of Oxford, Oxford, UK

27
28 12 ³Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan

29
30 13 ⁴Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna, India

31
32 14 ⁵Indian Council of Medical Research (ICMR), New Delhi, India

33
34 15 ⁶Drugs for Neglected Diseases initiative, Geneva, Switzerland

35
36 16 ⁷Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical
37
38 17 Sciences, Banaras Hindu University, Varanasi, India

39
40 18 ⁸Médecins Sans Frontières, Amsterdam, The Netherlands
41
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44

45 21 [§]Correspondence: prabin.dahal@ndm.ox.ac.uk & musaam2003@yahoo.co.uk
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22 **Authors' email:**

23 AbM abdalla.munir@iddo.org
24 PD prabin.dahal@iddo.org
25 RK rishikeshkumar@live.in
26 SSP sauman.singh@iddo.org
27 NAS niyamatalisiddiqui@yahoo.com
28 CN caitlin.naylor@iddo.org
29 JW james.wilson@iddo.org
30 GB gemma.buck@iddo.org
31 MR drmanjurahi@gmail.com
32 FA falves@dndi.org
33 PM paritosh_malaviya@yahoo.com
34 SS drshyamsundar@hotmail.com
35 KR koert.ritmeijer@amsterdam.msf.org
36 KS kasia.stepniewska@iddo.org
37 KP drkrishnapandey@yahoo.com
38 PJG philippe.guerin@iddo.org
39 AhM musaam2003@yahoo.co.uk

43 **Abstract**

44
45 **Introduction:** Visceral leishmaniasis (VL) is a parasitic disease with an estimated 30,000 new
46 cases occurring annually. Despite anaemia being a common haematological manifestation of
47 VL, the evolution of different haematological characteristics following treatment remains
48 poorly understood. An individual participant data meta-analysis (IPD-MA) is planned to
49 characterise the haematological dynamics in VL patients.

50
51 **Methods and analysis:** The Infectious Diseases Data Observatory (IDDO) VL data platform is
52 a global repository of individual participant data from therapeutic studies identified through
53 a systematic search of published literature (PROSPERO registration: CRD42021284622). The
54 platform currently holds datasets from clinical trials standardised to a common data format.
55 Corresponding authors and principal investigators of the studies indexed in the IDDO VL
56 data platform meeting the eligibility criteria for inclusion in this IPD-MA were invited to be
57 part of the collaborative IPD-MA. Mixed effects multivariable regression models will be
58 constructed to identify determinants of haematological parameters by taking clustering
59 within study-sites into account.

60
61 **Ethics and dissemination:** This IPD-MA meets the criteria for waiver of ethical review as
62 defined by the Oxford Tropical Research Ethics Committee (OxTREC) granted to IDDO, as the
63 research consists of secondary analysis of existing anonymised data (exempt granted on
64 29th March 2023, OxTREC REF: Infectious Diseases Data Observatory (IDDO)). Ethics
65 approval was granted by the ICMR-Rajendra Memorial Research Institute of Medical
66 Sciences ethics committee (Letter no: RMRI/EC/30/2022) on 04-07-2022. The results of this

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3 67 analysis will be disseminated at conferences, IDDO website and peer-reviewed publication
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6 68 in open-access journals. Findings of this research will be critically important for the control
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8 69 programmes at regional/global levels, policy makers and groups developing new VL
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10 70 treatment.

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15 72 **PROSPERO registration:** The systematic review component of the IPD-MA has the following
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17 73 PROSPERO registration: CRD42021284622.
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23 75 **Keywords**

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25 76 Visceral Leishmaniasis; Kala-azar; Statistical Analysis Plan, meta-analysis, individual
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27 77 participant data; IPD-MA; anaemia, haemoglobin; platelets, white blood cells, red blood
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29 78 cells
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35 80 **Word count: 2841 (main text); abstract (265)**
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3 81 ***Strengths and limitations of this study***
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- 6 82 • An individual participant data meta-analysis (IPD-MA) is proposed to characterise the
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8 evolution of haemoglobin and other haematological parameters during the study period;
9 83
10 this will overcome the limitations due to sample size issues.
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16 86 • The identification of studies has been made through a comprehensive literature search
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18 of all published studies since 1980 with a pre-defined inclusion-exclusion criterion.
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23 89 • A major strength of this IPD-MA is that it uses individual participant data hosted at
24
25 Infectious Diseases Data Observatory which has harmonised raw data to a common
26 90
27 standard based on an extensive consultation with the VL research community.
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33 93 • Retrieval of data from trials published prior to 2000 can be a major challenge.
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94 Introduction

95 Anaemia is a common haematological manifestation of Visceral Leishmaniasis (VL).[1]
96 In patients with VL, anaemia can arise due to one or more associated factors: sequestration
97 and haemolysis of red blood cells in the spleen associated with hypersplenism, bone marrow
98 suppression caused by nutritional deficiencies such as iron, vitamin B12 and folate
99 deficiencies, or due to clotting dysfunction leading to blood loss [1–5]. At the time of clinical
100 diagnosis, haemoglobin levels are often around 7–10 g/dL but can be as low as 4 g/dL.[1] The
101 severity of anaemia depends on the duration of clinical illness and can be exacerbated by
102 comorbidities and iron deficiency [6, 7].

103 After treatment with an antileishmanial drug, haematological improvement generally
104 occurs within a few weeks with significant recovery expected within 4–6 weeks [3]. The
105 trajectory of haematological recovery may be affected by patients' age or initial parasite load
106 [8], but the influence of drug regimen and other patient characteristics remains poorly
107 understood. Haematological safety of the VL treatment remains an important concern [9].
108 Therefore, characterisation of haematological profile and identification of drivers associated
109 with haematological recovery can help in optimal case management. The present individual
110 participant data meta-analysis (IPD-MA) aimed to characterise the haematological changes in
111 VL patients after treatment, and understand the role of host, parasite and drug related
112 characteristics.

113 Objectives

114 The objectives of this IPD-MA are:

- 115 1. To identify the determinants of haemoglobin concentration at enrolment, at initial
116 cure assessment and at definitive cure assessment

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3 117 2. To characterise the haemoglobin dynamics following treatment
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6 118 3. To identify the determinants of anaemia or severe anaemia at enrolment, at initial
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8 119 cure assessment and at definitive cure assessment
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10 120 4. To describe white blood cells and platelets dynamics following treatment (data
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13 121 permitting)
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15 122 5. To identify predictors of blood transfusion during treatment or follow-up (if
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17
18 123 available)

21 124 **Methods and analysis: Patients, interventions and outcomes**

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24 125 **Elements of the research aim (PICOT)**

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27 126 *Population:* Any patient enrolled in a prospective efficacy studies with a confirmed or
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29 127 suspected diagnosis of VL defined by serological and/or parasitological testing.

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32 128 *Interventions:* Any antileishmanial therapy.

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35 129 *Comparator:* Not restricted by the use of a comparator drug.

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38 130 *Outcome:* At least one of the following outcomes reported: anaemia (or haemoglobin
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41 131 measurements) at enrolment, anaemia and other haematological measurements at any
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43 132 post-baseline time-points.

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46 133 *Time:* Studies published on or after 1980.

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52 135 **Criteria for study eligibility**

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55 136 Studies in the IDDO VL data platform [10] will be deemed eligible for the purpose of this IPD-
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57 137 MA if they meet the following criteria:
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3 138 • Prospective efficacy studies on patients with confirmed or suspected VL either using
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5 139 microscopy/serology/molecular method (i.e. clinical diagnosis followed by a
6
7 140 confirmatory method)
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10 141 • Information is available on treatment regimen including drug, dose, and duration of
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12 142 regimen
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14 143 • Data on anaemia (or haemoglobin/haematocrit concentration) measured at
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16 144 enrolment
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21 145 ***Desirable criteria***

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23 146 • Methodology used for haematological quantification, e.g. name of device used
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25 147 • Information regarding if blood transfusion were given before or after treatment
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27 148 initiation
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29 149 • Information on co-administration of iron supplement
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34 150 ***Criteria for participant eligibility***

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37 151 The minimum information required for participants from each of the identified studies for
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39 152 inclusion in the IPD-MA analysis are listed below:
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43 153 • Details of antileishmanial treatment(s) administered
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45 154 • Baseline information on age and gender
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47 155 • Outcome is recorded
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53 157 **Outcomes**

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56 158 ***Outcomes and definitions:***
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3 159 **Primary outcome:** The primary outcome is the haemoglobin concentration measured at any
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6 160 time-period during the treatment and follow-up phase.
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9 161 **Secondary outcomes:** The following endpoints are identified as secondary:
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- 11
12 162 • Anaemia and severe anaemia at baseline, at the time of initial cure assessment and
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14 163 at the time of definitive cure assessment
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17 164 • White blood cells (WBC) at baseline and at any time during the treatment and
18
19 165 follow-up
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22 166 • Platelets count at baseline and at any time during the treatment and follow-up
23
24 167 • Requirement of blood transfusion during treatment or follow-up (if available)
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27 168 Anaemia will be defined following the WHO guidelines [11]. The timing of initial cure
28
29 169 assessment would typically take place within 28 days of treatment completion but this
30
31 170 varies slightly across studies; assessments undertaken between 15 to 60 days will be
32
33 171 considered as time of initial cure assessment. Similarly, the timing of definitive cure
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35 172 assessment will vary according to study design and hence assessments made between 5 to 7
36
37 173 months will be considered as 6 months.
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45 175 **Statistical methods**

48 176 **Identification of relevant studies using IDDO VL library**

51 177 We searched all the articles indexed in the open-access Infectious Diseases Data
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53 178 Observatory visceral leishmaniasis clinical trials library (IDDO VL library).[12] The IDDO VL
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55 179 library is continually updated and follows the Preferred Reporting Items for Systematic-
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58 180 Reviews and Meta-Analyses (PRISMA) guidelines [13]. The IDDO VL library indexes
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3 181 publications identified from the following databases: PubMed, Embase, Scopus, Web of
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5 182 Science, Cochrane, clinicaltrials.gov, WHO ICTRP, Global Index Medicus, IMEMR, IMSEAR,
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8 183 and LILACS. For this current review, the search includes all clinical trials published between
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10 184 1st of Jan 1980 and 2nd of May 2021. Details of the search strategy adopted is described
11
12
13 185 elsewhere [13]. The search details are presented in online supplemental file 1. Studies
14
15 186 indexed in the IDDO VL library will be eligible for inclusion in this review if they meet the
16
17
18 187 inclusion and exclusion criteria outlined above. This review is not limited by language.
19
20

21 188 **Collating IPD: IDDO VL data platform**

22
23 189 Principal investigators and the corresponding authors of the eligible studies
24
25
26 190 identified from IDDO VL library were invited to share IPD. At least two emails will be sent
27
28 191 out in case of non-response. Researchers agreeing to the terms and conditions of the
29
30 192 submission were invited to upload anonymised IPD to the IDDO repository through a secure
31
32
33 193 web portal [10]. Data in the IDDO VL platform are fully pseudonymised to protect personal
34
35 194 information and patient privacy.
36
37

38 195 **Data management**

39
40
41 196 Raw data from individual studies shared with IDDO are currently being standardised
42
43 197 using the Clinical Data Interchange Standards Consortium (CDISC) compliant curation
44
45
46 198 standards [14]. Investigators will be further contacted for validation or clarification, if
47
48 199 required, and individual study protocols will be requested. On standardisation, the data will
49
50
51 200 be stored in a relational database of several tables containing information on drug regimen,
52
53 201 parasitological, clinical, and haematological assessments and therapeutic outcomes.
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202 **Statistical methods for analysis of primary and secondary outcomes**

203 **Descriptive summary of the studies included**

204 Summary of included studies will be presented with respect to study location, years
205 of study, study population, duration of follow-up, drug regimen, methodology for diagnosis,
206 supervision of drug administration or treatment adherence (if available), methods and
207 devices used for haematology measurements.

208 **Summary of the participants included**

209 Summary of baseline characteristics of the participants included in the analysis will
210 be presented for each study, by region and overall. The following will be presented: age;
211 weight, parasite grade on enrolment, presence of fever (body temperature > 37.5 degrees
212 Celsius), haemoglobin (or haematocrit), anaemia or severe anaemia as defined using the
213 WHO definitions [11], spleen size, treatment, total mg/kg dose, and supervision of drug
214 administration. The number of available patients will be summarised for all variables,
215 proportions will be used for categorical or binary variables, and mean and standard
216 deviation (or median and interquartile range) will be used for continuous variables.

217 **Analysis of the primary endpoint**

218 Separate linear mixed effect regression models will be undertaken to identify
219 predictors of haemoglobin concentration at baseline, at the time of initial cure assessment
220 and at the time of assessment of definitive cure in a one-stage IPD meta-analysis. The
221 regression models constructed at the time of initial and final cure assessments will adjust for
222 the baseline measurements of haemoglobin as covariates along with the drug regimen.

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3 223 If repeated haemoglobin measurements are available for more than 3 time-points, the
4
5
6 224 longitudinal haemoglobin profile will be characterised using a linear mixed effects regression
7
8 225 model by considering time as a continuous variable. Fractional polynomials will be used to
9
10 226 explore any non-linear relationship in the evolution of haemoglobin concentration. If there
11
12
13 227 are few numbers of time-points, then time will be considered as a discrete variable (three
14
15 228 time-points: baseline, day 30, and day 180) in the regression model. If frequent data is
16
17
18 229 available, then the haemoglobin concentration will be summarised at weekly time-points
19
20 230 during the follow-up period.
21
22

23 231 The difference between haemoglobin concentration at baseline and at the time of
24
25 232 evaluation of definitive cure status (usually at day 180) will be used to quantify the absolute
26
27
28 233 mean disease attributed haematological fall. The difference between haemoglobin
29
30 234 concentration at baseline and at the end of the active treatment phase (day 30) will be used
31
32
33 235 to gauge if there is any drug attributed haematological fall.
34
35

36 236 Further description of candidate predictors and multivariable modelling is described
37
38
39 237 next.
40
41

42 238 **Candidate predictors and core set**

43
44

45 239 The following variables will be considered for inclusion in the analysis of primary and
46
47 240 secondary endpoints.
48
49

50 241 The following host variables are considered: age, gender, body weight, nutritional status,
51
52 242 comorbidity status (such as HIV), and duration of illness prior to study enrolment.
53
54

55 243 Nutritional status in children under 5 years of age will be assessed using standardised age,
56
57 244 weight, height and gender specific growth reference standards according to the WHO 2006
58
59
60

1
2
3 245 recommendations using igrowup Stata package (or equivalent library will in R be used) [15].
4

5 246 Anthropometric indicators include weight-for-age (WAZ), height-for-age (HAZ), and weight-
6
7

8 247 for-height (WHZ). The nutritional status of a child will be given as a Z-score and classified as
9

10 248 stunted, underweight or wasted as defined in the WHO guidelines [15].
11
12

13 249 The following parasite related baseline factors will be considered: parasite load and
14

15 250 information regarding the nature of infection (primary vs. previously treated cases). Any
16
17

18 251 cases described as previously untreated (or “fresh”) cases for leishmaniasis will be
19

20 252 considered as primary VL.
21
22

23 253 The following drug related variables will be considered for inclusion in analysis: drug
24

25 254 regimen, mg/kg total dose (or target dose), concomitant infections.
26
27

28 255 The following study or arm level variables will be considered in the analysis of primary and
29
30

31 256 secondary endpoints: geographical region, country, study site, and calendar year of the
32
33

34 257 study conduct.
35
36

37 258 The following covariates will be examined in the regression model and considered as core
38
39

40 259 predictor set: age, sex, baseline parasite density, HIV co-infection, geographical region,
41

42 260 baseline haematological measurements. These variables along with the drug regimen will
43
44

45 261 form the minimal adjustment set for assessment of other risk factors and will be kept in the
46
47

48 262 regression model regardless of statistical significance[16].
49

50 263 **Considerations for multivariable model construction**

51
52

53 264 Multivariable model construction will follow the recommendations of Heinze et al
54

55 265 (2017) [17]. Nested models will be compared by assessing the change in log-likelihood
56
57

58 266 estimates and Akaike’s information criterion (AIC) will be used for comparing competing
59
60

1
2
3 267 non-nested models. The functional form of the continuous variables will be determined
4
5
6 268 using multivariable fractional polynomials [18] or restricted cubic splines [16]. Stability
7
8 269 investigations will be undertaken to account for uncertainty introduced in multivariable
9
10 270 modelling through bootstrap resampling [17].
11
12

13 271 **Handling missing data**

14
15
16 272 To assess the impact of missing data, sensitivity analysis will be performed to see if
17
18 273 the main conclusions are affected by the exclusion of patients with missing data using
19
20 274 multiple imputation [19]. The imputation model will include all the variables in the target
21
22 275 analysis and additional auxiliary variables. The target analysis will be carried out in each of
23
24 276 the completed (observed plus imputed) datasets and the estimates will be combined across
25
26 277 the imputed datasets using Rubin's combination rules [19].
27
28
29
30
31

32 278 **Sensitivity analyses**

33
34 279 Two sensitivity analyses will be carried out using resampling techniques to assess
35
36 280 model stability. Bootstrap resampling will be used to assess the robustness of the derived
37
38 281 estimates and its variance using the recommendations in Heinze et al (2017) [17]. In the
39
40 282 second analysis, model will be refitted with one study excluded at a time, and a coefficient
41
42 283 of variation around the parameter estimates will be calculated. This would identify any
43
44 284 influential studies, that is, studies with unusual results (due to variations in underlying
45
46 285 methodology to measure outcomes, patient population, and so on) that affect the overall
47
48
49
50 286 pooled analysis findings.
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3 287 **Analysis of secondary endpoints**
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6 288 **Anaemia and severe anaemia at baseline, at test of initial cure and at end of the study**
7
8
9 289 **follow-up**
10

11
12 290 A mixed effects logistic regression model will be constructed to identify the
13
14 291 predictors associated with anaemia (or severe anaemia) at baseline using a one-stage IPD-
15
16
17 292 MA. Random effects for the study sites will be used to adjust for study-site effect [20].
18
19 293 Potential non-linear relationships between continuous predictors and the outcome will be
20
21
22 294 investigated using multivariable fractional polynomials [21]. Similar analysis will be
23
24 295 undertaken for anaemia (or severe anaemia) at the time of initial cure, and at the end of the
25
26
27 296 study follow-up. Multivariable model construction will be undertaken as outlined for the
28
29 297 primary endpoint.
30

31
32 298 **WBC and Platelets (if data are available)**
33
34

35 299 The distribution of WBC and platelets will be summarised at baseline, at the time of
36
37
38 300 initial cure assessment and at the time of the definitive cure assessment. If data are
39
40 301 available, predictors associated with WBC (or platelet counts) will be carried out using a
41
42
43 302 mixed effects linear regression (appropriate transformation will be used if distribution is
44
45 303 skewed). Statistical modelling will be undertaken as for the primary endpoint.
46
47

48 304 **Blood transfusion during the study follow-up (if data are available)**
49
50

51 305 Predictors associated with requirement of blood transfusion at any stage during the
52
53
54 306 study period will be identified (if data are available) using a mixed effects logistic regression.
55
56 307 Multivariable regression modelling will be undertaken as described for the other endpoints.
57
58

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309 **Subgroup analyses**

310 Patients living with HIV who are treated for VL typically have worse outcomes and
311 higher mortality risk than those who are not living with HIV [22]. A separate sub-group
312 analysis will be carried out among patients with defined VL-HIV coinfections (data
313 permitting). There is a known regional variation in treatment response in VL along with
314 differences in patient characteristics and treatment guidelines [23]. Therefore, a separate
315 analysis will be undertaken within each geographical region to construct the univariable and
316 multivariable regression models for the primary and secondary outcomes.

317 Longitudinal haemoglobin profile will be stratified by the transfusion status at any
318 stage of the study.

319 **Risk of bias assessment in included studies**

320 To examine the risk of bias in IPD-MA, the first four domains of the quality In
321 prognosis studies (QUIPS) tool and the first three domains of the prediction model risk of
322 bias assessment tool (PROBAST) will be considered as recommended in Riley et al
323 (2021)[16]. The relevant domains from the QUIPS checklist are study participation, study
324 attrition, prognostic factor measurement, and outcome measurement, and the relevant
325 domains from PROBAST checklist are participant selection, prognostic factors, and
326 outcomes. Two reviewers will independently assess the risk of bias in the studies included in
327 the analysis.

328 Risk of bias results will be incorporated into analyses by conducting subgroup
329 analyses among studies with overall low risk of bias or by conducting formal interaction
330 analyses with risk of bias responses [24].

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3 331 **Assessment of risk of potential bias in missing studies**
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6 332 Despite best possible efforts, it is anticipated that raw data from all the identified
7
8 333 studies will not be available. The characteristics of patient population and study meta-data
9
10 334 from the missing studies will be summarised to explore if the missing studies are
11
12 335 systematically different from the studies that are included in the IPD-MA. A two-stage IPD-
13
14 336 MA will be considered if sufficient details (or any covariate adjustment) are reported in the
15
16 337 original studies.
17
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21 338 **Software**
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24 339 All the analysis will be carried out using R software or Stata 17 software [25, 26]. Use
25
26 340 of any other data analysis tools will not change the statistical analysis plan.
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30 341 **Dissemination plans**
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33 342 **Ethics and dissemination**
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36 343 This IPD-MA meets the criteria for waiver of ethical review as defined by the Oxford
37
38 344 Tropical Research Ethics Committee (OxTREC) granted to IDDO, as the research consists of
39
40 345 secondary analysis of existing anonymised data. Ethics approval was granted by the ICMR-
41
42 346 Rajendra Memorial Research Institute of Medical Sciences ethics committee (Letter no:
43
44 347 RMRI/EC/30/2022) on 04-07-2022. Ethical approval was granted to each study included in
45
46 348 this pooled analysis by their respective ethics committees. This IPD-MA will address
47
48 349 research questions similar to that of included studies. Findings of this IPD-MA will be
49
50 350 reported in open-access, peer-reviewed journals following the PRISMA-IPD guidelines [27].
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3 352 **Patient and public involvement**
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6 353 The design and development of this IPD-MA were done by the study authors only and no
7
8 354 patient was involved at any stage. Patients and/or the public were not involved in the
9
10 355 design, or conduct, or reporting or dissemination plans of this research. The research
11
12 356 questions considered in this IPD-MA is based on a research agenda developed by the global
13
14 357 VL research community [28].
15
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19 358 **Further development of statistical analysis plan**
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22 359 The main analysis is planned as described above. Modification or additional analyses
23
24 360 may be required as the data collection progresses. Any modifications of the analysis will be
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26 361 documented and made publicly available on the IDDO study group website [29].
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362 Discussion

363 Anaemia is a common haematological manifestation of visceral leishmaniasis.
364 Despite this, the impact of treatment on different haematological parameters remains to be
365 fully understood. The aim of this IPD meta-analysis is to explore the dynamics of different
366 haematological parameters during the treatment and the convalescence phase of the
367 disease. IPD-MA is being used increasingly to explore factors affecting treatment outcomes
368 which otherwise would not be possible through standard aggregate data meta-analysis [30].

369 This IPD-MA will provide critical information regarding the trajectory of
370 haematological recovery among VL patients. The assessment of the host, parasite and drug
371 determinants that influence the haematological response can provide evidence-based
372 guidance for optimal case management and monitoring drug safety. The IDDO VL library,
373 which is a comprehensive library of all published studies since 1980 has been used for
374 identification of the studies eligible for this IPD-MA which has led to the construction of the
375 IDDO VL data platform. A major strength of this study is that data from several studies will
376 be harmonised to a common standard (in the IDDO VL data platform) based on an extensive
377 consultation with the VL research community [14], thus allowing us to address some of the
378 methodological sources of heterogeneity.

379 A major challenge is that a substantial proportion of the studies in the IDDO library
380 were conducted prior to the year 2000; the retrieval of data from historical trials is a major
381 challenge [31, 32]. Another limitation is that the exploration of the predictors of anaemia
382 and severe anaemia at baseline will be influenced by the underlying eligibility criteria
383 adopted in clinical trials as clinical trials frequently exclude patients with very low
384 haemoglobin at presentation [33]. Compared to non-pregnant patients, pregnant women

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3 385 are more at an increased risk of requiring blood transfusion [34]. However, pregnancy is
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6 386 often adopted as exclusion criteria in VL trials [33] and the haematological consequences in
7
8 387 this important patient group couldn't be explore despite the clear recognition of anaemia
9
10 388 and transfusion needs.

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14 389 This IPD-MA will characterise the haematological profile of VL patients at enrolment
15
16 390 and at the time-points of initial and definitive cure assessments. Findings of this research
17
18 391 will generate important information regarding the evolution of different haematological
19
20
21 392 characteristics.

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3 393 **Authors' contributions**
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5

6 394 Study conception: AbM, PD, RK, SS-P, NAS, CN, JW, GB, MR, FA, PM, SS, KR, KS, KP, PJG and
7
8
9 395 AhM. Project supervision: PD, AbM, KS, and PJG. Methodology: PD, NAS, SS-P, JW, FA, PJG
10
11 396 and KS. Data curation: AbM, SS-P, PM, JW, PD and GB. Project administration: SS-P and CN.
12
13
14 397 Funding acquisition: PJG. Resources: SS-P, FA and PJG. Writing-original draft: AbM, RK, SSP,
15
16 398 KS, PJG, AhM, and PD. Writing- review and editing: All authors were involved in reading and
17
18
19 399 critical revision of the initial draft and approved the final manuscript.
20

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22
23

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25
26
27 402 Data Observatory, Oxford University, UK (Recipient: Prof. Philippe Guerin; ref: INV-004713).
28
29
30 403 Funding agency had no role in developing the protocol.
31

32
33 404 **Conflict of Interest**
34

35
36 405 None
37

38
39 406 **Data Availability**
40

41
42 407 N/A as this is an analysis protocol.
43
44

45 408 **Patient consent for publication**
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47
48 409 Not required.
49

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51 410 **Supplemental file**
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54 411 Supplemental file 1: Search Details
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Example of Search Strategies: The dates will be different for future searches

Pubmed

((((((((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR clinical trials as topic[mesh:noexp] OR randomly[tiab] OR trial[ti] NOT (animals[mh] NOT humans [mh]))) AND Clinical Trial[ptyp])) AND (((("Leishmaniasis, Visceral"[Mesh]) OR visceral leishmaniasis[Title/Abstract]) OR kala azar[Title/Abstract]) OR black fever[Title/Abstract])) OR (((((((((((("Pentamidine"[Mesh]) OR "liposomal amphotericin B" [Supplementary Concept]) OR "Amphotericin B"[Mesh]) OR "Paromomycin"[Mesh]) OR "miltefosine" [Supplementary Concept]) OR "Sodium"[Mesh]) OR "8-aminoquinoline" [Supplementary Concept]) OR "Ketoconazole"[Mesh]) OR "Azoles"[Mesh]) OR "Allopurinol"[Mesh]) OR ("Atovaquone"[Mesh] OR "atovaquone, proguanil drug combination" [Supplementary Concept]))) OR ((pentamidine[Title/Abstract] OR ambisome[Title/Abstract] OR amphotericin[Title/Abstract] OR paromomycin[Title/Abstract] OR miltefosine[Title/Abstract] OR pentavalent[Title/Abstract] OR sodium[Title/Abstract] OR sitamaquine[Title/Abstract] OR azole*[Title/Abstract] OR allopurinol[Title/Abstract] OR atovaquone[Title/Abstract] OR ketoconazole[Title/Abstract] OR fluconazole[Title/Abstract] OR metronidazole[Title/Abstract]))) AND (((("Leishmaniasis, Visceral"[Mesh]) OR visceral leishmaniasis[Title/Abstract]) OR kala azar[Title/Abstract]) OR black fever[Title/Abstract])) NOT (((animals not humans)))
Filters activated: Publication date from 2016/01/01 to 2020/12/31.

- This search strategy uses the PubMed RCT filter for sensitivity- and precision-maximising version (2008). <https://work.cochrane.org/pubmed>

Database: Embase 1974 to present

Search Strategy:

- 1 exp visceral leishmaniasis/ (9675)
- 2 "black fever".ti,ab. (17)
- 3 "kala azar".ti,ab. (2227)
- 4 "visceral leishmaniasis".ti,ab. (9360)
- 5 1 or 2 or 3 or 4 (12383)
- 6 exp randomized controlled trial/ (603493)
- 7 Controlled clinical study/ (464274)
- 8 Random\$.ti,ab. (1531396)
- 9 randomization/ (86779)
- 10 intermethod comparison/ (260073)
- 11 placebo.ti,ab. (305154)
- 12 (compare or compared or comparison).ti. (507638)
- 13 ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ab. (2100417)
- 14 (open adj label).ti,ab. (78868)
- 15 ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab. (231216)
- 16 double blind procedure/ (172220)
- 17 parallel group\$.ti,ab. (25399)
- 18 (crossover or cross over).ti,ab. (104779)

- 1
2
3 19 ((assign\$ or match or matched or allocation) adj5 (alternate or group\$1 or intervention\$1 or patient\$1 or
4 subject\$1 or participant\$1)).ti,ab. (328145)
5 20 (assigned or allocated).ti,ab. (386517)
6 21 (controlled adj7 (study or design or trial)).ti,ab. (346624)
7 22 (volunteer or volunteers).ti,ab. (245818)
8 23 trial.ti. (298535)
9 24 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
10 (4650673)
11 25 5 and 24 (1506)
12 26 (pentamidine or ambisome or amphotericin or paromomycin or miltefosine or pentavalent or sodium or
13 sitamaquine or azole* or allopurinol or atovaquone or ketoconazole or fluconazole or metronidazole).mp.
14 (1012481)
15 27 5 and 26 (3701)
16 28 25 or 27 (4786)
17 29 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)
18 (6396469)
19 30 28 not 29 (3730)
20 31 30 (3730)
21 32 limit 31 to yr="2016 -Current" (774)

- 22
23
24 ➤ This search strategy uses the Cochrane RCT filter for Embase.

25 <https://www.cochranelibrary.com/central/central-creation>

26 Scopus

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28
29
30 ((TITLE-ABS-KEY ("visceral leishmaniasis" OR "kala azar" OR "black fever")) AND ((TITLE-ABS-KEY (random* OR rct OR placebo OR allocat* OR crossover* OR "cross
31 over" OR trial OR (doubl* W/1 blind*) OR (singl* W/1 blind*))) OR (TITLE-ABS-KEY (control* W/1 trial*)) OR (TITLE-ABS-KEY (cohort*)))) OR ((TITLE-ABS-KEY ("visceral
32 leishmaniasis" OR "kala azar" OR "black fever")) AND (TITLE-ABS-KEY (pentamidine OR
33 ambisome OR amphotericin OR paromomycin OR miltefosine OR pentavalent OR
34 sodium OR sitamaquine OR azole* OR allopurinol OR atovaquone OR ketoconazole OR
35 fluconazole OR metronidazole))) AND (EXCLUDE (EXACTKEYWORD , "Animals") OR
36 EXCLUDE (EXACTKEYWORD , "Animal") OR EXCLUDE (EXACTKEYWORD , "Animal
37 Experiment") OR EXCLUDE (EXACTKEYWORD , "Mouse") OR EXCLUDE (EXACTKEYWORD
38 , "Mice") OR EXCLUDE (EXACTKEYWORD , "Dogs")) AND (LIMIT-TO (LIMIT-TO (PUBYEAR , 2020) OR (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016)))

39 Web of Science Core Collection

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49
50 1. TOPIC: ("visceral leishmaniasis" OR "kala azar" OR "black fever")
51 2. TOPIC: (random* OR rct OR placebo OR allocat* OR crossover* OR "cross over" OR trial OR
52 (doubl* near/1 blind*) OR (singl* near/1 blind*))
53 3. TOPIC: (control* near/1 trial*)
54 4. TOPIC: (cohort*)
55 5. #4 OR #3 OR #2
56 6. #5 AND #1
57 7. TOPIC: (pentamidine OR ambisome OR amphotericin OR paromomycin OR miltefosine OR
58 pentavalent OR sodium OR sitamaquine OR azole* OR allopurinol OR atovaquone OR
59 ketoconazole OR fluconazole OR metronidazole)
60

8. #7 AND #1
9. #8 OR #6
10. #8 OR #6
11. Refined by: PUBLICATION YEARS: (2020 OR 2019 OR 2018 OR 2017 OR 2016)

Cochrane Central Register of Controlled Trials

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#1 MeSH descriptor: [Leishmaniasis, Visceral] explode all trees 39

#2 "black fever" 1

#3 "kala azar" 119

#4 "visceral leishmaniasis" 242

#5 #1 or #2 or #3 or #4 283

Custom Range: 2016 to 2020

World Health Organization Global Index Medicus <https://www.globalindexmedicus.net/>

tw:(tw:(("visceral leishmaniasis" OR "kala azar" OR "black fever")) AND (instance:"ghl") AND (year_cluster:("2016" OR "2017" OR "2018" OR "2019" OR "2020"))) AND (instance:"ghl")

ClinicalTrials.gov Advanced Search <https://clinicaltrials.gov/ct2/search/advanced?>

Condition or disease: visceral leishmaniasis OR kala azar OR black fever

WHO International Clinical Trials Registry Platform <http://apps.who.int/trialsearch/>

visceral leishmaniasis OR kala azar OR black fever – Trials at ALL stages

2016 – date

NOTE: THIS PLATFORM IS UNAVAILABLE ON 14/05/2020

PRISMA-P (preferred reporting items for systematic review and meta-analysis protocols) 2015 checklist: recommended items to address in a systematic review protocol

Section and topic	Item No	Checklist item
Administrative information		
Authors: All page numbers relates to the clear revised submission version of the manuscript submitted On the 10 th Oct 2023.		
Title:		
Identification	1a	A protocol for individual participant data meta-analysis; page 1; line 2-3.
Update	1b	Not applicable
Registration	2	PROSPERO registration: CRD42021284622; page 4; lines 72-73.
Authors:		
Contact	3a	Name with institutional affiliations of all authors and e-mail address corresponding authors; pages 1-2; lines 9-39. E-mail address of all authors; page 2; lines 22-39
Contributions	3b	Authors' contribution; page 21; line 388-394.
Amendments	4	Not applicable
Support:		
Sources	5a	Funding; page 21; line 395-398.
Sponsor	5b	Funding; page 21; line 395-398.
Role of sponsor or funder	5c	Funding; page 21; line 395-398.
Introduction		
Rationale	6	Introduction; page 6; lines 95-112.
Objectives	7	Study objectives; pages 6-7; lines 113-123.
Methods		
Eligibility criteria	8	Elements of PICOT & study and patient eligibility criteria: Pages 7-8; Lines 124-155
Information sources	9	Identification of relevant studies using IDDO VL library Pages 9-10: Lines 176-186
Search strategy	10	Identification of relevant studies using IDDO VL library

Section and topic	Item No	Checklist item
		Pages 9-10: Lines 176-186
Study records:		
Data management	11a	Collating IPD & Data management; page 10; lines 187-200.
Selection process	11b	Identification of relevant studies using IDDO VL library Pages 9-10: Lines 176-186
Data collection process	11c	Collating IPD & Data management; page 10; lines 187-200.
Data items	12	Statistical methods for primary and secondary outcomes; page 11-12; lines 201-261
Outcomes and prioritization	13	Outcomes and definitions; page 8-9; lines 157-173.
Risk of bias in individual studies	14	Risk of bias assessment in included studies page 16; lines 318-328
Data synthesis	15a	Handling missing data; page 14; lines 27-276.
	15b	Assessment of risk of potential bias in missing studies; pages 16-17; line 329-335.
	15c	Subgroup analyses: Page 16; lines 308-317 Sensitivity analyses; page 14; lines 277-285.
	15d	Assessment of risk of potential bias in missing studies; pages 16-17; line 329-335.
Meta-bias(es)	16	Risk of bias assessment in included studies page 16; lines 318-328
Confidence in cumulative evidence	17	Risk of bias assessment in included studies page 16; lines 318-328