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Klinische Tropenmedizin in globalem Kontext -

Epidemiologie, Diagnostik und Therapie tropentypischer Krankheiten in den Tropen, sowie bei Migranten und Reiserückkehrern in Deutschland

Kumulative Habilitationsschrift
zur Erlangung der Venia Legendi für das Fach Tropenmedizin

vorgelegt von
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1. Zusammenfassung der Habilitationsarbeit

Klinische Tropenmedizin in globalem Kontext - Epidemiologie, Diagnostik und Therapie tropentypischer Krankheiten in den Tropen, sowie bei Migranten und Reiserückkehrern in Deutschland

1.1. Einleitung: Klinische Tropenmedizin im 21. Jahrhundert

Klinische Tropenmedizin im 21. Jahrhundert umfasst verschiedenartige Teilbereiche: die klassische Tropenmedizin, die Ausübung medizinischer Fachdisziplinen unter ressourcenknappen Bedingungen in den Tropen, die Versorgung erkrankter Tropenrückkehrer, und die Reisemedizin. Wer das Fach als Hochschullehrer[1] vertritt, muss einen guten Überblick über all diese Bereiche haben:

Klinische Tropenmedizin beinhaltet zunächst ein Verständnis der in den Tropen endemischen Erkrankungen. Hierzu zählen z.B. Malaria, afrikanische Trypanosomiasis, Chagas oder die viralen hämorrhagischen Fieber. Das Auftreten tropenspezifischer Krankheiten ist an klimatische Gegebenheiten und an das Vorkommen bestimmter Vektoren und/oder zoonotischer Reservoire geknüpft.

Neben den tropenspezifischen Infektionen werden klinisch tätige Tropenmediziner intensiv mit global vorkommenden infektiologischen Krankheitsbildern wie Tuberkulose, HIV, Meningitiden und Blutstrominfektionen konfrontiert, die sie erkennen, und entsprechend den ortsspezifischen Ressourcen diagnostizieren und behandeln können müssen.

Zum klinischen Alltag in den Tropen zählen jedoch nicht nur Infektionen, sondern auch durch sozioökonomische, ökologische und kulturelle Faktoren bedingte Krankheitsbilder. Zu nennen wären z.B. Unter- und Mangelernährung, Schlangenbisse, Vergiftungen, weibliche Genitalverstümmelung oder die Folgen einer Behandlung beim traditionellen Heiler. Im Umgang mit den Patienten sind hierbei neben medizinischen auch ökologische, ethnologische und anthropologische Kenntnisse erforderlich.

Mit steigender Lebenserwartung und der Verbreitung eines "westlichen Lebensstils" wird auch bei der Arbeit in den Tropen die Diagnostik und Therapie von nicht-übertragbaren Krankheiten wie z.B. Diabetes mellitus oder arterieller Hypertonie und deren Folgeerkrankungen bedeutsam. Auch wenn diese Erkrankungen den im "Globalen Norden" ausgebildeten Ärzten aus ihrer klinischen Tätigkeit gut bekannt sind, gibt es doch im tropenmedizinschen Kontext einige Besonderheiten. Hierzu zählt der Umgang mit weit fortgeschrittenen Krankheitsbildern und ein Verständnis der pragmatischen Tätigkeit unter knappen Ressourcen. Im Gegensatz zur eigentlichen "Tropenmedizin" geht es hier also um das Praktizieren von "Medizin in den Tropen". Gefordert sind neben einem breiten Fachwissen auch Pragmatismus und Improvisationsbereitschaft.

[1] Die in der vorliegenden Habilitationsschrift zur besseren Lesbarkeit gewählte männliche Form schließt stets auch weibliche und diverse Personen mit ein.

Ressourcenknappheit bedeutet dabei nicht nur ein eingeschränktes Angebot an diagnostischen oder therapeutischen Möglichkeiten, sondern auch Mangel an medizinischem Fachpersonal. Dies erfordert bei der klinischen Tätigkeit in den Tropen ein fächerübergreifendes Verständnis von Medizin und die Bereitschaft, auch außerhalb der eigenen Kernkompetenz tätig zu werden. Die Fertigkeit zum interdisziplinären Agieren ist integraler Bestandteil der klinischen Tropenmedizin.

Die globale Vernetzung bewirkt, dass Infektionskrankheiten mit großer Schnelligkeit um die Welt transportiert werden.
Eine wichtige Funktion tropenmedizinisch tätiger Ärzte ist es, importierte Infektionen zeitnah zu erkennen und ggf. eine Weiterverbreitung zu verhindern. Dem Tropenmediziner kommt hier eine Wächterfunktion der öffentlichen Gesundheit zu. In der Tropen- und Reisemedizin hat sich der Begriff "Travelers as sentinels" etabliert (22, 26, 30, 33, 46, 48, 49).

Die Versorgung von Migranten aus tropischen und subtropischen Ländern ist aus dem medizinischen Alltag in Deutschland nicht mehr wegzudenken.
Eine kulturell sensible Versorgung von Migranten geht dabei über die Erkennung und Behandlung von Infektionskrankheiten weit hinaus. Migration bedeutet oftmals körperliches und seelisches Trauma und posttraumatischen Stress. Dies zu erkennen, und den betroffenen Patienten einen Zugang zu einer angemessenen medizinischen Versorgung und psychosozialen Unterstützung zu gewährleisten, sind weitere wichtige Aufgaben im tropenmedizinischen Alltag in Deutschland.

Auch die Reisemedizin, die sich mit der Beratung und prophylaktischen Versorgung von Reisenden vor der Ausreise befasst, ist Aufgabe der Tropenmediziner. Sie dient nicht nur der Gesunderhaltung individueller Reisender, sondern beugt auch dem Import und der Weiterverbreitung von Infektionen vor.
Die Reisemedizin wird in ihrer Bandbreite und Tragweite oft unterschätzt: In der reisemedizinischen Praxis geht es nicht nur um die Verabreichung von Schutzimpfungen und die Verordnung einer Malaria-Chemoprophylaxe, sondern auch z.B. um Vermittlung von Informationen zu Aufenthalten in großen Höhen und unter anderweitig extremen klimatischen Bedingungen, die angemessene Beratung von Langzeitreisenden, Schwangeren oder Familien mit Kleinkindern. Immer extremere Winkel der Welt werden bereist, ein steigender Anteil der Reisenden sind immunsupprimiert oder hochbetagt. Junge Menschen reisen im Rahmen eines Bundesfreiwilligendienstes für ein ganzes Jahr in abgelegenste Regionen tropischer Länder und leben dort unter einfachsten Bedingungen, junge Familien nutzen die Elternzeit, um mit ihren Säuglingen noch vor Abschluss des Standardimpfprogrammes Fernreisen zu unternehmen. All diese Personengruppen verdienen eine kompetente Beratung. Praxisorientierte Forschung dient dazu, relevante Beratungsinhalte herauszuarbeiten und die Qualität der Beratung zu verbessern.

Die Tropenmedizin unterliegt vor dem Hintergrund der sich verändernden Epidemiologie einem kontinuierlichen Wandel: Neue Erkrankungen treten auf, bekannte Krankheiten bieten neuartige Komplikationen, wie z.B. die schweren embryofetalen Fehlbildungen bei Zika-

Virusinfektion, die im Zuge der Ausbreitung von Zika in die westliche Hemisphäre beobachtet wurden (16, 29).

Globalisierung, zunehmende globale Vernetzung und Klimawandel tragen dazu bei, dass ursprünglich tropische Vektoren und Pathogene auch in gemäßigten Zonen Ausbreitung finden und Tropenkrankheiten auch außerhalb der Tropen auftreten können. Hierzu gibt es inzwischen zahlreiche Beispiele, so wie die Pandemie an Affenpocken (MPOX) 2022, das Auftreten von autochthonen Dengue- (20, 28, 32, 55), und Chikungunya-Infektionen (18, 73, 75) in Südeuropa oder von hämorrhagischem Krim-Kongo-Fieber in Spanien (36, 61, 62). In Korsika werden seit einigen Jahren Fälle der Tropenkrankheit Schistosomiasis (Bilharziose) nachgewiesen, wobei die erste Diagnose nicht vor Ort, sondern bei einem Reiserückkehrer nach Deutschland erfolgte (43), ein weiteres Beispiel der Bedeutung von "travellers as sentinels".

Auch Migration ist nicht statisch: Unterschiedliche Migrationswellen bringen Menschen aus den verschiedensten Ecken der Welt entlang immer neuer Migrationsrouten zu uns nach Europa: Flucht vor Bürgerkrieg in Syrien, Sudan oder dem Jemen, Flucht vor dem Taliban-Regime in Afghanistan, vor Milizionären und unterdrückenden religiösen Gruppen in Zentral- und Westafrika, vor erbarmungslosem Militärdienst in Eritrea - all diese Situationen bringen Geflüchtete mit unterschiedlichsten epidemiologischen, kulturellen und psychosozialen Hintergründen in unser Land, wo sie angemessen versorgt werden müssen.
Die medizinische Versorgung von Migranten ist jedoch schon lange keine Aufgabe, die auf Großstädte mit Tropeninstituten und Universitätskliniken beschränkt bleibt: Mitbürger aus den Tropen oder Subtropen leben inzwischen überall in Deutschland, auch in kleinen Städten und ländlichen Gemeinden. Hier ist es die Aufgabe des Hausarztes, mögliche Tropenerkrankungen zu erkennen und ggf. zu behandeln.

Tropenmedizin ist somit kein "Orchideenfach" mehr, welches auf wenige elitäre akademische Zentren in Metropolen des Landes beschränkt ist. Tropenmedizinisches Wissen muss viel mehr integraler Bestandteil der Ausbildung von Medizinstudierenden und Medizinern aller Fachdisziplinen sein. Hierfür kommt dem universitären Tropenmediziner eine wichtige Rolle als Aus- und Weiterbilder zu.
Hochschullehrer, die das Fach klinische Tropenmedizin abbilden, müssen somit nicht nur über ein breites medizinisches Fachwissen verfügen, sondern sich auch mit einem hohen Maß an Aufmerksamkeit und Flexibilität den stetig wandelnden Anforderungen dieses Fachs stellen.

Im folgenden Exposé und im anhängigen Schriftenverzeichnis möchte ich Beispiele meiner eigenen Forschungstätigkeit in klinischer Tropenmedizin in den oben skizzierten Kontexten vorstellen.
Ich hoffe, dass ich in dieser kumulativen Habilitationsarbeit einen Überblick sowohl über meine eigene Arbeit, als auch über die vielfältigen Facetten der modernen Tropenmedizin bieten, und die Grundlage meiner Lehrbefähigung für dieses faszinierende Fach unter Beweis stellen kann.

1.2. Ausgewählte Arbeiten zur Medizin in den Tropen

HIV/AIDS in Afrika

Rund 39 Millionen Menschen weltweit waren 2022 mit HIV infiziert. 67% der Menschen mit HIV/AIDS lebten in Afrika südlich der Sahara. Die 10 Länder mit der höchsten HIV-Prävalenz lagen allesamt auf dem afrikanischen Kontinent (85).

Wer in Subsahara-Afrika klinisch tätig ist, muss sich mit HIV/AIDS und den assoziierten opportunistischen Infektionen (OI) und Neoplasien intensiv beschäftigen. HIV und OI sind im klinischen Alltag für Morbidität und Mortalität von weitaus größerer Bedeutung als viele "klassische" Tropenkrankheiten.
Während die antiretrovirale Therapie inzwischen dank internationaler Geber-Konsortien weit verfügbar ist, und einen ähnlichen Standard hat wie im Globalen Norden, bleiben Diagnostik und Therapie vieler opportunistischer Infektionen eine Herausforderung.
Ein Beispiel dafür ist die Kryptokokkenmeningitis:

Die Kryptokokkenmeningitis ist die häufigste Form der adulten Meningitis in Regionen von Subsahara-Afrika mit hoher HIV-Prävalenz. Schätzungen zufolge gibt es jedes Jahr mehr als 220 000 Fälle und über 135 000 Todesfälle an Kryptokokkenmeningitis. Rund 15% aller AIDS-assoziierten Todesfälle weltweit sind durch diese Infektion verursacht (71).

Die zwei wichtigsten Erreger der Kryptokokkeninfektion sind die bekapselten Pilze *Cryptococcus (C.) neoformans* und *C. gattii*. Sie kommen weltweit vor und wurden insbesondere aus mit Vogelmist kontaminiertem Erdboden isoliert. Eine Kryptokokkose kann neben dem ZNS auch nahezu alle anderen Organe und Gewebe befallen, z.B. auch Lunge, Haut, Prostata, Peritoneum, Knochen und Auge (68).

Als therapeutischer Goldstandard für die Kryptokokkenmeningitis gilt eine Therapie aus liposomalem Amphotericin B und Flucytosin über 14 Tage (sog. Induktionsphase), gefolgt von einer Konsolidierungsphase mit Fluconazol 400-800mg über 6-8 Wochen und einer Erhaltungstherapie mit Fluconazol 200mg bis zur Immunrekonstitution (21, 69).
Eine adäquate Induktionstherapie mit zwei potenten fungiziden Medikamenten, die zu einer raschen Senkung der Erregerkonzentration im Liquor führen, ist prognostisch entscheidend: Aus Ländern des globalen Nordens wurde unter dieser Therapie eine 10-Wochen-Letalität von unter 10% berichtet (42).
Anders stellt sich die Situation in Subsahara-Afrika dar, wo zugleich mehr als 70% aller weltweiten Fälle von Kryptokokkenmeningitis vorkommen: Liposomales Amphotericin B ist in der Regel nicht verfügbar. Auch (nicht-liposomales) Amphotericin B ist schwer erhältlich. Zudem fehlt es an therapeutischen Überwachungsmöglichkeiten für das potenziell toxische Medikament. Flucytosin ist teuer und meist nicht vorhanden.
Lediglich das allein fungistatisch wirksame Fluconazol ist in vielen afrikanischen Ländern weit verfügbar, dank eines Spendenprogrammes der Herstellerfirma.

Vor dem Hintergrund der mangelnden Verfügbarkeit von Amphotericin B und Flucytosin war in vielen afrikanischen Ländern eine Monotherapie mit Fluconazol über lange Zeit die einzige verfügbare therapeutische Option:
In einer Studie aus Sambia erhielten 130 Patienten mit HIV-assoziierter Kryptokokkenmeningitis nach einer initialen Einzeldosis von Fluconazol 400mg (IV oder p.o.) eine orale Erhaltungstherapie mit Fluconazol 200mg. Eine Vergleichsgruppe erhielt lediglich Palliativtherapie. Die mediane Überlebenszeit lag in der Fluconazol-Gruppe bei 19 Tagen (1-164 Tage), in der unbehandelten Gruppe bei 10 Tagen (0-42 Tage) (58). Die Letalität nach 6 Monaten war 100% in beiden Gruppen.

In einer retrospektiven südafrikanischen Studie zeigte sich eine längere Überlebenszeit als in der sambischen Studie von median 76 Tagen unter Fluconazol-Monotherapie, eine weitere Dosissteigerung von Fluconazol auf 400mg brachte jedoch keine Verbesserung von Überlebenszeit oder Letalität (80).
Eine frühe US-amerikanische Arbeit demonstrierte eine Verringerung der 10-Wochen Letalität bei Dosissteigerung von 800mg (89% Letalität) auf 2000mg Fluconazol pro Tag (38% Letalität). Das Hinzufügen von Flucytosin zu unterschiedlichen Dosierungen von Fluconazol brachte jeweils eine weitere Verbesserung der Ergebnisse, bei allerdings sehr kleinen Fallzahlen (53).

A Prospective Longitudinal Study of the Clinical Outcomes from Cryptococcal Meningitis following Treatment Induction with 800mg Oral Fluconazole in Blantyre, Malawi

In einer prospektiven Beobachtungsstudie an 60 Patienten mit laborbestätigter HIV-assoziierter Kryptokokkenmeningitis in Blantyre, Malawi, untersuchten wir das Behandlungsergebnis unter dem nationalen malawischen Therapiestandard dieser Zeit von 800mg Fluconazol in der Induktionsphase, gefolgt von Fluconazol 400mg täglich für 6 Wochen, und einer Erhaltungstherapie von 200mg (78).
Eingeschlossen wurden HIV-Patienten mit Nachweis von *C. neoformans* aus dem Liquor mittels India-Ink-Färbung und/oder Pilzkultur. Tests auf Kryptokokken-Antigen (CrAg) standen nicht zur Verfügung. Primäre Endpunkte waren die Letalität nach 4, 10 und 52 Wochen. Abb. 1 zeigt die Kaplan-Meier-Überlebenskurve bis 52 Wochen nach Krankenhausaufnahme.

Die 4-Wochen Letalität lag in dieser Kohorte bei 43%, die 10-Wochen Letalität bei 55%. Am Ende der 52 Wochen waren nur 13 von 60 Patienten am Leben, entsprechend einer Letalität von 78%. Alle Patienten konnten bis zum Studienende nachverfolgt werden.
Als prädiktiver Faktor für ein negatives Behandlungsergebnis ließ sich eine Bewusstseinsminderung bei Krankenhausaufnahme herausarbeiten (Glasgow-Coma-Scale (GCS) <14). Die Kaplan-Meier-Überlebenskurve in Abhängigkeit vom GCS bei Aufnahme ist in Abb. 1B dargestellt.

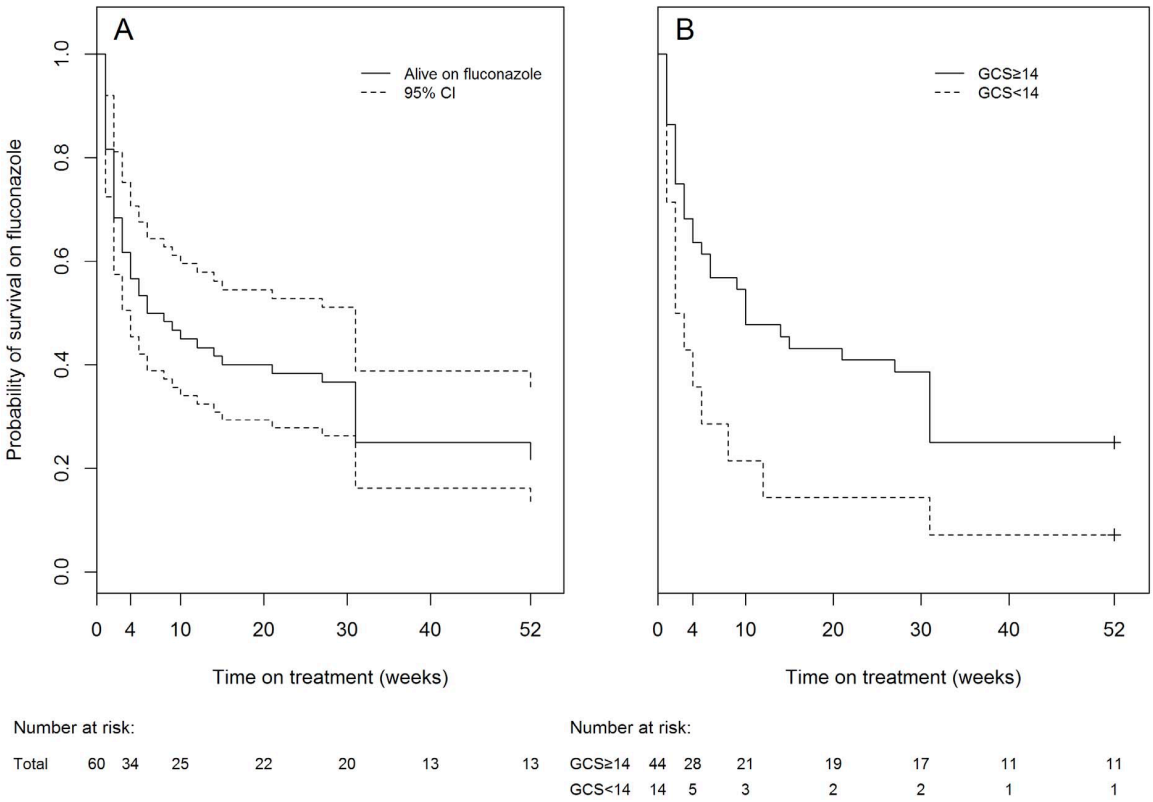

Abb.1: (A): Überlebenswahrscheinlichkeit von malawischen HIV-Patienten mit laborbestätigter Kryptokokkenmeningitis unter Fluconazol-Monotherapie (Induktionsphase mit 800mg), (B): Überlebenswahrscheinlichkeit in Abhängigkeit vom Glasgow-Coma-Score (GCS) bei Krankenhausaufnahme (78).

Infolge der Studie wurden die nationalen Therapieempfehlungen für die Kryptokokkenmeningitis in Malawi angepasst. Empfohlen wurde eine Dosissteigerung auf 1200mg Fluconazol in der Induktionsphase.

A Prospective Study of Mortality from Cryptococcal Meningitis following Treatment Induction with 1200mg Oral Fluconazole in Blantyre, Malawi

Wir führten daraufhin eine weitere prospektive Beobachtungsstudie unter der neuen Dosisempfehlung durch und schlossen 47 Patienten mit HIV-assoziierter Kryptokokkenmeningitis in die Studie ein (37). Die 10-Wochen-Letalität unter Induktionstherapie mit Fluconazol 1200mg (primärer Endpunkt) betrug 55% und ergab somit keinen Unterschied zur 800mg-Gruppe aus der vorangegangenen Studie (78). Ein Überlebensvorteil durch die Dosis-Steigerung war somit nicht nachweisbar.

Unsere Studienergebnisse passen zu den Befunden von *Longley et al*, die in einer Studie an 60 Patienten in Uganda primär die Konzentrationen von *C.neoformans* im Liquor unter einer Monotherapie mit Fluconazol 800mg versus 1200mg in der Induktionsphase verglichen. Es zeigte sich unter der höheren Fluconazol-Dosis zwar eine signifikant schnellere Elimination von *C. neoformans* aus dem Liquor, im klinischen Behandlungsergebnis waren beide Dosisgruppen jedoch gleich: die 2-Wochen Letalität lag im Median bei 30%, die Letalität

zum Zeitpunkt 10 Wochen bei 54%, die Unterschiede zwischen den Dosisgruppen waren nicht signifikant, bei insgesamt kleiner Fallzahl (51).

Nach diesen enttäuschenden Ergebnissen unter Fluconazol-Monotherapie stellte sich die Frage, ob es andere Behandlungsoptionen zur Verbesserung der Therapieergebnisse bei Kryptokokkenmeningitis mit Eignung für den klinischen Alltag im Globalen Süden geben könnte. Eine Kombination der antimykotischen Therapie mit Corticosteroiden brachte bei der HIV-assoziierten Kryptokokkenmeningitis keinen Vorteil: eine große Multicenterstudie mit Studienstandorten in Afrika und Asien musste abgebrochen werden, weil das Behandlungsergebnis im Corticosteroid-Arm (in Kombination mit einer antifungalen Induktionstherapie aus Amphotericin B und Fluconazol) signifikant schlechter war als in der Kontrollgruppe ohne Steroide (8).

Ein Problem beim Einsatz von Amphotericin B sind Komplikationen wie Hämato-, Nephro- und Phlebotoxizität. Unter ressourcenknappen Bedingungen stellt dies eine besondere Herausforderung dar, weil es an Personal und an Laborkapazität zur Überwachung von potenziellen Nebenwirkungen fehlt. Die Toxizität von Amphotericin B ist jedoch abhängig von der kumulativen Dosis und tritt daher in der Regel erst in der zweiten Behandlungswoche auf.
Vorangegangene Phase-2-Studien zeigten eine gute Verträglichkeit von Amphotericin B bei Verkürzung der Therapiedauer auf nur eine Woche (44, 57). Auch eine orale Kombinationstherapie von Fluconazol mit dem fungiziden Flucytosin erschien eine Option, die zudem eine ambulante Therapie möglich machen würde: In einer vorangegangenen kleinen südafrikanischen Studie hatte die orale Kombinationstherapie gegenüber einer Fluconazol-Monotherapie eine Verbesserung der 10-Wochen Letalität von 43% gegenüber 58% gezeigt (65).

Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa

Zusammen mit dem Malawi-Liverpool Wellcome Trust konnten wir im Rahmen des Studienkonsortiums "Advancing Cryptococcal Meningitis Treatment for Africa" (ACTA) an der Planung und der Initialphase einer prospektiven, randomisierten open-Label Phase-III-Studie mitwirken, die der Frage einer nachhaltigen Therapie der Kryptokokkenmeningitis für den Globalen Süden weiter nachging (54).

In dieser multizentrischen klinischen Studie mit insgesamt neun Studienstandorten in vier afrikanischen Ländern (Malawi, Tansania, Sambia und Kamerun) wurden erwachsene Patienten mit HIV-assoziierter Kryptokokkenmeningitis gemäß der Art der Induktionstherapie in drei Haupt-Therapiearme randomisiert:

1. Orale Kombinationstherapie mit Fluconazol 1200mg/d und Flucytosin 100mg/kg/d,
2. Amphotericin B-Kurztherapie (1mg/kg KG/d IV) für 1 Woche in Kombination mit jeweils Fluconazol oder Flucytosin per os,
3. Amphotericin B für zwei Wochen in Kombination mit jeweils Fluconazol oder Flucytosin per os.

Bewiesen werden sollte die Nichtunterlegenheit der oralen Induktionstherapie und des Amphotericin B-Kurzschemas im Vergleich zum Goldstandard einer zweiwöchigen Induktionsphase mit Amphotericin B bei Erwachsenen mit HIV-assoziierter Kryptokokkenmeningitis. Primärer Endpunkt war die Gesamtmortalität nach 2 Wochen. Sekundäre Endpunkte waren die Gesamtmortalität zum Zeitpunkt 4 und 10 Wochen, die Konzentration von *C. neoformans* im Liquor und die Verträglichkeit.

Es wurden insgesamt 678 Patienten eingeschlossen. Die 2-Wochen-Mortalität lag bei 18,2% für das orale Induktionsregime, bei 21,9% für Amphotericin B (einwöchig) im Vergleich zu 21,4% für 2 Wochen Amphotericin B (Tab. 1). Die Unterschiede waren nicht-signifikant. Es konnte somit eine Nichtunterlegenheit der beiden alternativen Therapieregimes gezeigt werden.
Auch für die Mortalität zum Zeitpunkt 4 und 10 Wochen waren beide Studien-Regimes dem Goldstandard nicht unterlegen.
Die Ergebnisse von 2-, 4- und 10-Wochen-Mortalität zeigt Tabelle 1.

Tab. 1: Gesamtmortalität an Kryptokokkenmeningitis in Abhängigkeit vom Therapieregime in der Induktionsphase; Intention-to Treat-Analyse, nach (54)

Outcome/Therapy	**Oral Regimen (n=225)**	**1-wk Amphotericin B (n=224)**	**2-wk Amphotericin B (n=229)**	**Difference (95% CI)**	
				Oral Regimen vs 2-wk Amphotericin B	**1-wk Amphotericin B vs 2-wk Amphotericin B**
Mortality at 2 weeks					
N° of deaths	41	49	49		
% (95% CI)	18.2 (13.2-23.3)	21.9 (16.5-27.4)	21.4 (16.1-26.7)	-3.18 (-10.50 to 4.15)	0.47 (-7.11 to 8.06)
Mortality at 4 weeks					
N° of deaths	56	66	77		
% (95% CI)	24.9 (19.2-30.5)	29.5 (23.6-35.5)	33.6 (27.5-39.7)	-8.74 (-17.06 to -0.41)	-4.16 (-12.71 to 4.39)
Mortality at 10 weeks					
N° of deaths	79	81	91		
% (95% CI)	35.1 (28.9-41.3)	36.2 (30-42.7)	39.7 (33.5-46.2)	-4.63 (-13.52 to 4.27)	-3.58 (-12.51 to 5.35)

Bei der Frage nach dem besten oralen Kombinationspartner für Amphotericin B zeigte sich eine signifikante Überlegenheit von Flucytosin gegenüber Fluconazol zu allen drei untersuchten Zeitpunkten (Tab. 2).

Tab. 2: Gesamtmortalität in Abhängigkeit vom Amphotericin B-Kombinationspartner, nach (54)

Outcome/Therapy	**Amphotericin B + Fluconazol (n=225)**	**Amphotericin B + Flucytosin (n=228)**	**Hazard Ratio Flucytosin vs Fluconazole (95% CI)**	**p-Wert**
Mortality at 10 wks				
N° of deaths	101	71	0.62 (0.45-0.84)	0.002
% (95% CI)	45.0 (38.5-51.5)	31.1 (25.3-37.3)		
Mortality at 2 wks				
N° of deaths	61	37	0.56 (0.37-0.85)	0.006
% (95% CI)	27.1 (21.3-32.9)	16.3 (11.5-21.1)		
Mortality at 4 wks				
N° of deaths	86	57	0.59 (0.42-0.83)	0.002
% (95% CI)	38.2 (31.9-44.6)	25.1 (19.4-30.7)		

Im Vergleich der fünf unterschiedlichen Regimes schnitt in Bezug auf die 10-Wochen-Gesamtmortalität die Kurztherapie aus Amphotericin B in Kombination mit Flucytosin am besten ab (24.2% (95% CI 16.2-32.1)), gefolgt vom oralen Induktionsregime aus Fluconazol und Flucytosin (35.1% (28.9-41.4%)). Eine Übersicht bietet Abb. 2.

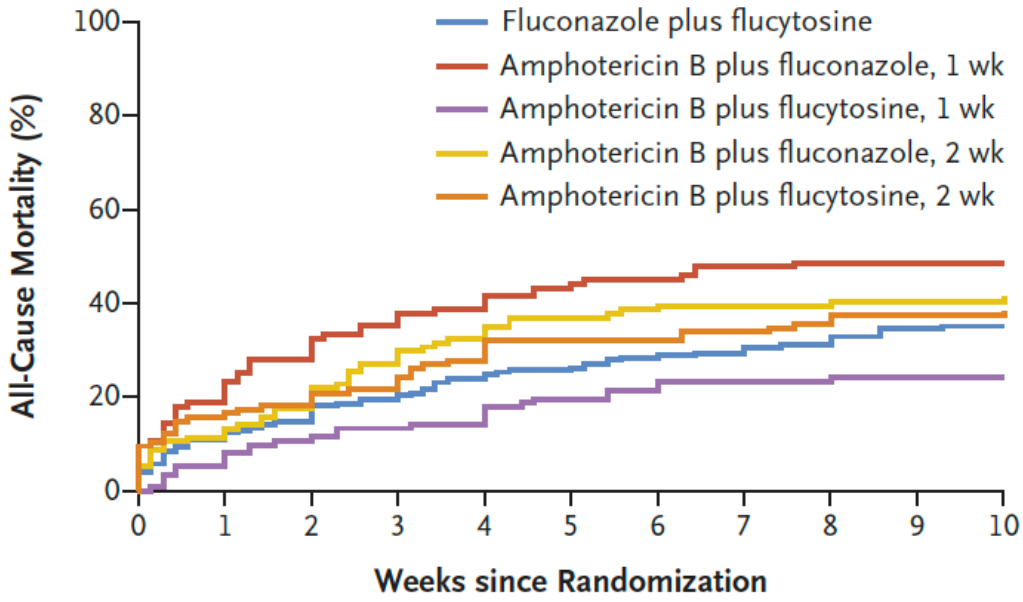

Abb. 2: Gesamtmortalität der HIV-assoziierten Kryptokokkenmeningitis in den fünf untersuchten Therapieregimes bis 10 Wochen nach Randomisierung (54).

Zusammenfassend erwies sich der international postulierte Goldstandard, der auf Empfehlungen der Infectious Diseases Society of America (IDSA) beruht, nicht als optimale Therapie für die HIV-assoziierte Kryptokokkenmeningitis unter ressourcenlimitierten Bedingungen. Das einwöchige Amphotericin Kurzregime und die orale Induktionstherapie aus Fluconazol und Flucytosin waren dem "Goldstandard" hinsichtlich der 10-Wochen-Gesamtmortalität unterlegen.

Infolge der Ergebnisse der ACTA-Studie änderte die WHO 2018 ihre Empfehlung zur Initialtherapie der Kryptokokkenmeningitis zugunsten eines einwöchigen Induktionsregimes mit Amphotericin B und Flucytosin gefolgt von 1200mg Fluconazol; die orale Induktionstherapie wurde als alternative Option empfohlen (91).

Um eine weitere Verkürzung der Therapie mit Amphotericin B zu ermöglichen, verglich die AMBITION-cm Phase-III-Studie das von der WHO neu empfohlene Induktionsregime (1 Woche Amphotericin B + Flucytosin) mit einer Einzelgabe liposomalen Amphotericin B in hoher Dosis (10mg/kg KG) in Kombination mit einem 2-wöchigen oralen Regime aus Fluconazol 1200mg/d plus Flucytosin 100mg/kg/d.
Bei Analyse der 10-Wochen-Gesamtmortalität zeigte sich eine Nichtunterlegenheit des Studienregimes gegenüber dem WHO-Standard bei zugleich besserer Verträglichkeit (45).

Das AMBITION-Regime wurde daraufhin 2022 in die WHO-Empfehlungen übernommen. Ob dieses Regime auch in ressourcenreichen Settings empfohlen werden sollte, war umstritten.

2024 wurden erstmalig globale Empfehlungen publiziert, die auf Unterschiede zwischen ressourcenreichen und ressourcenknappen klinischen Standorten bei der Therapie der Kryptokokkose eingehen (21): Für ressourcenreiche Settings wird eine Induktionstherapie mit liposomalem Amphotericin 3-4mg/kg/d in Kombination mit Flucytosin 25mg/kg/d 4x täglich für zwei Wochen empfohlen.
Für ressourcenlimitierte Standorte empfiehlt man eine Induktionsphase gemäß der Dreifachtherapie der AMBITION-cm Studie bzw. der WHO-Empfehlungen von 2022 (92). Die weitere Therapie ist für beide Szenarien gleich (in der Konsolidierungsphase Fluconazol 400-800mg für 8 Wochen, in der Erhaltungsphase 200mg für 12 Wochen).

1.3. Ausgewählte Arbeiten zu importierten Infektionen

SARS-CoV2

Eine der Aufgaben der Tropenmedizin ist es heute, die Eintragung von (neuartigen) Infektionen nach Deutschland zeitnah zu detektieren und daran mitzuwirken, ihre Weiterverbreitung zu verhindern. Längst beschränkt sich diese Wächterfunktion nicht nur auf klassische Tropen-Erkrankungen, wie zu Beginn der Corona-Pandemie deutlich wurde:

Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany

Als zu Beginn 2020 die ersten Fälle der neuartigen Coronavirusinfektion SARS-CoV2 detektiert wurden, stellte sich die Frage nach der Übertragung des neuen Virus. Diskutiert wurde nicht nur der Transmissionsmodus (z.B. Tröpfchen- oder Aerosole), sondern auch die Frage, ab welchem Zeitpunkt der Infektion eine Infektiosität vorläge. Beide Aspekte waren essenziell für die Implementierung effektiver Infektionsschutzmaßnahmen.

Zunächst ging man davon aus, dass SARS-CoV2 hinsichtlich seiner Transmission dem SARS-Virus ähneln würde, welches in den Jahren 2002/03 eine Pandemie verursacht hatte. Die Zahl der SARS-Fälle blieb damals beschränkt, trotz seiner raschen und weiträumigen Verbreitung in 25 Länder und auf 5 Kontinente. Es gab rund 8000 Fälle mit 774 Todesopfern. Ausschlaggebend für die im retrospektiven Vergleich geringe Anzahl von SARS-Fällen war, dass nur symptomatisch infizierte Personen ansteckend waren und in den ersten fünf Tagen der Infektion nahezu keine Übertragung stattfand (50). Auch gänzlich asymptomatisch verlaufende Infektionen waren bei SARS selten.
Wer ansteckend war, wusste somit von seiner Infektion und war auch klinisch leicht identifizierbar. Dies bedingte, dass sich SARS durch vergleichsweise einfache Maßnahmen, wie klinische Falldefinitionen, Temperaturmessungen und die Isolation von Erkrankten gut eindämmen ließ und die SARS-Pandemie bereits im Juli 2003 beendet war (67).

Als wir am 27.01.2020 im Tropeninstitut München den ersten deutschen Fall der neuartigen Coronavirusinfektion diagnostizierten, war bereits anhand unserer klinischen Beobachtungen klar, dass sich das neue Virus anders verbreiten würde als sein Vorläufer SARS:
Der erste deutsche Patient, ein Mitarbeiter einer Firma mit Sitz in Starnberg bei München, hatte sich bei einer Kollegin aus Shanghai angesteckt, die selbst augenscheinlich keine Krankheitssymptome wie Husten, Schnupfen und Heiserkeit aufwies. Auf telefonische Nachfrage bei der chinesischen Indexpatientin berichtete diese selbst, dass sie sich während ihres Aufenthaltes in Deutschland nicht krank gefühlt habe; sie sei erst bei Rückkehr nach China mit hohem Fieber und respiratorischen Symptomen erkrankt. Einige Tage darauf wurde bei ihr eine COVID-19 Infektion festgestellt (Abb. 3).

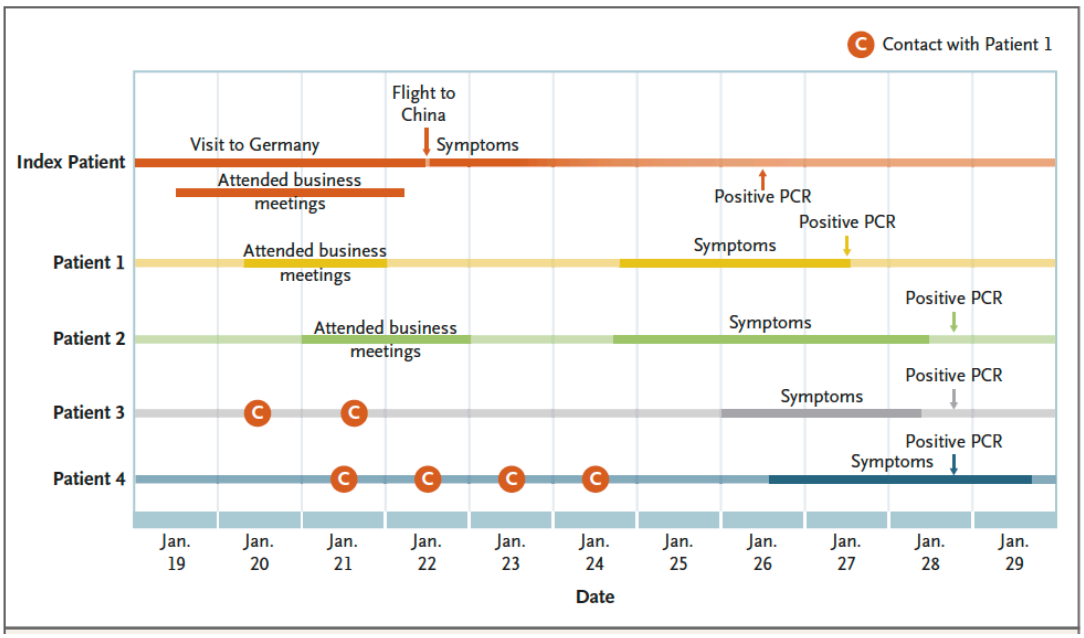

Abb. 3 Zeitlicher Verlauf der ersten 2019-CoV-Infektionen in Deutschland nach Exposition zur asymptomatisch infizierten Indexpatientin, einer chinesischen Geschäftspartnerin aus Shanghai (aus (77))

Die ersten beiden deutschen Patienten, die sich in unserer Ambulanz vorstellen, waren 2-3 Tage nachdem sie sich in Meetings mit der klinisch unauffälligen chinesischen Kollegin aufgehalten hatten, an respiratorischen Symptomen erkrankt. Die PCR auf das neuartige Virus war positiv, obgleich die Symptome bereits im Wesentlichen abgeklungen waren.
Zwei weitere Firmenmitarbeiter (Pat. 3 und 4 in Abb. 3) erkrankten, ohne der chinesischen Indexpatientin begegnet zu sein. Sie hatten jedoch engen Kontakt zu einem später erkrankten Kollegen gehabt (Pat. 1 in Abb.1).
In diesem ersten deutschen Cluster fanden sich somit zwei unabhängige Belege dafür, dass eine präsymptomatische Transmission von SARS-CoV2 möglich war (77): Pat. 1 und 2 hatten sich von der präsymptomatischen Kollegin aus China angesteckt, Pat. 3 und 4 vom präsymptomatischen Kollegen, Pat. 1.

Dieses erste deutsche COVID-19-Cluster umfasste im Verlauf insgesamt 16 Infektionen, von denen fünf (31%) auf eine präsymptomatische Transmission zurückzuführen waren. Eine

Infektion verlief gänzlich asymptomatisch und wurde lediglich laborchemisch nachgewiesen (13).
Die Beobachtungen am ersten deutschen Cluster waren hochrelevant; sie machten klar, dass Isolationsmaßnahmen, die lediglich auf klinischen Falldefinitionen beruhten, zur Eindämmung dieser Epidemie nicht ausreichen würden.
In den folgenden Wochen mehrten sich Berichte von Beobachtungen präsymptomatischer Transmissionen von SARS-CoV2 (3, 23, 40, 41, 89). Analysen von Transmissionsclusters zeigten in ihren Berechnungen, dass das serielle Intervall kürzer war, als die Inkubationszeit. Damit war die Möglichkeit einer präsymptomatischen Transmission auch mathematisch erwiesen (40, 64).
Es sollte jedoch noch über ein halbes Jahr dauern, bis auch die Weltgesundheitsorganisation (WHO) die Relevanz eines prä- oder asymptomatischen Transmissionswegs einräumte.

Therapierefraktäre *Giardia lamblia* Infektion bei Reiserückkehrern

Die Giardiasis ist die häufigste parasitäre Ursache für Durchfall bei Reiserückkehrern aus den Tropen und Subtropen (83). Sie wird durch das intestinale Protozoon *Giardia (G.) lamblia* (Syn: *G. intestinalis* oder *G. duodenalis)* verursacht. Die Infektion erfolgt fäkal-oral, entweder durch kontaminierte Nahrungsmittel und Getränke oder direkt von Mensch zu Mensch. Nach einer Inkubationszeit von 7-10 Tagen treten breiiger Durchfall, Übelkeit, starke Blähungen und Aufstoßen auf. Selbst nach erfolgreicher Therapie kann eine Laktoseintoleranz persistieren.
Die Erstlinientherapie der Giardiasis erfolgt in der Regel mit 5-Nitroimidazolen, in Deutschland mit Metronidazol 400-500mg, 3x täglich für 5-7 Tage.
In der klinischen Praxis spielt die therapierefraktäre Giardiasis mit Versagen der 5-Nitroimidazol-Erstlinientherapie bei Reiserückkehrern eine zunehmende Rolle (19, 56, 59).

Risk Factors for and Management of Metronidazole-refractory Giardiasis in International Travellers: A Retrospective Analysis

Wir führten eine retrospektive Kohortenstudie bei Reiserückkehrern und Migranten mit parasitologisch gesicherter Giardiasis durch. Einbezogene Variablen waren neben Alter und Geschlecht die Reisedauer und die Art der Erst- bis maximal Drittlinientherapie, jeweils mit Therapieergebnis.
Wir untersuchten den Anteil von Therapieversagen bei leitliniengerechter Erstlinientherapie mit Metronidazol, Risikofaktoren für ein Therapieversagen und die Art der effektivsten Zweitlinientherapie im Falle eines Versagens der Metronidazol-basierten Erstlinientherapie (70).
Über einen Analysezeitraum von 10 Jahren (2007 - 2016) wurden 339 Patienten mit Giardiasis eingeschlossen. Nahezu die Hälfte (46,3%) kam aus Südasien (Indien, Nepal, Pakistan, Bangladesch). 26,8% kamen aus Afrika, 13,3% aus Südamerika, 3,2% aus den übrigen Regionen der Welt.

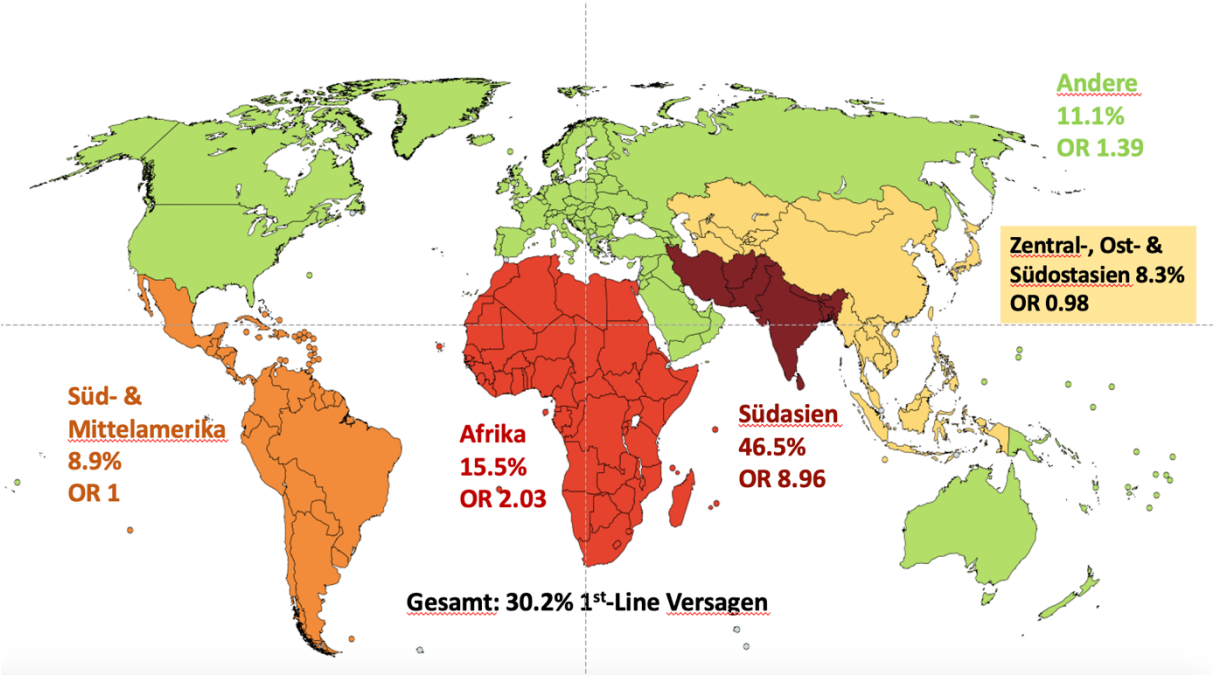

Abb. 4: Prozentuales Versagen der Erstlinientherapie mit Metronidazol bei parasitologisch gesicherter Giardiasis nach wahrscheinlichem Infektionsort; OR in Bezug auf Mittel- und Südamerika als Referenzregion (nach (70)).

Insgesamt versagte die Erstlinientherapie der Giardiasis bei 30,2% der Patienten.
Als einziger Risikofaktor für ein Therapieversagen ließ sich die Herkunftsregion der Infektion herausarbeiten. Alter, Geschlecht oder Reisedauer stellten hingegen keine Risikofaktoren dar. Die bereiste Weltregion mit dem häufigsten Therapieversagen war Südasien (46,5%, OR 8,96 im Vergleich zur Referenzregion Mittel- und Südamerika), gefolgt von Afrika (15,5%). In Südostasien lag die Rate eines Therapieversagens lediglich bei 8,3% (Abb. 4).
Die Zweitlinientherapie (n=93) versagte in insgesamt 46,2% der Fälle (Abb. 5).

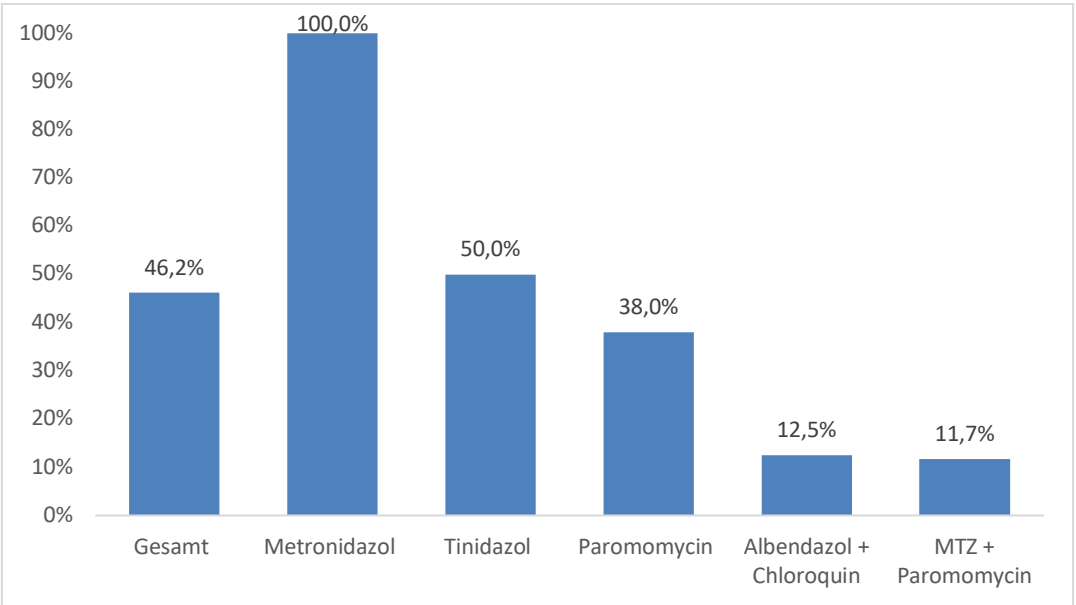

Abb. 5: Therapieversagen (%) bei Zweitlinientherapie der Giardiasis nach Primärtherapie mit Metronidazol (MTZ) (70).

Bei Analyse unterschiedlicher Zweitlinienregimes, erwies sich eine Wiederholung der Metronidazol-Monotherapie als nicht erfolgversprechend (100% Therapieversagen). Am besten schnitten eine Kombination aus Metronidazol plus Paromomycin ab (88,2% Heilungsrate), sowie eine Kombination aus Albendazol und Chloroquin (87,5%), bei allerdings geringen Fallzahlen (n=17 bzw. n=8).

Efficacy and Tolerability of Quinacrine Monotherapy and Albendazole Plus Chloroquine Combination Therapy in Nitroimidazole-Refractory Giardiasis: A TropNet Study

Eine potenziell vielversprechender Therapiealternative bei therapierefraktärer Giardiasis ist Quinacrin (Mepacrin). Quinacrin wurde während des 2. Weltkriegs zur Malariatherapie eingesetzt und war das erste Medikament dessen Wirksamkeit auf *Giardia lamblia* systematisch untersucht wurde (87). Der Wirkmechanismus von Quinacrin ist nicht bekannt. Aufgrund seiner potenziell schwerwiegenden neuropsychiatrischen Nebenwirkungen verlor Quinacrin nach dem 2. Weltkrieg an Bedeutung, als besser verträgliche Therapiealternativen verfügbar wurden: Chloroquin zur Malariatherapie und Metronidazol, später auch andere 5-Nitroimidazole, zur Therapie der Lamblieninfektion.
Quinacrin wurde in den letzten Jahren als Therapiealternative bei therapierefraktärer Giardiasis wiederentdeckt. Mehrere, allerdings kleine Studien belegen eine hundertprozentige Wirksamkeit von Quinacrin bei Nitroimidazol-refraktären Fällen (19, 56, 59, 72). In einer kubanischen Studie etwa, versagte die Metronidazol-Erstlinientherapie bei 208/456 der Patienten mit Lamblieninfektion (46%). Alle 15 Patienten, die nach Versagen von drei weiteren Therapieregimes schließlich in der Fünftlinientherapie Quinacrin erhielten, wurden geheilt (19).
Unklarheiten bestehen bezüglich der Bewertung der Sicherheit von Quinacrin, welches dosisabhängig u.a. zentralnervöse Nebenwirkungen verursachen kann. Rund 0,4% der Soldaten, die im 2. Weltkrieg mit Quinacrin bei Malaria behandelt wurden, entwickelten eine toxische Psychose (38). Die Häufigkeit toxischer Nebenwirkungen scheint mit der Höhe der kumulativen Dosis zu korrelieren, dabei ist die im Rahmen der Therapie einer Giardiasis erreichte Kumulativdosis (1,5g) deutlich geringer als Dosen, die bei der konventionellen Malariatherapie erreicht werden (2,1-2,8g). Die Schwere von möglichen Nebenwirkungen ist jedoch im Kontext einer nicht vital bedrohlichen Lamblieninfektion anders zu bewerten als bei einer potenziell tödlichen Malaria tropica.

Mit Partnern des europäischen Netzwerks TropNet führten wir eine Studie zur Wirksamkeit und Verträglichkeit von Quinacrin bei therapierefraktärer Giardiasis durch (63).
In einer prospektiven, nicht-randomisierten, multizentrischen, open-label Beobachtungsstudie erhielten 106 Reiserückkehrer mit parasitologisch bestätigter Lamblieninfektion nach Versagen der Metronidazol-Erstlinientherapie entweder Quinacrin (100mg 3x täglich p.o.) oder eine Kombination aus Albendazol (400mg 2x täglich p.o.) und Chloroquin (155mg 2x täglich) für eine Dauer von jeweils 5 Tagen. Endpunkte waren klinisches und parasitologisches Behandlungsergebnis 2-5 Wochen nach Therapie, sowie die Verträglichkeit.

Klinisches Befinden und mögliche Nebenwirkungen wurden bei allen Patienten durch Telefoninterviews 4-5 Wochen nach Therapie strukturiert erfasst. Eine parasitologische Verlaufskontrolle wurde bei allen Patienten durchgeführt, die über eine Persistenz von Symptomen berichteten; bei Beschwerdefreiheit war sie optional.

106 Patienten mit Giardiasis und sowohl klinischem als auch parasitologischem Versagen einer Nitroimidazol-basierten Erstlinientherapie wurden in die Studie eingeschlossen.
47% der Patienten waren weiblich, das mediane Alter lag bei 33 Jahren (21-68 Jahre), bei den männlichen Patienten bei 34 Jahren (8-59 Jahre). 57% der Studienteilnehmer hatten ihre Lamblieninfektion in Indien erworben.

73/106 Patienten erhielten Quinacrin, 33 weitere die Kombinationstherapie aus Albendazol und Chloroquin. 9 Patienten erhielten nach Versagen der Zweitlinientherapie aus Albendazol und Chloroquin eine Cross-Over Therapie mit Quinacrin. Die Ergebnisse sind in Tabelle 3 dargestellt.

Tab. 3: Parasitologisches und klinisches Behandlungsergebnis nach Zweitlinientherapie mit Quinacrin bzw. Albendazol/Chloroquin-Kombinationstherapie bei Reiserückkehrern mit therapierefraktärer Giardiasis, nach (63)

	n	**Parasitological cure**	**Clinical cure**	**Clinical improvement**	**Clinical failure**
Quinacrin	73	100% 56/56*	81% 59/73	19% 14/73	0% 0/73
Albendazol + Chloroquin	33	42% 12/28*	36% 12/33	55% 18/33	9% 3/33
Quinacrin cross-over	9	100% 9/9	100% 9/9	0% 0/9	0% 0/9

* Die parasitologische Heilung wurde nicht bei allen Patienten überprüft; im Falle einer klinischen Heilung war ein parasitologisches Follow-Up optional.

Unter Therapie mit Quinacrin kam es bei 100% der Patienten zu einer parasitologischen Heilung; 81% berichteten über ein Sistieren der Beschwerden, 19% über eine klinische Besserung (Tab. 3).
Bei Patienten, die eine Kombinationstherapie mit Albendazol/Chloroquin erhielten, kam es bei nur 42% zu einer parasitologischen Heilung, auch wenn 55% über eine klinische Besserung berichteten. Insgesamt 9/33 (27%) der Patienten aus der Albendazol/Chloroquin-Therapiegruppe entschlossen sich für eine Cross-Over Drittlinientherapie mit Quinacrin. Bei allen diesen Patienten kam es zu einer parasitologischen und zu einer klinischen Heilung.

45% der Studienteilnehmer, die Quinacrin erhalten hatten und 30% der Patienten mit Albendazol/Chloroquin, berichteten über Nebenwirkungen. Zwei Patienten brachen die Therapie vorzeitig ab, beide hatten Quinacrin eingenommen. Einer der beiden Patienten hatte schwere neuropsychiatrische Nebenwirkungen erlitten, u. a. Schlafstörungen, Alpträume, psychotische Symptome und Halluzinationen, die eine kurzzeitige Hospitalisation nötig machten. Nach Absetzen der Therapie sistierten die Symptome komplett. Der andere Patient entwickelte ein Nierenversagen; Quinacrin wurde aus Sicherheitsbedenken abgesetzt, auch wenn die durch die Diarrhö bei Lamblieninfektion verursachte Dehydratation als

wahrscheinlichere Ursache angenommen wurde. Trotz vorzeitigem Therapieabbruch wurden beide Patienten von ihrer Giardiasis geheilt.

Auch wenn die Studie methodologische Einschränkungen aufweist, da sie nicht randomisiert und verblindet war, ergeben sich deutliche Hinweise auf die Bedeutung von Quinacrin als wirksame Alternative bei therapierefraktärer Lambliasis.
Neben dem aktuell noch nicht abschließend zu bewertenden Nebenwirkungspotential wird der Einsatz von Quinacrin aktuell durch schlechte Verfügbarkeit und hohen Preis limitiert. Quinacrin ist für die Therapie der Lambliasis nicht zugelassen, sein Einsatz daher off-label. In Deutschland ist das Medikament nicht auf dem Markt und muss aus dem Ausland importiert werden (63). In der 2023 aktualisierten AWMF-Leitlinie "Gastrointestinale Infektionen" wird Quinacrin jedoch erstmals als Therapiealternative bei Versagen der Metronidazol-Erstlinientherapie aufgeführt (52).

Die Studienergebnisse veranschaulichen überdies, dass eine klinische Beschwerdepersistenz nach Therapie bei Lambliasis nicht notwendigerweise auf ein parasitologisches Therapieversagen zurückzuführen ist. Eine parasitologische Bestätigung empfiehlt sich vor Einleitung einer weiteren antiparasitären Therapie.

Autochthone Schistosomiasis auf Korsika

Auch vor Erkrankungen mit komplexen Übertragungszyklen macht die Ausbreitung von Tropenerkrankungen nicht halt: so wurden in den letzten Jahren wiederholt autochthone Infektionen von Schistosomiasis (Bilharziose) berichtet, die auf der französischen Mittelmeerinsel Korsika erworben worden waren.

Schistosomiasis wird durch eine Infektion mit Egeln (Trematoden) verursacht. Die adulten Schistosomen leben als "Pärchen-Egel" in den Venen des menschlichen Urogenital- oder Gastrointestinaltrakts. Das Weibchen produziert täglich viele hunderttausend Eier, die der menschliche Endwirt, je nach *Schistosoma* Spezies, im Urin bzw. Stuhl ausscheidet (25). Gelangen die Eier in Süßwasser, schlüpfen die Larven. Diese benötigen zur weiteren Entwicklung eine aquatische Schnecke als Zwischenwirt. In der Schnecke entwickelt sich ein weiteres Larvenstadium des Parasiten, sog. Gabelschwanz-Zerkarien, die ins Wasser abgegeben werden. Der Entwicklungszyklus schließt sich, wenn Menschen in Kontakt zu Süßwasser geraten, welches mit Zerkarien infestiert ist.

Schistosomiasis zählt zu den vernachlässigten Tropenerkrankungen (Neglected Tropical Diseases, NTDs). Rund 230 Millionen Menschen weltweit sind infiziert (25). Wichtigstes Verbreitungsgebiet ist Afrika, daneben kommen Transmissionsherde auf der arabischen Halbinsel, in Südamerika und Südostasien vor. Chronische Schistosomiasis geht mit einer erheblichen Morbidität einher, die für die tropischen Endemiegebiete im klinischen Alltag von großer Relevanz ist (25, 34) und auch bei der Versorgung von Migranten in Deutschland eine wichtige Rolle spielt.

Die wichtigsten humanpathogenen Parasiten sind *S. haematobium* (Urogenitalbilharziose), und *S. mansoni* (hepatointestinale Bilharziose). Daneben existieren auch zoonotische Erreger mit einem breiten Wirtsspektrum, welches Wild- und Nutztiere umfasst.
In den letzten Jahren wurden wiederholt hybride Infektionen aus humanen und zoonotischen Parasiten detektiert (9, 15, 88). Dies ist von großer praktischer Relevanz, da eine Eradikation der Schistosomiasis beim Menschen im Falle einer weiten Verbreitung von Hybridinfektionen mit zugleich zoonotischen und menschlichen Endwirten nahezu unmöglich wäre.

In Europa galt die humane Schistosomiasis bislang nicht als endemisch. In einigen Gebieten Südeuropas sind jedoch die zur Transmission benötigten aquatischen Schnecken-Zwischenwirte heimisch, sodass ein Übertragungspotential besteht. Im 20. Jahrhundert kam es in der portugiesischen Algarve vorübergehend zum Auftreten autochthoner Fälle von Schistosomiasis; der Fokus konnte in den 1970er Jahren eradiziert werden (14, 27). Der Ursprung der Erreger blieb unklar, man vermutete jedoch eine Eintragung aus ehemaligen portugiesischen Kolonien in Afrika. Auch aus Südspanien wurde ein autochthoner Ausbruch an Schistosomiasis mitgeteilt (81). Mehrere Länder Europas berichteten zudem über Fälle von zoonotischer Schistosomiasis bei Tieren.

Der erste Fall von autochthoner Schistosomiasis aus Korsika wurden 2013 bei einem jungen deutschen Reiserückkehrer mit Makrohämaturie diagnostiziert (43). Der Patient hatte nie tropische Endemiegebiete für Schistosomiasis bereist. Kurz nach Bekanntwerden dieses Falles wurden weitere Fälle bei französischen Korsika-Touristen bekannt (11, 17, 66).
Das GeoSentinel Netzwerk konnte weitere elf Fälle bei internationalen Reisenden nachweisen, die Mehrzahl von ihnen waren zum Zeitpunkt der Diagnose asymptomatisch (39).
Informationskampagnen und Screening-Untersuchungen der französischen Gesundheitsbehörden führten zur Detektion von über 120 weiteren Fällen (14). Als Infektionsquelle wurde der Fluss Cavu im Südosten Korsikas angenommen (11, 17, 43, 66), in dem alle Patienten der ersten Fallserien gebadet hatten. Bereits in der GeoSentinel-Serie fiel jedoch auf, dass 4 von 11 Reisenden mit Schistosomiasis nicht in diesem Fluss gebadet hatten (39).
Nach dem ersten großen Ausbruch wurden in den folgenden Jahren wenige weitere Fälle bekannt. Ein Screening von über 3000 aquatischen Schnecken am Cavu fiel negativ aus (10). Man ging seitens der französischen Behörden davon aus, dass das Problem behoben sei.

Developing Endemicity of Schistosomiasis, Corsica, France

2020 stellte sich in unserer Ambulanz ein Patient vor, bei dem extern eine Zystoskopie bei Makrohämaturie durchgeführt worden war. Histologisch war der Verdacht auf eine Blasenbilharziose geäußert worden. Der 49-jährige Deutsche war nie außerhalb von Europa gewesen, hatte jedoch im Jahr zuvor Korsika bereist. Er hatte in mehreren Flüssen gebadet, nicht aber im Cavu. Die Badestellen ließen sich anhand von GPS -Daten auf dem Smartphone genau rekonstruieren.

Im Urin fanden sich makroskopisch Eier, die Eiern von *S. haematobium* ähnelten, und die PCR auf *S.haematobium* war positiv.
Im Rahmen einer DNA-Extraktion konnten wir jedoch zeigen, dass es sich um ein zoonotisches Hybrid aus *S. haematobium* und *S. bovis* handelte: die nukleären Marker (995 Basenpaare, bp) zeigten eine typische Signatur von *S. haematobium,* wohingegen die mitochondrialen Marker (873bp) charakteristische Merkmale von *S. bovis* aufwiesen. Dieses Hybrid, dessen Ursprung in Westafrika vermutet wird, war auch bei vorangegangenen Schistosomiasis-Fällen aus Korsika nachgewiesen wurden (14).

Wir konnten somit belegen, dass die Transmission von Schistosomiasis auf Korsika weitergeht und zudem nicht auf den Cavu-Fluss beschränkt ist. Als wahrscheinlichster Infektionsort wurde der Fluss Solenzara angenommen, der dem Cavu benachbart ist, aber nicht mit ihm kommuniziert (79).

In der Folge wurden weitere Transmissionsfälle von Schistosomiasis am Solenzara-Fluss bekannt, wiederum anhand von Beobachtungen an deutschen Reiserückkehrern (90).
Es ist daher anzunehmen, dass die Tropenkrankheit inzwischen auf der Mittelmeerinsel als endemisch gelten muss.

Wie der Parasit nach Korsika gelangt ist, und ob primär das zoonotische Hybrid importiert wurde, oder es sich erst vor Ort aus zoonotischen Erregern und *S. haematobium* rekombiniert hat, ist noch Gegenstand wissenschaftlicher Debatte.
Unabhängig von seinem Ursprung könnte sich ein zoonotisches Hybrid aus *S.haematobium* und *S. bovis* auch in tierischen Reservoiren etablieren. Dies könnte die anhaltende Transmission erklären und würde eine Elimination erschweren.

Der Zwischenwirt der urogenitalen Schistosomiasis, die Süßwasserschnecke *Bulinus truncatus*, ist in mehreren Ländern Südeuropas heimisch. Die Schnecken tolerieren Temperaturen von 2°C bis 40°C. Malakologische Untersuchungen haben gezeigt, dass *S.haematobium* sich in den Bulinus-Schnecken ab Temperaturen von 20°C entwickeln kann (12), optimal für die Entwicklung der Parasiten und die Abgabe der Larven ins Wasser sind jedoch höhere Temperaturen von 26-31°C (1). Hier könnte ein Anstieg der Wassertemperaturen im Kontext des Klimawandels eine Rolle spielen und die Transmission auch in Europa begünstigen (14).
Das Zusammentreffen aus Klimawandel und Massenmigration von Menschen aus Schistosomiasis-endemischen Ländern, wie sie aktuell insbesondere Länder Südeuropas betrifft, macht ein weiteres Auftreten autochthoner Fälle von Schistosomiasis in Europa auch in Zukunft sehr wahrscheinlich (4).

Importierte *Plasmodium knowlesi*-Malaria

Plasmodium knowlesi ist ein zoonotischer Malaria-Erreger, der ausschließlich in Südost-Asien vorkommt. Sein tierisches Reservoir findet sich in Makaken. *P. knowlesi* ist auch humanpathogen. Schwere Krankheitsverläufe, wie bei Falciparum-Malaria, kommen vor.

In Malaysia ist die *P. knowlesi*-Malaria inzwischen mit über 3000 Fällen pro Jahr die häufigste Malariaform und verursacht von allen Malaria-Spezies im Land die meisten Todesfälle (5).

Der Erreger wurde 1931 in Kalkutta in Makaken entdeckt (60). Seine Fähigkeit, auch Menschen zu infizieren wurde 1932 durch Knowles und Das Gupta unter Studienbedingungen demonstriert (47), lange war jedoch nicht klar, welche Bedeutung der Malaria-Erreger für den Menschen unter natürlichen Bedingungen haben würde.
Erst 1965 wurde der erste natürlich erworbene Fall von *P. knowlesi* -Malaria bei einem Menschen nachgewiesen (24). Der Parasit kann mikroskopisch leicht mit *P. malariae* verwechselt werden, dem Verursacher der klinisch in der Regel leicht verlaufenden Malaria quartana, die gleichfalls in Asien endemisch ist (7, 24). Aufgrund der Schwierigkeiten in der konventionellen Diagnostik wurde die epidemiologische Bedeutung des Erregers in Südostasien erst nach Einführung der PCR erkannt (82).

P. knowlesi hat den kürzesten asexuellen Replikationszyklus von allen Malariaparasiten (<24h) und kann so rasch hohe Parasitämien verursachen und schwere Verläufe der Malaria hervorrufen, die unbehandelt zum Tod führen (6).
Wir wollten der Rolle nachgehen, die *P.knowlesi* bei Reisenden in Deutschland spielt:

Retrospective Clinical Case Series Study in 2017 identifies *Plasmodium knowlesi* as Most Frequent Plasmodium Species in Returning Travellers from Thailand to Germany

Wir führten daraufhin zusammen mit anderen großen deutschen Tropeninstituten eine retrospektive Analyse von importierten *P.knowlesi*-Malaria Fällen durch (35).
Im Zeitraum von 2012 bis Januar 2017 wurden insgesamt sechs Fälle von *P. knowlesi* Malaria identifiziert. Zum Vergleich wurden im Rahmen einer retrospektiven Studie des GeoSentinel Netzwerkes aus über 65 tropenmedizinschen Spezialambulanzen im Analysezeitraum von 14 Jahren (2003-16) lediglich drei Fälle von *P.knowlesi*-Malaria registriert (2).

Alle sechs im Rahmen unserer Studie erfassten Fälle von *P.knowlesi* Malaria traten bei erwachsenen Reisenden auf (medianes Alter 53 (45-73) Jahre; 4/6 männlich).
Der Malaria-Schnelltest war in 4 von 5 Fällen, in denen ein Schnelltestergebnis vorlagen, falsch negativ (80%). Die Mikroskopie als diagnostischer Goldstandard war in allen Fällen positiv, bei allerdings oft sehr geringer Parasitämie. Alle Fälle waren PCR-positiv. Einer der Patienten wies einen schweren Verlauf auf und musste bei Multiorganversagen intensivmedizinisch behandelt werden. Alle Patienten hatten die Westküste Thailands bzw. Inseln in der Andamanensee bereist, sodass diese Region als Ursprung der Malaria anzunehmen ist.
Laut einer Surv-Stat Abfrage von RKI Daten wurden zwischen 2018 und 2023 fünf weitere Fälle an *P.knowlesi* Malaria in Deutschland diagnostiziert (74).

Auch wenn es sich um eine kleine Fallserie handelt, so illustriert sie doch anschaulich die diagnostischen Herausforderungen der *P.knowlesi* Malaria. Es ist davon auszugehen, dass

diese Malariaform unterdiagnostiziert wird, was die im Vergleich zu unserer Arbeit geringe Anzahl von Fällen im GeoSentinel Netzwerk erklärt.
Immunchromatographische Malaria-Schnelltests, die gerade in nicht-spezialisierten Einrichtungen bei der primären Malariadiagnostik eine große Rolle spielen, sind bei der *P.knowlesi* Malaria nicht verlässlich. Wir konnten auch zeigen, dass diese "seltene" zoonotische Malaria inzwischen die häufigste nach Deutschland importierte Malariaform aus Thailand ist, einer der wichtigsten Reisedestinationen der Deutschen.
Als praktische klinische Konsequenz ergibt sich, dass bei Fieber nach Aufenthalt in Südostasien auch an eine *P. knowlesi* Malaria zu denken. Bei negativem Schnelltest und unklarem mikroskopischen Befund sollte frühzeitig eine Malaria-PCR angestrebt werden, da die *P. knowlesi*-Malaria letal verlaufen kann.

1.4. Ausgewählte Arbeiten zur Reisemedizin

Jedes Jahr werden rund 800-1000 Malariafälle nach Deutschland importiert (31). Empfehlungen zur Malariaprävention für Reisende gibt die Deutsche Gesellschaft für Tropenmedizin, Reisemedizin und Globale Gesundheit (DTG) jährlich heraus (76); sie werden durch DTG-Mitglieder ehrenamtlich erarbeitet. Offizielle nationale Empfehlungen gibt es in Deutschland nicht.

Zur Malaria-Prävention wird neben wirksamem Mückenschutz für Malaria-Hochrisikogebiete auch die dauerhafte Einnahme einer Malaria-Chemoprophylaxe empfohlen.
Für Gebiete mit mittlerem Malariarisiko, z.B. für bestimmte Regionen in Süd- und Südostasien und Südamerika, wird das Mitführen einer sog. Standby-Therapie empfohlen: Die Reisenden werden gebeten, ein Fieberthermometer mitzuführen und im Falle von Fieber binnen 24h medizinische Hilfe aufzusuchen, um eine Malaria auszuschließen. Ist keine medizinische Hilfe verfügbar, so sollen die erkrankten Reisenden sich vorsorglich selbst auf eine mögliche Malaria behandeln. Analog zur Therapie einer unkomplizierten Malaria erfolgt die Selbstbehandlung entweder mit Atovaquon/Proguanil oder mit Artemether/Lumefantrin für eine Dauer von drei Tagen.
So lautet die Empfehlung - aber was passiert tatsächlich vor Ort? -

Response to Fever and Utilization of Standby Emergency Treatment (SBET) for Malaria in Travellers to Southeast Asia: A Questionnaire-based Cohort Study

In einer prospektiven Beobachtungsstudie wurden Reisende eingeschlossen, die in Gebiete in Südostasien mit mittlerem Malaria-Risiko zu reisen beabsichtigten (n=876)(86).
Die Studienteilnehmer wurden entsprechend den Empfehlungen der DTG (s.o.) zum Verhalten bei Auftreten von Fieber und zur Einnahme einer Notfallselbsttherapie beraten. Das Rezept für eine Stand-By-Malariatherapie wurde den Reisenden mitgegeben. Nach der Reise wurden die Studienteilnehmer per Telefoninterview befragt, ob sie das Medikament tatsächlich besorgt und mitgeführt hätten, ob Fieber aufgetreten sei und welche Maßnahmen die Reisenden in diesem Falle ergriffen hätten.

714 Reisende waren nach der Reise telefonisch kontaktierbar, 130 (18.2%) berichteten über Fieber auf der Reise oder binnen 14 Tagen nach Reiserückkehr. 100/130 Reisenden mit Fieber hatten die rezeptierte Malaria-Notfalltherapie mitgenommen (76,9%) (Abb. 6).

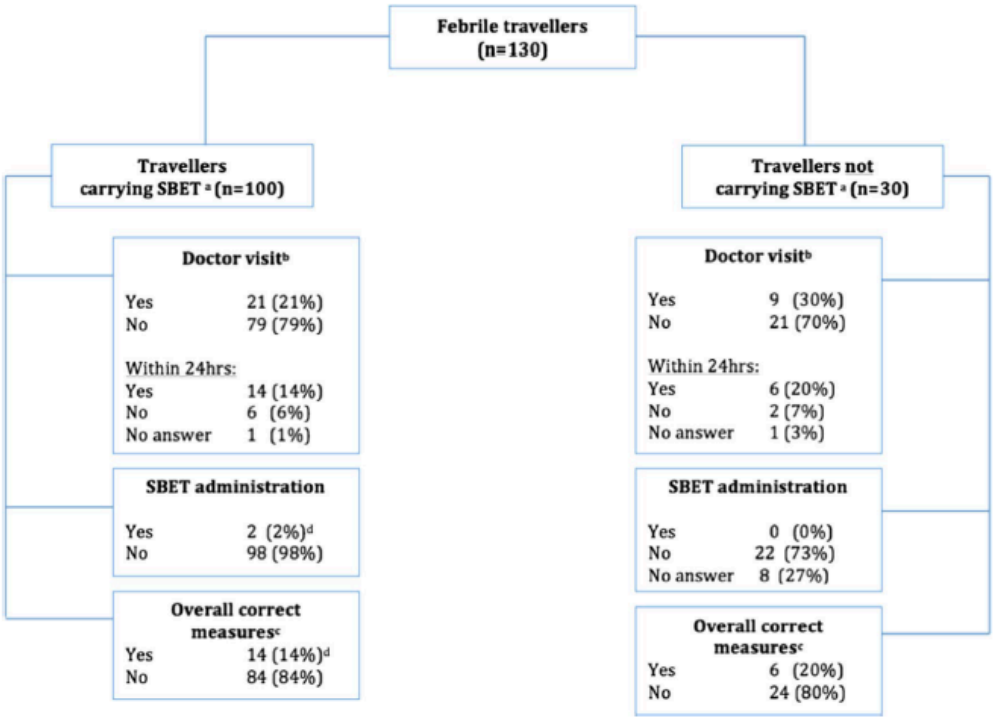

Abb. 6 Reaktion von Reisenden auf Fieber während eines Aufenthaltes in einem Malariagebiet. [a]SBET = Stand-By Emergency Treatment, Notfallselbsttherapie einer vermuteten Malaria, [b]Doctor Visit = Arztbesuch im Reiseland, [c]Correct measures = korrekt durchgeführte Selbstmedikation mit einem Stand-By Medikament oder Arztbesuch binnen 24h. [d]Beide Reisende, die SBET einnahmen, verabreichten sich diese falsch und wurden daher nicht als "korrekt" gewertet.

30/100 Reisenden mit Fieber suchten die empfohlene medizinische Einrichtung auf, 20 zeitnah binnen 24 Stunden. Nur 2 Reisende mit Fieber (2%) nahmen die Notfall-Therapie ein, beide allerdings inkorrekt. Malaria-Episoden traten bei den kontaktierten Reisenden nicht auf. Insgesamt handelten nur 20/130 Reisenden mit Fieber (15.3%) den Empfehlungen entsprechend korrekt.

Unsere Studie verdeutlicht, dass nur wenige Reisende die in der reisemedizinischen Beratung empfohlene Strategie der Notfall-Selbsttherapie umsetzten. Was die Ursachen dafür waren (Verständnis, fehlendes Risikobewusstsein, Bequemlichkeit...), ließ sich nicht eruieren. Malaria-Episoden traten bei den befragten Reisenden nicht auf, wobei ein Selektionsbias nicht auszuschließen ist, da nicht alle Teilnehmer kontaktiert werden konnten.

Die Ergebnisse unserer und anderer Studien belegen eine schlechte Compliance bei touristisch Reisenden in Bezug auf das Konzept der notfallmäßigen Selbsttherapie der Malaria (84). Gleichzeitig sinkt die Malariainzidenz in vielen Teilen der Welt außerhalb des tropischen Afrika; insbesondere in Asien und Lateinamerika (93). Zugleich verbessert sich vielerorts die Infrastruktur, sowohl in Bezug auf die medizinischen Versorgung als auch auf den

öffentlichen Nahverkehr, was eine zeitnahe Abklärung von Fieberepisoden vor Ort zunehmend erleichtert.
Die unselektierte Verordnung von Malariamedikamenten für alle Reisenden in Regionen mit mittlerem Malaria-Risiko erscheint somit nicht länger gerechtfertigt und stellt eine Ressourcenverschwendung dar.
Vor dem Hintergrund der Studienergebnisse hat die DTG ihre Empfehlungen modifiziert: Das Mitführen einer Stand-By Therapie wird nunmehr nur noch für Reisen in sehr abgelegene Gebiete empfohlen, in denen davon auszugehen ist, dass binnen 48h keine medizinische Hilfe aufgesucht werden kann (76). Dies ist für den Großteil der touristisch bereisten Regionen in Asien und Lateinamerika nicht länger der Fall.

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3. In der Habilitationsschrift diskutierte eigene Arbeiten

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4. Erklärungen

Erklärung über bisher eingereichte Habilitationsprojekte

Es wurden durch mich bisher keine weiteren Habilitationsprojekte an einer anderen Hochschule eingereicht. Ebenso wurde mir bislang kein akademischer Grad entzogen; ein entsprechendes Verfahren ist nicht anhängig.

München, 28.03.2024 Dr. med. Camilla Rothe

Versicherung an Eides statt

Hiermit versichere ich an Eides statt, dass ich die schriftliche Habilitationslistung selbständig verfasst und die Herkunft des verwendeten oder zitierten Materials ordnungsgemäß kenntlich gemacht habe.

München, 28.03.2024 Dr. med. Camilla Rothe

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PLOS ONE

A Prospective Longitudinal Study of the Clinical Outcomes from Cryptococcal Meningitis following Treatment Induction with 800 mg Oral Fluconazole in Blantyre, Malawi

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Abstract

Introduction: Cryptococcal meningitis is the most common neurological infection in HIV infected patients in Sub Saharan Africa, where gold standard treatment with intravenous amphotericin B and 5 flucytosine is often unavailable or difficult to administer. Fluconazole monotherapy is frequently recommended in national guidelines but is a fungistatic drug compromised by uncertainty over optimal dosing and a paucity of clinical end-point outcome data.

Methods: From July 2010 until March 2011, HIV infected adults with a first episode of cryptococcal meningitis were recruited at Queen Elizabeth Central Hospital, Blantyre, Malawi. Patients were treated with oral fluconazole monotherapy 800 mg daily, as per national guidelines. ART was started at 4 weeks. Outcomes and factors associated with treatment failure were assessed 4, 10 and 52 weeks after fluconazole initiation.

Results: Sixty patients were recruited. 26/60 (43%) died by 4 weeks. 35/60 (58.0%) and 43/56 (77%) died or failed treatment by 10 or 52 weeks respectively. Reduced consciousness (Glasgow Coma Score $<$14 of 15), moderate/severe neurological disability (modified Rankin Score $>$3 of 5) and confusion (Abbreviated Mental Test Score $<$8 of 10) were all common at baseline and associated with death or treatment failure. ART prior to recruitment was not associated with better outcomes.

Conclusions: Mortality and treatment failure from cryptococcal meningitis following initiation of treatment with 800 mg oral fluconazole is unacceptably high. To improve outcomes, there is an urgent need for better therapeutic strategies and point-of-care diagnostics, allowing earlier diagnosis before development of neurological deficit.

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Introduction

Cryptococcal meningitis, caused by the encapsulated saprophytic yeast *Cryptococcus neoformans*, occurs most commonly in HIV infected patients with CD4 counts of 100 cells/μl or less [1,2]. As a consequence of the HIV epidemic, it is the most common cause of meningitis amongst adults in Sub Saharan Africa (SSA) [3–5]. Of an estimated global incidence of 957,900 cases per year, 75% occur in SSA [6].

Unacceptable differences in mortality exist between developed and developing countries. Ten week case fatality rates have been estimated at 9% in Western Europe and North America, but as high as 70% in SSA [6]. There is no evidence that mortality in SSA has improved since the roll-out of antiretroviral therapy (ART) and uncertainty over optimal treatment regimens for resource limited settings is partially responsible for this. ''Gold-standard'' therapy involves a two week induction phase with the fungicidal combination amphotericin B and flucytosine prior to consolidation and maintenance therapy with fluconazole [7]. However, the cost, complexities of administration and side effects prevent the routine use of this regimen in resource poor settings. Standard treatment in Africa usually consists of fluconazole monotherapy.

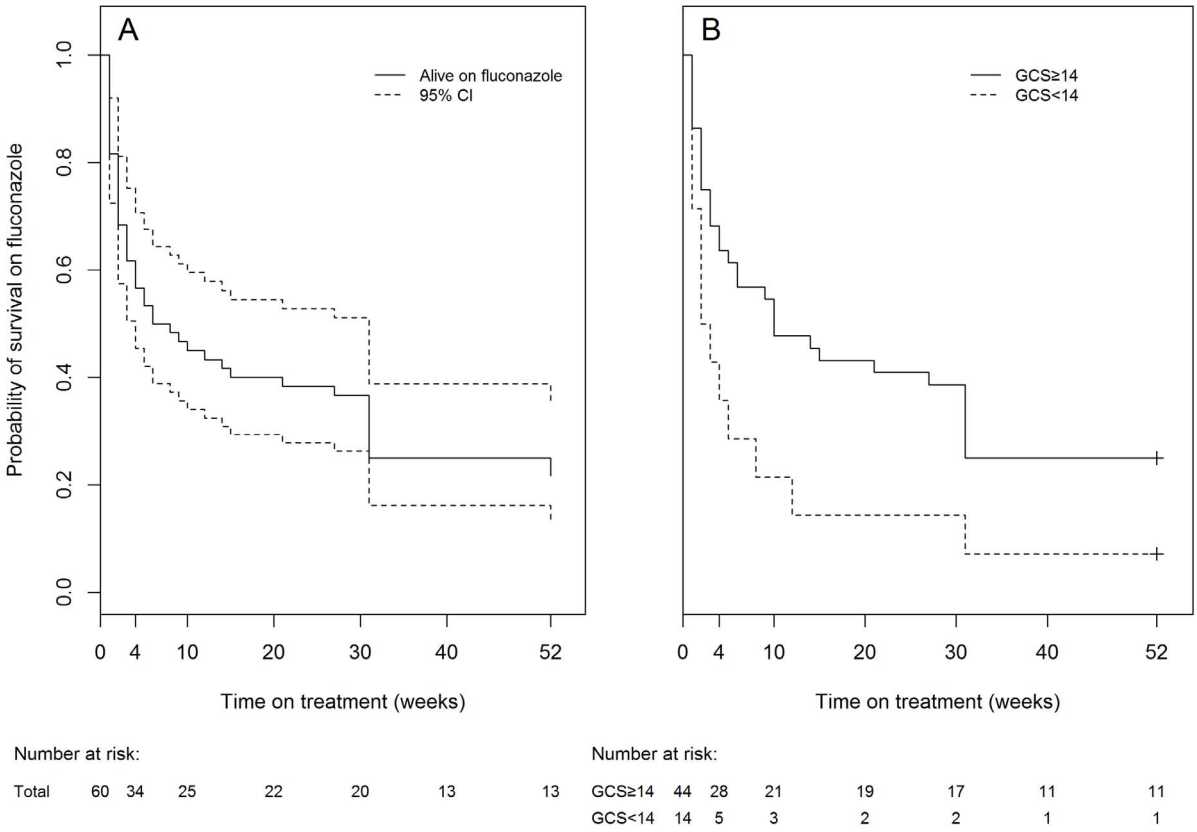

Figure 1. A shows survival on fluconazole therapy for all 60 patients. The solid line shows estimated proportion of survivors and the dotted lines show 95% confidence intervals. Amongst the 47 patients who did not survive on fluconazole to 52 weeks, two had positive CSF cultures at 10 weeks and were switched to fluconazole. The remaining 45 died. **B** shows survival on fluconazole stratified by baseline GCS. 2 patients (one who died in the first week and one who survived to 52 weeks) were excluded from this analysis as no baseline GCS was recorded. The solid line represents patients with baseline GCS≥14 and the dotted line represents patients with baseline GCS<14. Hazard ratio for death or treatment failure by 52 weeks: 2.09, 95% CI: 1.07–4.06 p = 0.030.
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Fluconazole is a fungistatic drug and past studies have shown poor clinical outcomes from monotherapy; in Uganda and Zambia administration of 200 mg/day resulted in 1–2 month survival of only 10–12% [8,9]and in South Africa doubling the dose to 400 mg did not improve in-hospital survival [10]. A recent study demonstrated that dose escalation to 1200 mg/day achieves faster fungal clearance from cerebrospinal fluid (CSF) without increased toxicity [11,12], but clinical end-point studies are lacking. At the time of the study, Malawian national guidelines stipulated the use of oral fluconazole at a dose of 800 mg [13].

Increasingly, early ART introduction has been advocated in patients suffering from opportunistic infections. Therefore, several African countries including Malawi now recommend commencing ART within a month of diagnosis of cryptococcal meningitis [13,14]. However, patients who initiate ART very early (i.e. 3 days after starting 800 mg oral fluconazole) are at risk of intracranial Immune Reconstitution Inflammatory Syndrome (IRIS), which is often severe and can be fatal [15]. It is possible that patients initiated on fungistatic therapy are at greater risk of IRIS as the pathogen persists for longer. Conclusive data are lacking on the optimal time for initiating ART in these patients.

Within the last 5 years evidence has emerged that regimens incorporating short courses of amphotericin B may be safely and effectively used in South Africa and Uganda [16–20]. Clinical trials are now proposed using different combinations of amphotericin B, flucytosine and fluconazole to establish the best approach to treatment in resource poor settings. While preparations for these trials are underway, accurate clinical outcome data from a fluconazole-monotherapy regimen are required to provide a meaningful comparison from a high incidence and resource-poor setting.

From July 2010 until March 2011, 60 consecutive patients with confirmed cryptococcal meningitis in Blantyre, Malawi, were recruited and treated according to national guidelines [13]. They were followed-up for one year in order to determine clinical outcomes from cryptococcal meningitis in a resource limited setting following treatment induction with 800 mg oral fluconazole as monotherapy.

Methods

Ethics Statement

The study was prospectively approved by the University of Malawi, College of Medicine Research Ethics Committee (COMREC protocol P.04/10/926). Cryptococcal meningitis may impair a patient's cognitive function to the extent that assistance of a guardian was required for discussions with the study team and therefore verbal consent was requested to approach all patients on diagnosis of the condition. Informed written consent to enter the study was obtained from patients or their guardians if unconscious. The study itself was completely observational: all procedures performed and medications commenced by the

Table 1. Baseline factors associated with treatment outcome by 4 weeks.

Variable	Total n = 60	Failed Treatment [1] n = 26	Survived n = 34	Hazard ratio (for death)		
				Hazard ratio	95% CI	p-value
Demographics and clinical presentation						
Age, years (median, IQR)	32 (29–39)	35 (28–40)	31 (29–37)	1.03	0.98–1.07	0.286
Male sex (n, %)	33 (55)	14 (54)	19 (56)	0.91	0.42–1.96	0.802
Headache duration, days (median, IQR)	14 (5–30)	14 (4–30)	17.5 (7–30)	1.00	0.99–1.02	0.707
Fever - temperature >37.5°C (n, %)	21 (37)	12 (52)	9 (27)	2.27	1.00–5.15	0.050
Cranial nerve palsy or localising neurological signs (n, %)	5 (10)	2 (8)	3 (9)	1.06	0.25–4.48	0.938
Overall neurological function						
Baseline GCS <14 (n, %)[2]	14 (24)	9 (36)	5 (15)	2.27	0.99–5.11	0.051
Modified Rankin Score (mRS) >3/5 (n, %)	24 (40)	16 (62)	8 (24)	3.00	1.36–6.63	0.007
Abbreviated Mental Test Score (AMTS) <8/10 (n,%)[3]	13 (30)	8 (57)	5 (17)	3.97	1.37–11.50	0.010
HIV Parameters						
On ART at baseline (n, %)[4]	13 (36)	6 (38)	7 (35)	1.21	0.44–3.34	0.709
CSF parameters						
≥5 White cells/mm^{3}in CSF (n, %)	44(76)	20 (77)	25 (76)	1.18	0.46–0.72	0.724
White cell count in CSF (median cells/mm^3, IQR)	20 (5–74)	18 (10–73)	29 (4–72)	1.00	1.00–1.00	0.787
CSF glucose below <2.2 mmol/l (n, %)[5]	27 (61.4)	14 (78)	13 (50)	3.13	1.03–9.53	0.045
CSF protein >0.4 g/L (n, %)[6]	39 (84.8)	16 (80)	23 (89)	0.71	0.24–2.14	0.546
CSF India Ink stain positive (n, %)	36 (61)	16 (61)	20 (61)	1.02	0.46–2.24	0.966

[1]All treatment failures in the first 4 weeks died.
[2]Baseline GCS known for 58 patients (25 failed, 33 survived).
[3]Baseline AMTS performed on 44 patients (14 failed, 30 survived).
[4]ART status known for 36 patients (n = 16 failed, n = 20 survived).
[5]CSF glucose result available from 44 patients (18 failed, 26 survived).
[6]CSF protein results available from 46 patients (20 failed, 26 survived).
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authors were consistent with their roles as attending physicians within the hospital. National and local guidelines and protocols for the management of cryptococcal meningitis and HIV were strictly adhered to by the study team in the routine care of the patients.

Setting

Queen Elizabeth Central Hospital (QECH) is a 1,500 bed hospital in Blantyre, Malawi. It serves a population of approximately 1 million in the Blantyre District and takes tertiary referrals from further afield. Inpatient prevalence of HIV has been estimated at 70% amongst adult patients [21]. Since 2000, the Malawi Liverpool Wellcome Trust Clinical Research Programme (MLW) has offered a routine, quality controlled, diagnostic microbiology service for samples of cerebrospinal fluid (CSF) taken from adults and children presenting with clinical features suggestive of meningitis.

Recruitment and Clinical Assessment

Patients were eligible for recruitment if they received a first microbiological diagnosis of cryptococcal meningitis following either positive India-Ink microscopy or positive culture of CSF. Malawian national guidelines for the treatment of Cryptococcal meningitis were followed; treatment was initiated with oral fluconazole 800 mg once daily (od) for 2 weeks, followed by 400 mg od for 6 weeks, then secondary prophylaxis with 200 mg od indefinitely [13].

Baseline demographic and clinical data were recorded including presence of fever (body temperature >37.5°C) and cranial nerve palsies or focal neurological signs. Prior use of ART and CD4 count at baseline were noted. Three aspects of overall neurological function were assessed; consciousness by Glasgow Coma Score (GCS), physical disability by modified Rankin Score (mRS) and cognition by Abbreviated Mental Test Score (AMTS). mRS is a 6 point scale (0 = No symptoms, 1 = No significant disability, 2 = Minor disability,3 = Moderate disability, 4 = Moderate-severe disability, 5 = Severe disability/bed-ridden), which has previously been used in Malawi for stroke patients [22]. AMTS is the sum of correct responses to 10 standard questions [23], adapted for Malawi by changing "When did World War I begin?" to "When did Malawi attain independence?" and replacing "Name the current UK monarch" with "Name the current Malawian president". AMTS has not been validated in Malawi and results may be subject to bias by cultural background and educational attainment [24], however there was no better method of assessing cognition available.

It was not possible to measure CSF pressure and LPs were only repeated if patients developed clinical features of raised intracranial pressure.

Patients were reviewed 4, 10 and 52 weeks after initiation of fluconazole, and encouraged to contact the study team at any time if clinical deterioration occurred. ART was commenced 4 weeks into fluconazole therapy. The study team ensured continuous access to fluconazole and ART.

Table 2. Baseline factors associated with treatment outcome by 10 and 52 weeks.

Variable	Treatment outcomes by 10 weeks Hazard ratio (for death or failure)			Treatment outcomes by 52 weeks Hazard ratio (for death or failure)		
	Hazard ratio	**95% CI**	**p-value**	**Hazard ratio**	**95% CI**	**p-value**
Demographics and clinical presentation						
Age, years (median, IQR)	1.01	0.96–1.05	0.758	1.01	0.96–1.05	0.803
Male sex (n, %)	0.69	0.35–1.34	0.273	0.70	0.39–1.29	0.254
Headache duration, days (median, IQR)	1.00	0.99–1.01	0.790	1.00	0.99–1.01	0.528
Fever - temperature >37.5°C (n, %)	1.47	0.72–2.98	0.295	1.27	0.66–2.41	0.469
Cranial nerve palsy or localising neurological signs (n, %)	0.76	0.18–3.18	0.709	1.78	0.62–4.94	0.285
Overall neurological function						
Baseline GCS <14	2.23	1.08–4.62	0.030	2.09	1.07–4.06	0.030
Modified Rankin Score (mRS) >3/5>3/5 (n, %)	2.28	1.17–4.47	0.011	1.76	0.96–3.22	0.068
Abbreviated Mental Test Score (AMTS) <8/10	2.96	1.28–6.85	0.011	2.41	1.12–5.19	0.024
HIV Parameters						
On ART at baseline (n, %)	1.46	0.62–3.42	0.385	1.47	0.68–3.19	0.326
CSF parameters						
>5 White cells/ml in CSF (n, %)	1.05	0.35–0.89-	0.984	0.93	0.49–1.77-	0.831
White cell count in CSF (cells/mm^3)	1.00	0.99–1.01	0.841	0.99	0.99–1.00	0.676
CSF glucose below <2.2 mmol/l (n, %)[6]	1.81	0.77–4.25	0.172	1.29	0.63–2.62	0.483
CSF protein >0.5 g/L (n, %)	0.66	0.25–1.78	0.414	0.60	0.26–1.40	0.239
CSF India Ink stain positive (n, %)	0.82	0.42–1.60	0.559	0.86	0.47–1.56	0.607

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Treatment outcomes were defined as survival or treatment failure (death or therapy changed to amphotericin B following clinical deterioration with persistently positive CSF cultures after one month of fluconazole). This pragmatic approach to refractory cases was necessitated by a limited supply of amphotericin B, a shortage of trained staff to administer the drug and a lack of routine diagnostic biochemistry to monitor side effects.

Laboratory Methods

All CSF diagnostic testing was performed at MLW. Cell counts were performed using a FastRead (Immune Systems) disposable counting chamber. India-Ink microscopy was performed on all unclotted samples of adequate volume. All samples were cultured on sheep blood and chocolate agar for 48 hours under aerobic and microaerophilic conditions and sub-cultured onto sabouraud agar and urea slopes if they were India-Ink positive or culture yielded yeasts. Bacteria and yeasts were identified using standard methods [25]. Neither cryptococcal antigen testing nor antifungal susceptibility testing was routinely available. Mycobacterial culture of CSF was not performed. The MLW laboratory participates in internationally recognised quality control programmes including NEQAS (UK) and the South African NHLS scheme. Full blood count and CD4 count were performed at the main QECH laboratory.

Statistics

Clinical and microbiological data was stored in a Microsoft Excel file and analysed using "R" version 2.12.1. Baseline characteristics and treatment outcomes were reported by simple descriptive statistics. A two-sample Wilcoxon test was used to assess the relationship between baseline CD4 count and prior ART. Kaplan-Meier plots were used to demonstrate survival on fluconazole by 52 weeks. Data from previous studies suggest that deaths within the first month of treatment are directly attributable to cryptococcal meningitis whilst later deaths are multi-factorial [26]. Therefore, analysis of variables associated with poor outcome was done using Cox proportional hazards regression modelling with an end-point of "time to death" during the first four weeks. Hazard Ratios (HRs) with 95% confidence intervals (CI) were presented. This analysis was repeated using alternative end-points of "time to death or treatment failure" during 10 and 52 weeks of follow-up. In all analyses, a p-value of <0.05 was considered statistically significant.

Results

Baseline Demographic and Clinical Features

Sixty patients with a first episode of cryptococcal meningitis were recruited. Thirty-three (55%) were male and the median age was 32 years (range: 15–62). All patients complained of headache at presentation, with a median duration of 14 days (range: 1–150). 21 (37%) were febrile.

Baseline GCS was <14 of 15 in 14/58 (24%) patients. mRS was >3 (moderate disability) in 24/60 (40%) patients. 13/44 (30%) patients who completed an AMTS on admission obtained a score of <8/10. Five (10%) patients had localising neurological signs (two with focal weakness, two with cranial nerve VI palsies and one with both of these abnormalities).

All patients were HIV-infected. Thirty-two had a CD4 count result available, the median result was 37cells/μl (range: 2–234 cells/μl). Thirteen (22%) patients were on ART at presentation. Their median CD4 count was 55 cells/μl (range: 2–234cells/μl). There was no association between CD4 count and prior ART (two-sample Wilcoxon test, $p = 0.69$).

Baseline CSF Characteristics

All 60 patients had evidence of cryptococcal infection in their CSF; 57 (95%) were culture positive and the remaining 3 (5%) had positive microscopy but no culture result available. White cells were seen in the CSF of 45 (75%) patients. The median white cell count was 20 cells/μl (range: 0–520).

Forty-six patients had a documented CSF protein result and 46 a documented CSF glucose. Median CSF protein was 0.78 g/L (range: 0.23–4.04, normal 0.15–0.40 g/L) and median CSF glucose was 1.97 mmol/L (range: 0.06–3.65, normal 2.22–3.88 mmol/L). 39/46 (85%) patients had high CSF protein and 27/44 (61%) had low CSF glucose values.

Treatment Outcomes

26/60 (43%) patients died in the first 4 weeks. A total of 33 (55%) died by 10 weeks, and a further 2 failed fluconazole monotherapy so were switched to intravenous amphotericin B between weeks 2 and 10. In total, 35/60 (58%) patients had fluconazole treatment failure by 10 weeks.

Between weeks 10 and 52, a further 8 patients died and 4 were lost to follow-up. There were only 13 known survivors one year after presentation. Of these, six were reviewed in person and seven were interviewed by telephone. The six presenting for review were taking both ART and secondary fluconazole prophylaxis. All had good neurological recovery (6 had AMTS = 10, 5 had mRS = 0, and one had mRS = 1). In total 43/56 (77%) of patients had failure of treatment of cryptococcal meningitis at one year following treatment induction with 800 mg oral fluconazole. Figure 1a shows that the probability of survival on fluconazole monotherapy for 4 weeks was 55% (95% CI: 44–70%), for 10 weeks was 43% (95% CI: 32–58%) and for one year was 22% (95% CI: 14–36%).

Baseline Clinical Factors Associated with Treatment Outcome

Table 1 shows univariate analysis of associations between baseline variables and time to death during the first 4 weeks. Of the clinical factors, mRS>3 (HR: 3.00, 95% CI: 1.36–6.63) and AMTS score <8/10 (HR: 3.97, 95% CI: 1.37–11.50) were associated with shorter survival and there were strong trends towards earlier death with fever (HR: 2.27, 95% CI: 1.00–5.15) and GCS<14 (HR: 2.27 95%CI: 0.99–5.11). Of the laboratory factors, CSF glucose <2.2 mmol/l (HR: 3.13, 95% CI: 1.03–9.53) was associated with earlier death. Multivariate analysis was precluded by a high degree of co-linearity between clinical variables describing overall neurological function (mRS, AMTS and GCS), and the number of missing data-points amongst some laboratory variables.

When the univariate analysis was repeated with end-points of treatment failure by 10 or 52 weeks (Table 2), the importance of low CSF glucose concentration and fever at baseline were lost. However, on 10 week analysis AMTS<8/10 (HR: 2.96, 95%CI: 1.28–6.85), mRS>3 (HR: 2.96, 95%CI: 1.17–4.47) and GCS<14 (HR: 2.23, 95% CI: 1.08–4.62) were significantly associated with shorter survival. On 52 week analysis AMTS<8/10 (HR: 2.41, 95% CI: 1.07–4.06) and GCS<14 (HR: 2.09, 95% CI: 1.07–4.06) remained significant. Figure 1b demonstrates the probability of survival on fluconazole over 52 weeks sub-divided by baseline GCS<14 or >14.

Prior ART was not associated with better outcomes. Of the 13 patients on ART at baseline, 9 (69%) had died at 10 weeks and 11 (84%) at one year. As HIV viral loads, quantitative CSF fungal culture and CSF mycobacterial culture were unavailable, it was impossible to distinguish cryptococcal IRIS, HIV treatment failure and other causes of cerebral infection or IRIS.

CD4 counts were unavailable for 28 patients, 11 of who died within the first 4 weeks so were not used in the analysis.

Discussion

Clinical outcomes from cryptococcal meningitis in HIV infected Malawian adults following treatment induction with 800 mg oral fluconazole were poor, with 43% mortality at 4 weeks, 58% treatment failure at 10 weeks and 77% treatment failure at 1 year. These data are consistent with prior studies of fluconazole monotherapy at both lower [8–10] and identical [11] doses in Africa and show only a modest improvement from the pre-ART and pre-fluconazole era. The 58% treatment failure rate at 10 weeks is similar to the 60% (18/30 patients) seen in a smaller cohort from a fluconazole dose response study in Uganda [11]. Malawi has since increased the induction phase dose of fluconazole to 1200 mg, although clinical end-point data from randomised controlled studies using this regimen are unavailable.

The contribution of baseline clinical and laboratory variables to poor outcomes by 4, 10 and 52 weeks was assessed. Although AMTS provides an imperfect assessment of cognitive function, a score of <8/10 was associated with earlier death or treatment failure at all time-points. Prior studies have reported similar results [26], suggesting that altered mental status at presentation is a persistently strong prognostic marker. Moderate or severe functional disability (mRS>3) and reduced consciousness (GCS<14), another marker of altered mental status were also associated with shorter survival by early and late time-points of analysis.

Fever (temperature >37.5°C) and low CSF glucose measurements (<2.2 mmol/l) were associated with poor outcomes by 4 weeks but were insignificant by later time-points. This finding is consistent with data suggesting the longer an individual survives, the less likely that subsequent death is due to cryptococcal meningitis [26]. Conversely, patients with impaired cognition, functional disability or reduced consciousness are at prolonged higher risk of poor adherence to medications and late treatment complications such as pressure sores, intercurrent infection and thrombosis. This may explain why baseline neurological deficit remained strongly linked to poor outcome after one year of follow-up.

The high incidence of altered mental status at baseline suggests that many patients presented with advanced disease. As the study contained no comparator arm, it is impossible to say whether better anti-fungal therapy would have improved outcomes in late presenters with worse neurological deficits. However, it is likely that public health interventions to encourage earlier presentation of patients with symptoms of meningitis will augment optimal anti-fungal treatments.

CD4 count data were incomplete, however several patients with extremely low CD4 counts survived to one year (median CD4 count amongst 13 long-term survivors: 13 (IQR 4–41) cells/μl). This illustrates the potential for full recovery from cryptococcal meningitis provided an adequate framework is in place to ensure uninterrupted supply of antifungals and timely commencement of ART. Without the intensive follow-up afforded by study participation, mortality in this group may have been higher.

Prior ART was not associated with improved survival and CD4 counts amongst ART-experienced patients were no higher than the rest of the cohort. This may represent initiation of ART during severe immune-suppression (i.e. CD4 count has risen from an unknown nadir) or ART failure. Irrespective, these data reinforce

the well documented association between late ART initiation and higher mortality [27,28] and emphasise the need for early HIV diagnosis and point of care cryptococcal antigen screening to detect early cryptococcal disease in high risk patients.

There were some limitations to this work. The sample size was small. As the study was conducted amidst routine clinical practice some data were incomplete, precluding multivariate analysis. Given the constraints of our setting, standardised management of high CSF pressure was not possible and it was difficult to characterise all co-morbidities, episodes of IRIS or causes of death after 4 weeks.

Conclusions

We have described clinical outcomes from cryptococcal meningitis in patients initiated on 800 mg oral fluconazole monotherapy. Despite timely ART initiation and uninterrupted drug supply through near complete follow up, there was unacceptably high mortality. There is an urgent need to assess the efficacy of faster acting, combination fungicidal regimens, particularly in light of recent work on those containing flucytosine [29,30]. In order to address this, a phase III trial assessing short-course amphotericin B and oral combination regimens will commence in 2013 [''Advancing cryptococcal meningitis treatment in Africa'' (ACTA) ISRCTN: 45035509]. Improvements in clinical outcomes from cryptococcal meningitis must also involve public health interventions to encourage early presentation and roll-out of rapid, point of care diagnostics [31]. Earlier HIV diagnosis and ART initiation is of equal importance to prevent all diseases of advanced immunosuppression [32].

Author Contributions

Conceived and designed the experiments: NF CR DJS RSH JvO TSH DGL TA. Performed the experiments: NF DJS CR PG JC BD. Analyzed the data: NF DJS MM CR. Contributed reagents/materials/analysis tools: NF BD RSH. Wrote the paper: NF CR DJS. MLW Laboratory Manager: BD.

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PLOS ONE

A Prospective Study of Mortality from Cryptococcal Meningitis following Treatment Induction with 1200mg Oral Fluconazole in Blantyre, Malawi

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Abstract

Objective: We have previously reported high ten-week mortality from cryptococcal meningitis in Malawian adults following treatment-induction with 800mg oral fluconazole (57% [33/58]). National guidelines in Malawi and other African countries now advocate an increased induction dose of 1200mg. We assessed whether this has improved outcomes.

Design: This was a prospective observational study of HIV-infected adults with cryptococcal meningitis confirmed by diagnostic lumbar puncture. Treatment was with fluconazole 1200mg/day for two weeks then 400mg/day for 8 weeks. Mortality within the first 10 weeks was the study end-point, and current results were compared with data from our prior patient cohort who started on fluconazole 800mg/day.

Results: 47 participants received fluconazole monotherapy. Despite a treatment-induction dose of 1200mg, ten-week mortality remained 55% (26/47). This was no better than our previous study (Hazard Ratio [HR] of death on 1200mg vs. 800mg fluconazole: 1.29 (95% CI: 0.77–2.16, p = 0.332)). There was some evidence for improved survival in patients who had repeat lumbar punctures during early therapy to lower intracranial pressure (HR: 0.27 [95% CI: 0.07–1.03, p = 0.055]).

Conclusion: There remains an urgent need to identify more effective, affordable and deliverable regimens for cryptococcal meningitis.

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Introduction

Cryptococcal meningitis is the commonest cause of meningitis in adults in sub Saharan African (SSA) countries with high HIV seroprevalence [1]. The global incidence of cryptococcal meningitis was estimated at 957,900 cases/year in 2009 and 75% of cases occur in SSA [2]. In Blantyre, Malawi, *C. neoformans* was responsible for 70% of adult CSF-culture positive meningitis presenting to a tertiary referral hospital from 2000 until 2012 [3]. This burden of cryptococcal meningitis has not changed despite a highly successful national programme of antiretroviral therapy (ART) roll-out since 2004 [3].

Current gold standard induction therapy is two weeks of amphotericin B and flucytosine [4,5]; however these drugs remain largely unavailable in SSA including Malawi [6]. Amphotericin B is not only expensive, but difficult to administer and associated with toxicities which are challenging to monitor in resource-poor settings. Consequently, high-dose oral fluconazole is widely used in SSA, but has significantly weaker early fungicidal activity than the gold standard regimen [4,7].

We have previously reported extremely poor outcomes from cryptococcal meningitis in Blantyre, when treated with 800mg daily oral fluconazole as induction therapy [8]. In 2011 the Malawian national treatment guidelines regarding the management of cryptococcal meningitis changed, increasing the initial dose of fluconazole at treatment induction from 800mg to 1200mg daily [9]. Many African health services elected to make this change following a study that demonstrated better early fungicidal activity

using 1200mg fluconazole than with 800mg [10]. We present a pragmatic, prospective observational study of clinical outcomes from cryptococcal meningitis using this dose, which is the current standard of care for many African countries.

Methods

Queen Elizabeth Central Hospital (QECH) Blantyre is the largest government hospital in Malawi and admits approximately 10,000 adult patients per year. All patients with clinical features of meningitis undergo diagnostic lumbar puncture (LP). Inclusion criteria were unchanged from the previous study [8].

Consecutive adult patients (age ≥16) with a first presentation of cryptococcal meningitis were recruited between September 2012 and May 2013. The diagnosis was confirmed by positive India-Ink microscopy of CSF or culture-confirmed *C. neoformans* from CSF. Cryptococcal antigen testing (CrAg), quantitative cryptococcal cultures and fluconazole resistance testing were unavailable. Subjects' clinical history, including HIV diagnosis and ART history were recorded. Patients without a recent HIV test were confidentially counselled and tested. Presence of focal neurological deficit, Glasgow Coma Score (GCS) and modified Rankin score (mRS) were recorded. mRS is a 6 point disability scale (0 = No symptoms, 1 = No significant disability, 2 = Minor disability, 3 = Moderate disability, 4 = Moderate-severe disability, 5 = Severe disability/bed-ridden). Patients were reviewed on admission to the study, on discharge home, at four weeks and ten weeks from diagnosis.

Patients were treated according to national guidelines with 1200mg fluconazole per day for two weeks at induction followed by 400mg/day for a further 8 weeks, then lifelong secondary prophylaxis at 200mg/day [9]. A small donated supply of Amphotericin B was sporadically available for readmitted patients with evidence of fluconazole failure and patients swapped to this agent were withdrawn from the study. Patients not already receiving ART were initiated 4 weeks after diagnosis. Although national guidelines recommend daily LPs to serially reduce intracranial pressure (ICP) during early therapy, this was impossible due to staffing limitations and a lack of CSF manometry equipment. Routine practice at QECH was to undertake therapeutic LP in the event of symptoms suggesting raised ICP (e.g. severe headache).

The study endpoint was mortality at 10 weeks from diagnosis.

Statistical analysis was undertaken using "R" (version 2.15.2). Clinical parameters of study participants were compared with those from our previous study [6] by a two sample Wilcoxon test for continuous variables or a χ^2-test for categorical variables. The study endpoint, and the relationships between prior ART or repeat LPs and mortality were assessed by survival analysis using Hazard Ratios (HRs) and Kaplan-Meier plots. The binomial exact test was used to calculate confidence intervals (CI) around death rates.

The study was prospectively approved by the University of Malawi College of Medicine Research Ethics Committee (COMREC no: P04/10/926). Informed written consent was obtained from patients to enrol in the study. Informed written consent was obtained from guardians if patients lacked mental capacity, due to advanced cryptococcal disease, to provide valid consent. The consent procedure and forms were reviewed and approved by COMREC, including the guardian consenting procedure. Two copies of the consent forms were signed per patient; one was retained by the patient and another by the study team.

Results

58 patients were screened for enrolment, 3 patients were excluded because *C. neoformans* was isolated from blood only. Five patients were lost to follow up and therefore not included in the analysis. 3 patients were withdrawn because they were switched to Amphotericin B therapy by their physician; 2 of whom survived. Data from the remaining 47 patients is presented here; 46 were CSF culture-positive for *C. neoformans* and one patient was culture negative but India-Ink microscopy positive.

The median age was 35 years (Inter-quartile range [IQR]: 32–40 years) and 51% (24/47) were male. All patients presented with headache, the median duration at presentation was 7 days (IQR: 7–17 days). 24% (11/46) had a GCS <14/15. mRS scores showed 24/50 (51%) subjects had moderate to severe disability (grade 3–5).

All patients were HIV infected with a median CD4 count of 36 cells/μl (IQR: 17–62 cells/μl). At baseline, 45% (21/47) were taking ART for a median duration of 63 days (IQR: 21–551 days). A further 17% (8/47) commenced ART during the course of the study and 6% (3/47) had previously commenced ART but defaulted treatment.

Mortality at 10 weeks was 55% (26/47). The median time to death was 16 days (IQR: 7–49 days). In the previous cohort, mortality at 10 weeks amongst patients who received fluconazole 800mg/day was 57% (33/58) and median time to death was 19 days (IQR: 6–61 days). Figure 1 shows Kaplan-Meier survival plots for patients initiated on fluconazole 800mg (1A) and fluconazole 1200mg (1B); there is no difference in survival between the two induction doses. The HR for death on 1200mg vs. 800mg was 1.29 (95% CI: 0.77–2.16, p = 0.332).

The only significant clinical differences between patients in this study and the previous cohort pertained to ART; a higher proportion of patients in the current study were taking ART at enrolment and the duration of prior ART was longer (see Table 1). In the current study, there was some evidence of improved survival in patients on ART prior to enrolment (HR for death in those who presented on ART vs. those not on ART: 0.48 [95% CI: 0.21–1.07, p = 0.071]). Combining all 105 patients

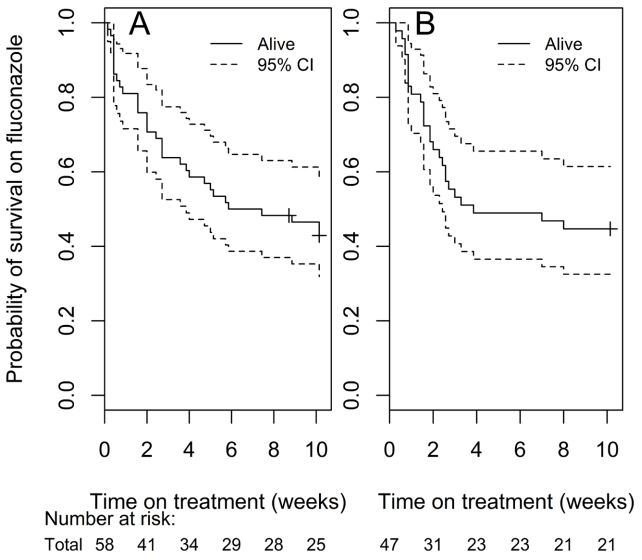

Figure 1. Kaplan-Meier Survival plot of patients on fluconazole. A: 800mg induction dose. B: Kaplan-Meier Survival plot of patients on fluconazole 1200mg induction dose.
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Table 1. Comparison of baseline variables between patients initiated on fluconazole 800mg and those initiated on fluconazole 1200mg.

Baseline variable	Patients initiated on *Fluconazole 800mg OD* N = 58 [8]	Patients initiated on *Fluconazole 1200mg OD* N = 47	p-value[a]
Age in years, median (IQR)	32 (29–39)	35 (32–40)	0.130
Male sex, n (%)	33 (55)	24 (51)	0.824
Headache duration in days, median (IQR)	14 (5–30)	7 (7–17)	0.508
Cranial nerve palsy/localising signs, n (%)[b]	5 (10)	12 (21)	0.225
GCS <14/15, n (%)	14 (25)	11 (24)	1
Modified Rankin Score>3/5, n (%)	24 (41)	23 (49)	0.564
HIV status known at recruitment, n (%)	35 (60)	35 (75)	0.187
CD4 count in cells/μl, median (IQR)	37 (11–58)	36 (17–62)	0.721
On ART at baseline, n (%)	13 (22)	21 (45)	0.027
Duration of prior ART, median (IQR)[c]	20 (5–67)	63 (21–511)	0.048

[a]Continuous variables analysed by Wilcoxon test, categorical variables analysed by χ^2-test test.
[b]Includes blindness, cranial nerve palsies or focal weakness.
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across both studies, mortality was not significantly affected by prior ART (HR for death: 0.84 [95% CI: 0.49–1.46, p = 0.550]). No other evidence of difference in clinical severity at presentation between the two cohorts was observed.

Repeat CSF drainage was performed on 11/23 (23%) patients in the current study and 16/58 (28%) in the previous cohort with symptoms suggestive of raised ICP. In the current study, there was weak evidence towards survival in patients who had repeat LPs (HR for death if LP repeated: 0.27 [95% CI: 0.07–1.03, p = 0.055]). When data from both studies were combined, this weak survival evidence persisted (HR for death: 0.52 [95% CI: 0.25–1.07, p = 0.077]).

This study was not designed as a prospective randomised controlled comparison two doses of fluconazole. Instead, we present prospective observational data revealing death from fluconazole in 55% (95% CI: 40–70%) patients treated with 1,200mg fluconazole and 57% (95% CI: 43–70%) patients treated with 800mg fluconazole. Both studies indicate that cryptococcal meningitis therapy based on induction with oral fluconazole, whether at 800 or 1200mg/day achieves unacceptably poor outcomes.

Discussion

A Ugandan study of fluconazole demonstrated higher EFA in CSF at a daily dose of 1200mg than 800mg without increased toxicity [10], and it was hoped that this would translate into improved clinical outcomes. However, a more recent pharmacokinetic-pharmacodynamic (PK-PD) model of treatment with 1200mg fluconazole predicted that only 67% of patients on this dose will obtain adequate CSF drug concentrations to achieve fungal stasis [11]. In our study, 10-week mortality from cryptococcal meningitis in Malawi has not improved following an increase in the induction dose of fluconazole from 800mg to 1200mg. The highest mortality occurred in the initial two weeks, suggesting that rapid fungal clearance is essential for a good clinical outcome.

Expanded access to ART in Malawi [12] meant that more patients had received ART before recruitment to the current cohort than in our previous study of fluconazole 800mg/day [8]. This raises the possibility that Immune Reconstitution Inflammatory Syndrome (IRIS) masked a benefit from the higher dose of fluconazole, however in the current study, there was slightly higher survival in patients on ART and in a combined analysis of both studies the effect of prior ART was non-significant. Secondly IRIS is unlikely as these patients were culture positive with CD4 counts <50 cells/uL at presentation. Overall, these data do not support a negative confounding effect from IRIS.

Despite improved ART provision, there remains a large population with advanced HIV in Blantyre, and there is an urgent need to protect them from cryptococcal meningitis. General strategies include earlier HIV diagnosis and treatment. A specific approach with increasing evidence of cost-effectiveness is CrAg screening and treatment of asymptomatic antigenaemia in the ART clinic [13,14,15].

Several studies have reported that serial LPs to lower ICP during treatment of cryptococcal meningitis may improve outcomes [16,17]. Although Malawian national guidelines suggest performing daily LPs during early therapy [9], there is neither adequate staffing nor the equipment for this to be possible. Without CSF manometers we could only repeat LPs on patients with symptoms of raised ICP. There was weak evidence of improved survival in this group. Importantly the individuals selected for additional procedures had the worst initial symptoms; these data support the importance of therapeutic CSF drainage. The benefit of serial LPs may be even greater if they are routinely guided by CSF pressure measurement.

The current and previous study recruited patients with similar characteristics in the same setting, however they have limitations; the studies were undertaken sequentially, not as part of a combined randomised controlled trial. Furthermore, there was some loss to follow up and it was different to monitor adherence to therapy. Serial CD4-counts and HIV-viral loads were unavailable preventing a formal diagnosis of IRIS. Nevertheless, the studies provide important information on the treatment of cryptococcal meningitis from an authentic high-burden setting.

Oral fluconazole is currently the only routinely available therapy in much of SSA, but mortality rates are unacceptably high. An increase in the recommended induction dose from 800mg to 1200mg daily has had no impact on clinical outcomes in Malawi. There are on-going studies with even higher doses of fluconazole, (ACTG study: http://clinicaltrials.gov/show/NCT00885703) and of novel therapeutic strategies ("Advancing

cryptococcal meningitis treatment in Africa'' [ACTA] ISRCTN: 45035509). Whilst the outcome of these studies is awaited, there remains an urgent need for expanded access to amphotericin B and flucytosine across SSA.

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Author Contributions

Conceived and designed the experiments: CR NAF TJA RSH DGL TSH. Performed the experiments: KMG CR RG PG. Analyzed the data: DSJ KMG NAF. Contributed reagents/materials/analysis tools: RSH CJ. Wrote the paper: KMG NAF DJS. Reviewed article: RSH DGL TJA TSH CR RG.

ORIGINAL ARTICLE

Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa

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Drs. Molloy, Kanyama, Heyderman, and Loyse and Drs. Jaffar and Harrison contributed equally to this article.

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ABSTRACT

BACKGROUND

Cryptococcal meningitis accounts for more than 100,000 human immunodeficiency virus (HIV)–related deaths per year. We tested two treatment strategies that could be more sustainable in Africa than the standard of 2 weeks of amphotericin B plus flucytosine and more effective than the widely used fluconazole monotherapy.

METHODS

We randomly assigned HIV-infected adults with cryptococcal meningitis to receive an oral regimen (fluconazole [1200 mg per day] plus flucytosine [100 mg per kilogram of body weight per day] for 2 weeks), 1 week of amphotericin B (1 mg per kilogram per day), or 2 weeks of amphotericin B (1 mg per kilogram per day). Each patient assigned to receive amphotericin B was also randomly assigned to receive fluconazole or flucytosine as a partner drug. After induction treatment, all the patients received fluconazole consolidation therapy and were followed to 10 weeks.

RESULTS

A total of 721 patients underwent randomization. Mortality in the oral-regimen, 1-week amphotericin B, and 2-week amphotericin B groups was 18.2% (41 of 225), 21.9% (49 of 224), and 21.4% (49 of 229), respectively, at 2 weeks and was 35.1% (79 of 225), 36.2% (81 of 224), and 39.7% (91 of 229), respectively, at 10 weeks. The upper limit of the one-sided 97.5% confidence interval for the difference in 2-week mortality was 4.2 percentage points for the oral-regimen group versus the 2-week amphotericin B groups and 8.1 percentage points for the 1-week amphotericin B groups versus the 2-week amphotericin B groups, both of which were below the predefined 10-percentage-point noninferiority margin. As a partner drug with amphotericin B, flucytosine was superior to fluconazole (71 deaths [31.1%] vs. 101 deaths [45.0%]; hazard ratio for death at 10 weeks, 0.62; 95% confidence interval [CI], 0.45 to 0.84; P=0.002). One week of amphotericin B plus flucytosine was associated with the lowest 10-week mortality (24.2%; 95% CI, 16.2 to 32.1). Side effects, such as severe anemia, were more frequent with 2 weeks than with 1 week of amphotericin B or with the oral regimen.

CONCLUSIONS

One week of amphotericin B plus flucytosine and 2 weeks of fluconazole plus flucytosine were effective as induction therapy for cryptococcal meningitis in resource-limited settings. (ACTA Current Controlled Trials number, ISRCTN45035509.)

Cryptococcal meningitis is the most common form of adult meningitis in many regions that have a high prevalence of human immunodeficiency virus (HIV) infection[1,2] and accounts for 10 to 20% of all HIV-related deaths, with more than 100,000 deaths each year.[3] This high burden is driven by a high case fatality rate, which in sub-Saharan Africa is estimated to be 70% at 3 months.[3,4]

Treatment of cryptococcal meningitis in resource-limited settings is challenging. The international standard induction treatment of 2 weeks of amphotericin B deoxycholate plus flucytosine[5] is not available in most African clinical centers. Amphotericin B requires intravenous administration and close laboratory monitoring and is associated with phlebitis, secondary infections, anemia, and renal impairment.[6] Flucytosine is currently unavailable, although the molecule is used widely as a constituent of emtricitabine, and generic manufacture is possible at low cost.[7] Most countries therefore rely on generic or donated fluconazole induction monotherapy; however, the rate of fungal clearance with fluconazole is slower than that with amphotericin B, even at an elevated dosage, and mortality associated with this treatment is 50 to 60% at 10 weeks and is higher than 70% at 1 year even in study cohorts.[8,9]

Phase 2 studies have defined several promising treatment strategies that are associated with fungal clearance similar to that with 2-week amphotericin B regimens and that have more favorable safety profiles. An oral combination of fluconazole and flucytosine was found to be associated with a rate of clearance of infection similar to that with amphotericin B alone and to be associated with higher survival rates than those with fluconazole alone.[10] Shorter-course amphotericin B had a more favorable side-effect profile than standard 2-week courses, with no diminution in the rate of clearance of infection in the second week, perhaps because of the long half-life of amphotericin B in brain tissue.[11,12] The efficacy of shorter-course amphotericin B treatment has also been shown in animal models.[13]

In addition, the drug of choice to combine with amphotericin B remains unclear. In a previous trial, amphotericin B plus flucytosine was associated with higher survival rates at day 70 than amphotericin B alone.[14] Amphotericin B plus flucytosine was not found to differ from amphotericin B plus fluconazole with regard to mortality at 10 weeks, but the results of a secondary analysis at 6 months favored flucytosine.[14] However, these results have been insufficient to drive wider availability of flucytosine.[15]

Therefore, we tested two new treatment strategies that could be more readily sustainable in African centers than 2 weeks of amphotericin B and more effective than fluconazole: oral therapy with higher-dose fluconazole plus flucytosine, and a shorter course (1 week) of induction therapy with amphotericin B–based treatment. These regimens were compared with a 2-week regimen of amphotericin B–based treatment. In addition, within the amphotericin B groups, we randomly assigned patients to receive either flucytosine or fluconazole as the partner drug.

METHODS

TRIAL DESIGN AND OVERSIGHT

We conducted an open-label, phase 3, randomized, noninferiority, multicenter trial (Advancing Cryptococcal Meningitis Treatment for Africa [ACTA]) to compare three treatment strategies (an oral combination regimen of fluconazole plus flucytosine, 1 week of amphotericin B, and the standard 2 weeks of amphotericin B) for the induction treatment of HIV-associated cryptococcal meningitis. Flucytosine and fluconazole were also evaluated as partner drugs with amphotericin B.

Participants were recruited from nine African centers: Queen Elizabeth Central Hospital, Blantyre, Kamuzu Central Hospital, Lilongwe, and Zomba Central Hospital, Zomba, Malawi; University Teaching Hospital, Lusaka, Zambia; Muhimbili, Amana, and Mwananyamala Hospitals, Dar Es Salaam, Tanzania; and Hôpital Central, Yaoundé, and Douala General Hospital, Douala, Cameroon. The protocol was approved by the London School of Hygiene and Tropical Medicine Research Ethics Committee and by all the site national research ethics committees and regulatory bodies. Written informed consent was obtained from all the patients or, in the case of patients with altered mental status, from the next of kin (consent was obtained from these patients after recovery).

Lateral-flow cryptococcal antigen tests were donated by or purchased from IMMY. Trial drugs were purchased from Bristol-Myers Squibb (am-

photericin B [Fungizone]), Meda Pharmaceuticals (flucytosine), and Cipla or Medopharm (fluconazole). In places where the Pfizer fluconazole donation program was running, donated fluconazole was used when available. The trial funders, suppliers, and drug manufacturers had no role in trial design; data collection, analysis, and interpretation; or manuscript preparation. The authors vouch for the accuracy and completeness of the data and for the adherence of the trial to the protocol, available with the full text of this article at NEJM.org.

TRIAL PARTICIPANTS

HIV-seropositive adults (≥18 years old) with a first episode of cryptococcal meningitis who tested positive on India ink staining, cryptococcal antigen assay, or both in cerebrospinal fluid (CSF) were included. Patients were excluded if they had previously received more than one dose of amphotericin B or more than one treatment dose (1200 mg) or more than seven low doses (200 mg) of fluconazole in the 2 weeks before screening, were pregnant or lactating, were taking contraindicated concomitant drugs, or had any previous adverse reactions to the trial drugs.

An alanine aminotransferase (ALT) level that was more than 5 times the upper limit of the normal range, a polymorphonuclear leukocyte count that was less than 500 per cubic millimeter, or a platelet count that was less than 50,000 per cubic millimeter were late-exclusion criteria (i.e., a patient who met one or more of these criteria at baseline was withdrawn from the trial). In addition, if an elevated creatinine level remained above 220 μmol per liter on the day after randomization despite the patient receiving rehydration, the patient was withdrawn from the trial.

Initially, patients were excluded if they had previously been exposed to antiretroviral therapy (ART). However, because it became clear that a large number of patients were presenting with cryptococcal meningitis while taking ART or with previous exposure to ART, soon after commencement of the trial (after 4% of total enrollment), a protocol amendment allowed the inclusion of these patients. Full details of the trial design can be found in the protocol and statistical analysis plan.

INTERVENTIONS AND RANDOMIZATION

We assessed three treatment strategies (the use of an oral regimen, a 1-week amphotericin B regimen, and a 2-week amphotericin B regimen), as well as two alternative partner drugs for amphotericin B (fluconazole or flucytosine). The oral regimen consisted of fluconazole (1200 mg per day) plus flucytosine (100 mg per kilogram of body weight per day) given orally for 2 weeks. The 1-week amphotericin B regimen consisted of amphotericin B (1 mg per kilogram per day administered intravenously) plus either fluconazole (1200 mg per day) or flucytosine (100 mg per kilogram per day) for 7 days, followed on days 8 through 14 by fluconazole (1200 mg per day). The 2-week amphotericin B regimen consisted of amphotericin B (1 mg per kilogram per day administered intravenously) plus either fluconazole (1200 mg per day) or flucytosine (100 mg per kilogram per day) for 14 days.

Patients underwent block randomization individually, stratified according to site, to one of the three treatment strategies and, for patients who were assigned to an amphotericin B regimen, to one of the two partner drugs. Overall, this strategy resulted in a 2:1:1:1:1 ratio of patients assigned to receive one of the five combinations of treatment strategy and partner drug with amphotericin B. For each site, a computer-generated randomization list with block sizes of 18, 24, and 30 was produced. The trial pharmacist and clinician were responsible for conducting the randomization by sequentially drawing sealed envelopes that contained the treatment assignment for each enrolled patient.

Patients who received amphotericin B were given 1 liter of normal saline intravenously daily in addition to usual fluid requirements and preemptive potassium and magnesium (glycerophosphate) supplementation.[16] Oral medications were given through a nasogastric tube if the patient was unable to swallow. Laboratory blood tests were performed regularly during the first 2 weeks of treatment. Baseline and day 7 electrocardiographic monitoring was discontinued at the advice of the data and safety monitoring committee after 100 paired electrocardiograms showed no evidence of clinically significant prolongation of the QT interval in association with fluconazole at a dose of 1200 mg per day. Lumbar punctures were performed at baseline and on days 7 and 14 for quantitative cultures.[17] In addition, patients with high CSF pressure underwent daily therapeutic lumbar punctures until the pressure was controlled.[5] Patients were followed

for 10 weeks after randomization. After 2 weeks, fluconazole was given at 800 mg per day until ART was started at 4 weeks (or restarted in those who had discontinued ART), at 400 mg per day until 10 weeks, and at 200 mg per day thereafter. ART was prescribed in accordance with national guidelines.

END POINTS

The primary end point for comparison of the two experimental treatment strategies with the standard therapy of 2 weeks of amphotericin B–based treatment was all-cause mortality at 2 weeks. Two weeks was chosen in view of the noninferiority design of the trial and the fact that mortality at 2 weeks is more likely than mortality at later time points to reflect deaths from cryptococcal meningitis.[18] Secondary end points included 4-week and 10-week all-cause mortality, the rate of decrease in the $\log_{10}$ CSF fungal count over 14 days, and clinical and laboratory-defined grade 3 and 4 adverse events.

For the comparison between partner drugs for the amphotericin B regimens, the primary end point was all-cause mortality at 10 weeks. The secondary end points were all-cause mortality at 2 weeks and 4 weeks, rate of clearance of infection, and adverse events.

STATISTICAL ANALYSIS

A target enrollment of 680 patients (226 per strategy) was set in order to achieve 90% power to show noninferiority with a 10-percentage-point noninferiority margin and under the assumption of 15% mortality at 2 weeks in the 2-week amphotericin B groups. For the comparison of the partner drugs with amphotericin B, with the use of a superiority design and under the assumption of a 10-week mortality of 40% with one partner treatment, the trial had 90% power to detect a 35% lower mortality with the alternative partner treatment.

The primary analysis was based on the intention-to-treat population. A generalized linear model with a binomial distribution and identity link function was used to calculate differences and upper limits of the one-sided 95% confidence interval for mortality. Post-hoc analysis of the primary end point with a one-sided 97.5% confidence interval, the upper limit of which is equivalent to the upper limit of a two-sided 95% confidence interval and the use of which is equivalent to applying a Bonferroni correction ($\alpha = 0.025$) for two comparisons, was also performed. Correction for multiple comparisons was not applied to the analyses of secondary outcomes. The per-protocol population excluded patients who missed more than 1 day of treatment within the first 2 weeks after randomization.

All-cause mortality at 2, 4, and 10 weeks was compared between the groups with the use of log-rank tests. Kaplan–Meier plots were also constructed, and Cox regression models with treatment as a predictor were used to derive hazard ratios and two-sided 95% confidence intervals. Analyses were also performed with adjustment for prespecified covariates: site, age, sex, Glasgow Coma Scale score, CD4+ cell count, CSF fungal count at baseline, and ART status at baseline. Sensitivity analyses of all-cause mortality were performed under the assumption that all the patients who were lost to follow-up had died.

The analysis of the $\log_{10}$ CSF fungal count over a period of 14 days from baseline was performed with a linear mixed-effects model. For comparison with previous studies,[17-19] linear regression was also used to calculate slopes of the decrease in CSF fungal count for each patient, and the mean slopes were compared between the groups.

All analyses were performed with the use of SAS software, version 9.3 (SAS Institute). Additional details are provided in the Supplementary Appendix, available at NEJM.org.

RESULTS

TRIAL POPULATION

From January 2013 through November 2016, a total of 721 patients underwent randomization (Fig. 1). Of these patients, 43 were excluded from all analyses: 30 met predefined late-exclusion criteria, 3 immediately withdrew consent, 7 were negative for cryptococcal meningitis, and 3 had had cryptococcal meningitis previously. A total of 16 patients were excluded from the per-protocol analysis: 14 missed more than 1 day of treatment within the 2-week induction period, and 2 did not receive the correct randomly assigned treatment. Baseline characteristics were similar in the treatment groups and reflected the severity of immunosuppression in the population (Table 1, and Tables S1 and S2 in the Supplementary Ap-

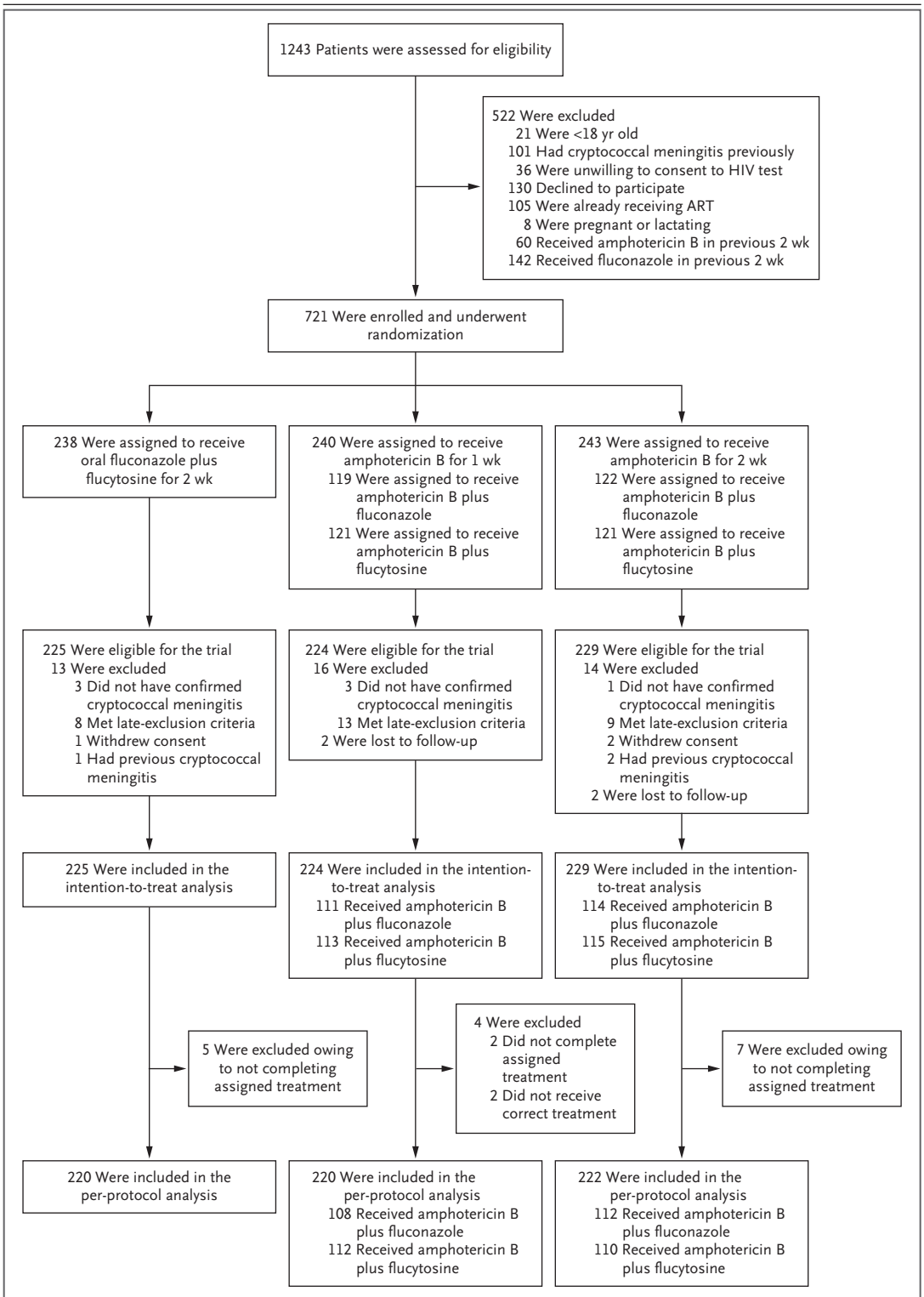

Figure 1. Screening, Randomization, and Analysis Populations.

Patients may have had more than one reason for exclusion. ART denotes antiretroviral therapy, and HIV human immunodeficiency virus.

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=229)
Male sex — no. (%)	119 (52.9)	137 (61.2)	134 (58.5)
Median age (IQR) — yr	36.0 (32.0–43.0)	38.5 (32.0–44.0)	37.0 (32.0–43.0)
Reported ART exposure — no. (%)†	128 (56.9)	119 (53.1)	134 (58.5)
Median weight (IQR) — kg‡	50 (46–60)	53 (47–60)	51 (46–60)
Current headache — no. (%)	221 (98.2)	221 (98.7)	226 (98.7)
Median duration of headache (IQR) — days§	14 (7–21)	14 (7–21)	14 (7–28)
Seizures within 72 hr before enrollment — no. (%)	40 (17.8)	43 (19.2)	36 (15.7)
Current fever — no. (%)	119 (52.9)	103 (46.0)	115 (50.2)
Current vision loss — no. (%)	17 (7.6)	17 (7.6)	22 (9.6)
Any cranial-nerve palsy — no. (%)	17 (7.6)	13 (5.8)	23 (10.0)
History of tuberculosis — no./total no. (%)	63/224 (28.0)	60/224 (26.8)	60/229 (26.2)
Glasgow Coma Scale score <15 — no. (%)¶	53 (23.6)	46 (20.5)	64 (27.9)
Abnormal mental status — no. (%)‖	101 (44.9)	90 (40.2)	107 (46.7)
Median CSF fungal count (IQR) — $\log_{10}$ CFU/ml**	5.0 (3.7–5.7)	5.0 (3.5–5.9)	5.0 (3.8–5.7)
Median CSF opening pressure (IQR) — cm††	22 (13–35)	24 (13–38)	25 (15–38)
CSF opening pressure >30 cm — no./total no. (%)††	69/218 (31.7)	78/211 (37.0)	80/215 (37.2)
Median CSF white-cell count (IQR) — cells/mm^3‡‡	4.0 (0.0–20.0)	4.0 (0.0–15.0)	3.0 (0.0–15.0)
Median CSF glucose level (IQR) — mmol/liter§§	2.0 (1.0–2.6)	2.0 (1.0–2.6)	2.0 (1.0–2.4)
Median CSF protein level (IQR) — mg/dl¶¶	113 (48–190)	102 (5–163)	99 (55–154)
Median hemoglobin level (IQR) — g/dl‖‖	10.7 (9.2–12.1)	11.0 (10.0–12.5)	10.9 (9.6–12.4)
Median creatinine level (IQR) — mg/dl***	0.7 (0.6–0.9)	0.8 (0.6–0.9)	0.7 (0.6–0.9)
Median baseline CD4+ cell count (IQR) — cells/mm^3†††	25 (10–63)	26.5 (12–63)	26 (10–64)

* ART denotes antiretroviral therapy, CFU colony-forming units, CSF cerebrospinal fluid, and IQR interquartile range.
† If only the patients who were enrolled after the ART amendment are considered, the proportions are 60.1% in the oral-regimen group, 55.6% in the 1-week amphotericin B groups, and 60.4% in the 2-week amphotericin B groups.
‡ Data were missing for 5 patients in the oral-regimen group, 6 in the 1-week amphotericin B groups, and 5 in the 2-week amphotericin B groups.
§ Data were missing for 4 patients in the oral-regimen group, 3 in the 1-week amphotericin B groups, and 3 in the 2-week amphotericin B groups.
¶ Scores on the Glasgow Coma Scale range from 3 to 15, with lower scores indicating lower levels of consciousness.
‖ Abnormal mental status was defined as having one or more of the following symptoms in the past 72 hours: drowsiness, behavioral change, or seizures.
** Data were missing for 10 patients in the oral-regimen group, 2 in the 1-week amphotericin B groups, and 8 in the 2-week amphotericin B groups. In total, 35 patients had CSF fungal burden of 0 at baseline: 9, 14, and 12 patients, respectively.
†† Data were missing for 7 patients in the oral-regimen group, 13 in the 1-week amphotericin B groups, and 14 in the 2-week amphotericin B groups.
‡‡ Data were missing for 4 patients in the oral-regimen group, 8 in the 1-week amphotericin B groups, and 10 in the 2-week amphotericin B groups.
§§ Data were missing for 27 patients in the oral-regimen group, 24 in the 1-week amphotericin B groups, and 26 in the 2-week amphotericin B groups.
¶¶ Data were missing for 24 patients in the oral-regimen group, 23 in the 1-week amphotericin B groups, and 32 in the 2-week amphotericin B groups.
‖‖ Data were missing for 1 patient in the oral-regimen group and 1 in the 2-week amphotericin B groups.
*** Data were missing for 4 patients in the oral-regimen group, 1 in the 1-week amphotericin B groups, and 1 in the 2-week amphotericin B groups. To convert values for creatinine to micromoles per liter, multiply by 88.4.
††† Data were missing for 16 patients in the oral-regimen group, 12 in the 1-week amphotericin B groups, and 18 in the 2-week amphotericin B groups.

Table 2. Unadjusted Analysis of Mortality and Rate of Fungal Clearance in CSF According to Treatment Strategy in the Intention-to-Treat Population.*

Outcome	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=229)	Difference (95% CI)†	
				Oral Regimen vs. 2-Wk Amphotericin B	1-Wk Amphotericin B vs. 2-Wk Amphotericin B
Mortality at 2 wk					
No. of deaths	41	49	49		
% (95% CI)	18.2 (13.2 to 23.3)	21.9 (16.5 to 27.4)	21.4 (16.1 to 26.7)	–3.18 (–10.50 to 4.15)	0.48 (–7.11 to 8.06)
Mortality at 4 wk					
No. of deaths	56	66	77		
% (95% CI)	24.9 (19.2 to 30.5)	29.5 (23.6 to 35.5)	33.6 (27.5 to 39.7)	–8.74 (–17.06 to –0.41)	–4.16 (–12.71 to 4.39)
Mortality at 10 wk					
No. of deaths	79	81	91		
% (95% CI)	35.1 (28.9 to 41.3)	36.2 (30.0 to 42.7)	39.7 (33.5 to 46.2)	–4.63 (–13.52 to 4.27)	–3.58 (–12.51 to 5.35)
Fungal clearance‡					
No. of patients	182	179	182		
Clearance rate — $\log_{10}$ CFU/ml/day	–0.26±0.18	–0.40±0.24	–0.42±0.25	0.10 (0.07 to 0.13)§	0.01 (–0.01 to 0.04)¶

* Plus–minus values are means ±SD. Patients who were lost to follow-up were included as alive in the analysis.
† Differences between mortality rates are given in percentage points. The upper limit of the two-sided 95% confidence interval is equivalent to that of the one-sided 97.5% confidence interval.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.
§ P<0.001 for the between-group difference.
¶ P=0.32 for the between-group difference.

pendix). In total, 59% of patients were taking or had previously taken ART. Patients who had never taken ART started the therapy at a median of 28 days (interquartile range, 27 to 34) after randomization.

MORTALITY

A total of 678 patients were eligible for inclusion in the intention-to-treat analyses. Of these patients, 1 was lost to follow-up within 2 weeks and 3 were lost to follow-up between 2 weeks and 10 weeks. Mortality was similar in the oral-regimen, 1-week amphotericin B, and 2-week amphotericin B groups: 18.2%, 21.9%, and 21.4%, respectively, at 2 weeks and 35.1%, 36.2%, and 39.7%, respectively, at 10 weeks (Table 2 and Fig. 2A). The upper limit of the one-sided 95% confidence interval for the difference in mortality at 2 weeks (primary end point) was 3.0 percentage points for the comparison of the oral-regimen group with the 2-week amphotericin B groups (P<0.001) and 6.8 percentage points for the comparison of the 1-week amphotericin B groups with the 2-week amphotericin B groups (P=0.007) (Fig. 2D). The upper limits of the one-sided 97.5% confidence intervals (the use of which is equivalent to applying a Bonferroni correction for the comparisons) both remained below the noninferiority margin (Table 2). The hazard ratios for death at 2 weeks, as compared with the 2-week amphotericin B groups, were 0.82 (95% confidence interval [CI], 0.54 to 1.25) in the oral-regimen group and 1.01 (95% CI, 0.68 to 1.51) in the 1-week amphotericin B groups; the corresponding hazard ratios for death at 10 weeks were 0.83 (95% CI, 0.61 to 1.13) and 0.89 (95% CI, 0.66 to 1.21) (Table S3 in the Supplementary Appendix). The results were similar in the per-protocol analysis, adjusted analysis, and sensitivity analyses (Fig. 2D, and Tables S4, S5, and S6 in the Supplementary Appendix).

As partner treatment with amphotericin B,

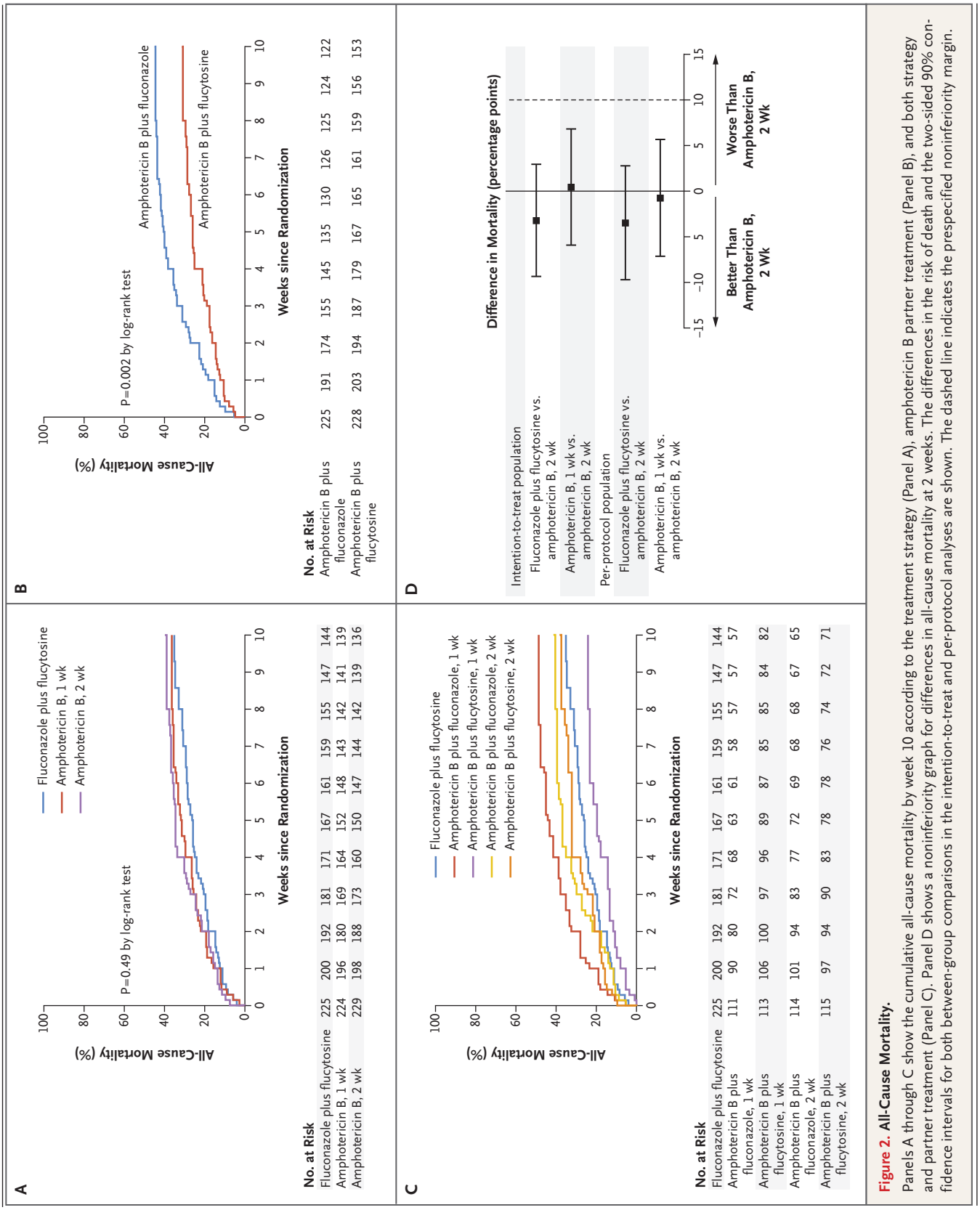

Figure 2. All-Cause Mortality.

Panels A through C show the cumulative all-cause mortality by week 10 according to the treatment strategy (Panel A), amphotericin B partner treatment (Panel B), and both strategy and partner treatment (Panel C). Panel D shows a noninferiority graph for differences in all-cause mortality at 2 weeks. The differences in the risk of death and the two-sided 90% confidence intervals for both between-group comparisons in the intention-to-treat and per-protocol analyses are shown. The dashed line indicates the prespecified noninferiority margin.

Table 3. Unadjusted Time-to-Event Analysis of Mortality and Rate of Fungal Clearance in CSF According to Partner Treatment with Amphotericin B in the Intention-to-Treat Population.*

Outcome	Amphotericin B + Fluconazole (N=225)	Amphotericin B + Flucytosine (N=228)	Hazard Ratio (95% CI)	P Value†
Mortality at 10 wk				
No. of deaths	101	71		
% (95% CI)	45.0 (38.5 to 51.5)	31.1 (25.3 to 37.3)	0.62 (0.45 to 0.84)	0.002
Mortality at 2 wk				
No. of deaths	61	37		
% (95% CI)	27.1 (21.3 to 32.9)	16.3 (11.5 to 21.1)	0.56 (0.37 to 0.85)	0.006
Mortality at 4 wk				
No. of deaths	86	57		
% (95% CI)	38.2 (31.9 to 44.6)	25.1 (19.4 to 30.7)	0.59 (0.42 to 0.83)	0.002
			Difference in Mean Clearance Rate (95% CI)	
Fungal clearance‡				
No. of patients	175	186		
Clearance rate — $\log_{10}$ CFU/ml/day	−0.36±0.23	−0.46±0.25	−0.06 (−0.03 to −0.08)	<0.001

* Plus–minus values are means ±SD. Missing values were not imputed.
† P values for the between-group differences in all-cause mortality were calculated with the use of a log-rank test.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.

flucytosine was superior to fluconazole (hazard ratio for death at 10 weeks with flucytosine vs. fluconazole, 0.62; 95% CI, 0.45 to 0.84; P=0.002) (Table 3 and Fig. 2B). This difference was driven by a difference in mortality between the 1-week amphotericin B–flucytosine group and the 1-week amphotericin B–fluconazole group. The results of separate analyses of the five groups are shown in Table 4 and Figure 2C, with the 2-week amphotericin B–flucytosine group as the comparator. The 1-week amphotericin B–flucytosine group had the lowest 10-week mortality (24.2%; 95% CI, 16.2 to 32.1), significantly lower than any other amphotericin B group (unadjusted hazard ratio, 0.56 [95% CI, 0.35 to 0.91], and adjusted hazard ratio, 0.59 [95% CI, 0.36 to 0.96], as compared with the 2-week amphotericin B–flucytosine group). The hazard ratio for death by 10 weeks, with the 1-week amphotericin B–flucytosine group used as the comparator, was 1.56 (95% CI, 1.01 to 2.42) in the oral-regimen group, 2.54 (95% CI, 1.60 to 4.05) in the 1-week amphotericin B–fluconazole group, and 1.97 (95% CI, 1.22 to 3.17) in the 2-week amphotericin B–fluconazole group (Table S7 in the Supplementary Appendix).

There was no significant difference in mortality between patients who had never taken ART and those who had previously been exposed to ART (Fig. S1 in the Supplementary Appendix).

RATE OF CLEARANCE OF INFECTION

The rate of clearance of infection (measured as the decrease in $\log_{10}$ colony-forming units per milliliter of CSF per day) was similar in the 1-week and 2-week amphotericin B groups and more rapid in the amphotericin B groups than in the oral-regimen group (Table 2). Flucytosine as the partner drug given with amphotericin B was associated with more rapid clearance than fluconazole (Table 3). Results were similar when linear regression was used, as in previous studies (Table S8 in the Supplementary Appendix).

SAFETY

Laboratory-defined side effects were less frequent in the oral-regimen group than in the 1-week or 2-week amphotericin B groups (Table 5, and Table S9 in the Supplementary Appendix). Grade 4 anemia developed in 0.9% of patients in the oral-regimen group, 4.9% of patients in the

Table 4. Unadjusted Time-to-Event Analysis of Mortality and Rate of Fungal Clearance in CSF According to Treatment Strategy and Partner Treatment with Amphotericin B in the Intention-to-Treat Population.*

Outcome	Oral Regimen (N=225)	1-Wk Amphotericin B + Fluconazole (N=111)	1-Wk Amphotericin B + Flucytosine (N=113)	2-Wk Amphotericin B + Fluconazole (N=114)	2-Wk Amphotericin B + Flucytosine (N=115)	Hazard Ratio vs. 2-Wk Amphotericin B + Flucytosine (95% CI)				P Value†
						Oral Regimen	1-Wk Amphotericin B + Fluconazole	1-Wk Amphotericin B + Flucytosine	2-Wk Amphotericin B + Fluconazole	
Mortality at 10 wk										
No. of deaths	79	54	27	47	44					
Mortality (95% CI) — %	35.1 (28.9 to 41.3)	48.6 (39.4 to 57.9)	24.2 (16.2 to 32.1)	41.3 (32.3 to 50.4)	38.3 (29.4 to 47.2)	0.87 (0.60 to 1.27)	1.42 (0.95 to 2.12)	0.56 (0.35 to 0.91)	1.10 (0.73 to 1.67)	0.001
Mortality at 2 wk										
No. of deaths	41	36	13	25	24					
Mortality (95% CI) — %	18.2 (13.2 to 23.3)	32.4 (23.7 to 41.1)	11.6 (5.7 to 17.5)	21.9 (14.3 to 29.5)	20.9 (13.4 to 28.3)	0.84 (0.50 to 1.39)	1.64 (0.97 to 2.78)	0.51 (0.26 to 1.00)	1.03 (0.59 to 1.82)	0.002
Mortality at 4 wk										
No. of deaths	56	46	20	40	37					
Mortality (95% CI) — %	24.9 (19.2 to 30.5)	41.4 (32.3 to 50.6)	17.8 (10.7 to 24.9)	35.1 (26.3 to 43.8)	32.2 (23.6 to 40.7)	0.74 (0.49 to 1.12)	1.41 (0.91 to 2.18)	0.50 (0.29 to 0.86)	1.10 (0.70 to 1.72)	<0.001
						Difference from 2 Wk Amphotericin B + Flucytosine in Mean Clearance Rate (95% CI)				
Fungal clearance‡										
No. of patients	182	81	98	94	88					
Clearance rate — $\log_{10}$ CFU/ml/day	−0.26±0.18	−0.36±0.23	−0.44±0.25	−0.37±0.24	−0.49±0.26	0.14 (0.11 to 0.17)§	0.08 (0.04 to 0.12)§	0.03 (−0.01 to 0.06)¶	0.06 (0.03 to 0.10)§	

* Plus–minus values are means ±SD. Missing values were not imputed.
† P values in this column pertain to the comparison of all five survival curves and were calculated with the use of the log-rank test.
‡ Data are from a mixed-effects model with treatment, day, and interaction between treatment and day as fixed effects, the log baseline measurement of fungal count as a covariate, and patient as a random effect.
§ P<0.001 for the difference from the 2-week amphotericin B–flucytosine group.
¶ P=0.16 for the difference from the 2-week amphotericin B–flucytosine group.

1-week amphotericin B groups, and 8.8% of patients in the 2-week amphotericin B groups; the median decrease from baseline in hemoglobin level over the first 2 weeks was 0.4, 1.8, and 2.7 g per deciliter, respectively, and 5.5%, 10.3%, and 20.2%, respectively, of patients in each group received a transfusion. A grade 3 or 4 increase in the serum creatinine level developed in 4.9% of patients in the oral-regimen group, 6.2% of patients in the 1-week amphotericin B groups, and 8.8% of patients in the 2-week amphotericin B groups. Grade 4 hypokalemia developed in only one patient, most likely because preemptive electrolyte replacement was provided for patients receiving amphotericin B. Grade 4 neutropenia was recorded in 3.2% of the patients who were taking a regimen that included 2 weeks of flucytosine, in 0.9% of those taking 1 week of flucytosine, and in 1.3% of those taking a flucytosine-free regimen. A grade 4 increase in the alanine aminotransferase level developed in only two patients, one of whom was taking fluconazole. Clinical adverse events were frequent with all regimens, which was reflective of the severe immunosuppression in this patient population.

DISCUSSION

In this trial, we recruited patients from centers in southern, eastern, and central Africa, where the burden of cryptococcal meningitis is highest. We found that combination oral therapy with higher-dose fluconazole plus flucytosine and shorter-course 1-week amphotericin B–based treatment were noninferior to 2 weeks of amphotericin B–based therapy and that, as the partner drug with amphotericin B, flucytosine was associated with lower mortality than fluconazole. This latter difference was driven by the superiority of flucytosine to fluconazole in the 1-week amphotericin B groups. Indeed, although caution is appropriate in interpreting these results, given the secondary nature of the comparisons involved, 1 week of amphotericin B plus flucytosine was associated with higher survival rates than the other regimens, although the difference only just met the criteria for significance in the comparison with the oral combination and, in an adjusted analysis, in the comparison with the 2-week amphotericin B–flucytosine regimen.

The results were consistent in the per-protocol and intention-to-treat analyses, as well as in adjusted and sensitivity analyses. Few patients (0.6%) were lost to follow-up. Patients with severe disease were not excluded, so that the study population reflected patients presenting at centers across Africa. Also supportive of the generalizability of the results was the finding that mortality in the 2-week amphotericin B–fluconazole group (41.3% at 10 weeks) was the same as that seen in the placebo group of the recent multicenter trial of adjunctive glucocorticoids in which the same antifungal regimen was used.[20]

The results were consistent with those in animal models and in our phase 2 studies[10-13] and may reflect, at least in part, a balance between the rate of clearance of infection and the drug-related side effects. Flucytosine as the partner drug with amphotericin B was associated with more rapid clearance of infection than fluconazole and had a similar side-effect profile, as was previously shown in a study in Vietnam.[14] Clearance of infection was as rapid in the 1-week amphotericin B groups as it was in the 2-week amphotericin B groups, and, as expected, the shorter regimens had fewer side effects, with, in particular, less anemia. Our results with 1 week of amphotericin B–flucytosine lend further support to the concept of prolonged efficacy after an initial loading of brain compartments with amphotericin B,[13,21] as was also recently shown with the use of a single high dose of liposomal amphotericin B.[22] Of note, we implemented full preemptive management and monitoring of amphotericin B toxic effects.[16] It is likely that in resource-limited settings, the challenges of transfusion and monitoring would further disadvantage the 2-week amphotericin B regimens, as evidenced by the continued very high mortality rates reported from African centers where 2 weeks of amphotericin B has been used.[23,24] The amphotericin B–free oral combination regimen had few laboratory-defined side effects and also had efficacy, despite slower clearance of infection.

All the best-performing regimens in our trial contained flucytosine. In particular, mortality in the 1-week amphotericin B–flucytosine group was significantly lower than that in the other amphotericin B groups, whereas mortality in the 1-week amphotericin B–fluconazole group was the highest. It may be that the more effective partner drug is particularly important in the context of shorter courses of amphotericin B. In addition to rapid fungicidal activity, flucytosine may

Table 5. Laboratory-Defined and Clinical Adverse Events That Occurred within 21 Days after Randomization, According to Treatment Strategy.*

Event	Oral Regimen (N=225)	1-Wk Amphotericin B (N=224)	2-Wk Amphotericin B (N=228)
Any adverse event — no. of patients (%)			
Grade 3 or 4†	129 (57.3)	128 (57.1)	154 (67.5)
Grade 3	60 (26.7)	60 (26.8)	74 (32.5)
Grade 4	69 (30.7)	68 (30.4)	80 (35.1)
Anemia — no. of patients (%)			
Grade 3‡	9 (4.0)	20 (8.9)	40 (17.5)
Grade 4§	2 (0.9)	11 (4.9)	20 (8.8)
Median change in hemoglobin level to day 14 (IQR) — g/dl¶	–0.4 (–1.0 to 0.4)	–1.8 (–2.8 to –0.9)	–2.7 (–4.0 to –1.6)
Neutropenia — no. of patients (%)			
Grade 3‡	14 (6.2)	14 (6.2)	17 (7.5)
Grade 4§	8 (3.6)	3 (1.3)	4 (1.8)
Hypokalemia — no. of patients (%)			
Grade 3‡	3 (1.3)	14 (6.2)	15 (6.6)
Grade 4§	0	0	1 (0.4)
Thrombocytopenia — no. of patients (%)			
Grade 3‡	1 (0.4)	5 (2.2)	3 (1.3)
Grade 4§	4 (1.8)	2 (0.9)	1 (0.4)
Elevated ALT — no. of patients (%)			
Grade 3‡	6 (2.7)	6 (2.7)	7 (3.1)
Grade 4§	0	1 (0.4)	1 (0.4)
Creatinine increase — no. of patients (%)			
Grade 3‡	6 (2.7)	13 (5.8)	16 (7.0)
Grade 4§	5 (2.2)	1 (0.4)	4 (1.8)
Median change in creatinine level to day 14 (IQR) — μmol per liter‖	0 (–8.8 to 13.0)	14.0 (0.0 to 33.0)	35.4 (12.0 to 65.0)
Grade 3 or 4 pneumonia — no. of patients (%)	2 (0.9)	3 (1.3)	6 (2.6)
Grade 3 or 4 diarrhea or vomiting — no. of patients (%)	6 (2.7)	2 (0.9)	3 (1.3)
Grade 3 or 4 bacteremia or sepsis — no. of patients (%)	9 (4.0)	9 (4.0)	14 (6.1)
Other grade 3 or 4 adverse event — no. of patients (%)	120 (53.3)	125 (55.8)	148 (64.9)

* One patient who died after randomization but before receiving the trial treatment was excluded from the safety analysis.
† The total number of grade 3 or 4 adverse events (at any time after randomization) was 377 in the oral-regimen group, 465 in the 1-week amphotericin B groups, and 658 in the 2-week amphotericin B groups.
‡ The definitions of grade 3 adverse events were as follows: anemia, a hemoglobin level of 6.5 to 7.4 g per deciliter; neutropenia, a neutrophil count of 500 to 749 per cubic millimeter; hypokalemia, a potassium level of 2.0 to 2.4 mmol per liter; thrombocytopenia, a thrombocyte count of 25,000 to 49,999 per cubic millimeter; elevated alanine aminotransferase (ALT), an ALT level of 178 to 350 U per liter; and an increase in creatinine, a creatinine level of 2.47 to 4.42 mg per deciliter (218 to 390 μmol per liter).
§ The definitions of grade 4 adverse events were as follows: anemia, a hemoglobin level of less than 6.5 g per deciliter; neutropenia, a neutrophil count of less than 500 per cubic millimeter; hypokalemia, a potassium level of less than 2.0 mmol per liter; thrombocytopenia, a thrombocyte count of less than 25,000 per cubic millimeter; elevated ALT, an ALT level of more than 350 U per liter; and an increase in creatinine, a creatinine level of more than 4.55 mg per deciliter (402 μmol per liter).
¶ Data were missing for 35 patients in the oral-regimen group, 49 patients in the 1-week amphotericin B groups, and 46 patients in the 2-week amphotericin B groups.
‖ Data were missing for 34 patients in the oral-regimen group, 46 patients in the 1-week amphotericin B groups, and 39 patients in the 2-week amphotericin B groups.

also have other properties, such as a more prolonged postantibiotic effect than fluconazole,[25] to help explain this difference.

Despite the fact that patients in resource-limited settings have access to ART, the incidence of cryptococcal meningitis is not decreasing in many centers.[26,27] Widespread availability of generic flucytosine is urgently needed as an essential part of global programs to reduce HIV-related mortality, as is continued investigation into new drug therapies. Efforts by international agencies to make flucytosine available are gaining momentum.[7,15,28,29]

In conclusion, in our trial, 1 week of amphotericin B plus flucytosine was the most effective option for induction therapy for patients with HIV-associated cryptococcal meningitis in resource-limited settings. Our results also suggest that in the absence of availability of amphotericin B or in conditions in which amphotericin B cannot be administered safely, the oral combination of fluconazole plus flucytosine provides an effective and sustainable alternative.

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APPENDIX

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CORRESPONDENCE

Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany

TO THE EDITOR: The novel coronavirus (2019-nCoV) from Wuhan is currently causing concern in the medical community as the virus is spreading around the world.[1] Since its identification in late December 2019, the number of cases from China that have been imported into other countries is on the rise, and the epidemiologic picture is changing on a daily basis. We are reporting a case of 2019-nCoV infection acquired outside of Asia in which transmission appears to have occurred during the incubation period in the index patient.

A 33-year-old otherwise healthy German businessman (Patient 1) became ill with a sore throat, chills, and myalgias on January 24, 2020. The following day, a fever of 39.1°C (102.4°F) developed, along with a productive cough. By the evening of the next day, he started feeling better and went back to work on January 27.

Before the onset of symptoms, he had attended meetings with a Chinese business partner at his company near Munich on January 20 and 21. The business partner, a Shanghai resident, had visited Germany between Jan. 19 and 22. During her stay, she had been well with no signs or symptoms of infection but had become ill on her flight back to China, where she tested positive for 2019-nCoV on January 26 (index patient in Fig. 1).

On January 27, she informed the company about her illness. Contact tracing was started, and the above-mentioned colleague was sent to the Division of Infectious Diseases and Tropical Medicine in Munich for further assessment. At presentation, he was afebrile and well. He reported no previous or chronic illnesses and had no history of foreign travel within 14 days before the onset of symptoms. Two nasopharyngeal swabs and one sputum sample were obtained and were found to be positive for 2019-nCoV on quantitative reverse-transcriptase–polymerase-chain-reaction

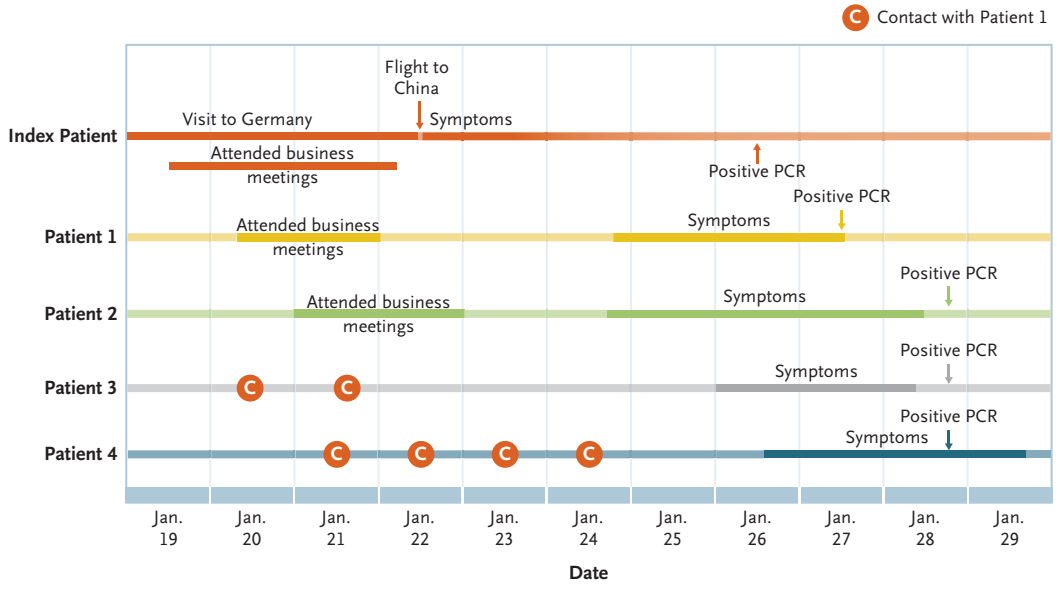

Figure 1. Timeline of Exposure to Index Patient with Asymptomatic 2019-CoV Infection in Germany.

(qRT-PCR) assay.[2] Follow-up qRT-PCR assay revealed a high viral load of 10^8 copies per milliliter in his sputum during the following days, with the last available result on January 29.

On January 28, three additional employees at the company tested positive for 2019-nCoV (Patients 2 through 4 in Fig. 1). Of these patients, only Patient 2 had contact with the index patient; the other two patients had contact only with Patient 1. In accordance with the health authorities, all the patients with confirmed 2019-nCoV infection were admitted to a Munich infectious diseases unit for clinical monitoring and isolation. So far, none of the four confirmed patients show signs of severe clinical illness.

This case of 2019-nCoV infection was diagnosed in Germany and transmitted outside of Asia. However, it is notable that the infection appears to have been transmitted during the incubation period of the index patient, in whom the illness was brief and nonspecific.[3]

The fact that asymptomatic persons are potential sources of 2019-nCoV infection may warrant a reassessment of transmission dynamics of the current outbreak. In this context, the detection of 2019-nCoV and a high sputum viral load in a convalescent patient (Patient 1) arouse concern about prolonged shedding of 2019-nCoV after recovery. Yet, the viability of 2019-nCoV detected on qRT-PCR in this patient remains to be proved by means of viral culture.

Despite these concerns, all four patients who were seen in Munich have had mild cases and were hospitalized primarily for public health purposes. Since hospital capacities are limited — in particular, given the concurrent peak of the influenza season in the northern hemisphere — research is needed to determine whether such patients can be treated with appropriate guidance and oversight outside the hospital.

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Original article

Risk factors for and management of metronidazole-refractory giardiasis in international travellers: A retrospective analysis

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ABSTRACT

Background: Giardia lamblia is a common cause of diarrhoea in returning travellers. Failure of the recommended first-line treatment, metronidazole, has frequently been observed. Recommendations for treatment of metronidazole-refractory giardiasis lack clarity and evidence.
Methods: We conducted a retrospective data analysis of returned travellers with confirmed giardiasis at the Bernhard-Nocht-Clinic in Hamburg, Germany, between 2007 and 2016.
Results: We identified 339 cases of giardiasis, mostly acquired in South Asia (n = 157). 308 patients received metronidazole as first-line treatment, leading to treatment failure in 93 cases. Statistical analysis suggested by far the highest risk of metronidazole treatment failure for travellers returning from South Asia (Odds Ratio 8.73). Second-line therapy consisted of various different therapy regimens. Combination therapy as second-line treatment seemed to be more effective than monotherapy. A repeat course of metronidazole proved to be futile.
Conclusion: This study reveals a strikingly low effectiveness of metronidazole, especially in patients returning from South Asia. Second-line treatment showed inconsistency of regimens and yielded unsatisfactory results. These findings require reconsideration of treatment strategies for giardiasis. Large prospective trials are urgently needed to assess new first-line treatment options and to help implement advice for effective, agreed second-line treatment strategies. Translational projects should be created to link the understanding of resistance mechanisms with epidemiological data and clinical outcome.

1. Introduction

Giardiasis is the most common parasitic cause of diarrhoea and gastrointestinal complaints in travellers returning from the tropics and subtropics. It is caused by an infection with the intestinal protozoon *Giardia lamblia* (syn. *Giardia intestinalis* or *Giardia duodenalis)* [1–3] hereinafter referred to as *Giardia*.

In developing countries *Giardia* is found endemically and is almost omnipresent in populations living in areas with poor sanitation [4].

The risk of contracting an infection while travelling to these regions is high: Studies show that 25–50% of travellers visiting low-resourced settings in the tropics suffer from diarrhoea [2,3,5]. In an analysis of the spectrum of disease in ill returned travellers, Freedman et al. noted that diarrhoea due to parasitic infection outnumbered bacterially induced diarrhoea in all regions except Southeast Asia. The most frequently found parasite was *Giardia lamblia* [2,3,6].

Destination, duration, purpose and mode of travel also seem to affect the probability of an infection with *Giardia* [2,5,7–9].

Jelinek and Löscher reported that the risk of infection increased with prolonged duration of travel [7]. Touristic travel correlates with a higher rate of infection compared to business travel or travel with the purpose of visiting friends and relatives (VFR) [2].

Studies also found links between age, sex, pre-travel medical advice and contracting an infection with *Giardia* [2,5,7,10].

Giardia exists in two developmental stages: a cystic form and a mobile flagellated trophozoite [11]. Infection occurs by ingestion of food or water contaminated with the environmentally resistant cysts, by person-to-person or person-to-animal contact with cyst-positive faeces

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[12]. 10–25 cysts suffice to cause an infection, making *Giardia* highly contagious [11].

Following ingestion, the trophozoite stage hatches from the cyst. It replicates actively and colonizes the small intestine. The cycle completes when trophozoites differentiate into cysts and are shed in the faeces.

Giardia can be considered as a species complex of eight genetic groups (termed assemblages A-H). Assemblages differ in host distribution and host specificity [13]. Assemblages A and B are commonly associated with human infection but may also cause zoonotic infection [14].

Characteristic symptoms caused by *Giardia* occur 7–10 days post-infection and include diarrhoea, bloating, abdominal cramps, increased acid regurgitation and nausea [7,11,15,16]. The severity and range of symptoms vary widely. While some patients experience severe impairment, others are completely asymptomatic. Furthermore, giardiasis ranges from being self-limited to protracted. It is known to cause weight loss and other signs of malabsorption. Children may exhibit growth retardation and cognitive impairment due to nutrient deficiency [11,16–18].

Chronic giardiasis is associated with immunoglobulin deficiencies, immunosuppression and malnutrition [19,20].

Even after parasite clearance, both immunocompromised as well as immunocompetent patients may develop chronic health issues like fatigue, abdominal complaints [7,15] and irritable bowel syndrome [21].

No vaccine against giardiasis is available and pharmacotherapy is the only available option to treat giardiasis. In low-prevalence settings treatment of confirmed cases of giardiasis is always recommended to cure symptoms, reduce the risk of post-infectious complications and limit the spread of the infection [22].

The first-line therapy for *Giardia* infection in many countries is metronidazole. The most commonly used regimen is 500mg tds for 5–10 days [23–25]. Metronidazole is widely available and listed as an essential drug by WHO.

The other 5-nitroimidazoles, tinidazole, secnidazole and ornidazole are also used as first-line therapy for giardiasis [26–29]. Their longer serum half-life permits to give them in a single dose for one or a few days. They have milder side effects than metronidazole and a slightly higher cure rate [30].

However, an increasing number of first-line nitroimidazole treatment failure has been observed. The precise mechanisms remain unclear [31–34].

Further drugs commonly used in the treatment of giardiasis are benzimidazoles (eg albendazole, mebendazole), paromomycin, chloroquin, mepacrine (quinacrine), nitazoxanide and furazolidone [25].

Recommendations for second-line treatment are hampered by the lack of data from comprehensive clinical trials [34,35]. Additionally, the availability of antiprotozoal drugs varies between different countries [36].

Therefore, the aim of this study was to analyse the giardia-infected patient collective in a group of returned travellers, to determine the rate of metronidazole failure and associated risk factors. The study also aimed to provide an overview of the implemented second-line therapies and their outcomes.

2. Methods

2.1. Study site and study population

We conducted a retrospective data analysis of patients with confirmed *Giardia* infection seeking medical attention at the Bernhard-Nocht-Clinic in Hamburg, Germany, between January 2007 and April 2016. The clinic is part of the Department of Medicine at the University Medical Center Hamburg-Eppendorf. It is a specialist clinic for tropical medicine and one of Germany's largest academic travel clinics. It offers pre-travel consultations, vaccinations and a clinic for returning travellers and migrants.

All cases of giardiasis included in our study had to be parasitologically confirmed. Confirmation was attained mainly by microscopy but also by antigen test or PCR. For inclusion of patients referred from other clinics or general practitioners, documentation of previous parasitological records and treatment were mandatory. Cases were compiled from the laboratory registry at the Bernhard-Nocht-Institute and from the clinic's database.

We reviewed medical records of the identified cases, including documentation of follow-up consultations and microbiological test results. Data for the following variables was extracted: patient's age at first attendance, sex, destinations and duration of travel; type, duration and outcome of antiprotozoal treatment and, if documented, parasitological technique used to confirm cure or treatment failure.

Some patients had travelled several countries. In that case, the one country where the infection was most likely acquired, considering onset of symptoms, incubation period and patient's assessment, was chosen for further analysis.

For analytical purposes destinations were organised in regions, based on the UN composition of macro geographical (continental) regions.

Accordingly, countries were grouped into „Africa", „South Asia", „Central and Southeast Asia" and „Central and South America". North America, Europe, Western Asia and Oceania were subsumed as „others" in consideration of the low prevalence of imported giardiasis from these regions.

Cases were classified as first-line treatment failure if they met the following criteria: patients previously treated for confirmed giardiasis at the clinic or elsewhere with a stool sample testing positive by one or more of the methods given above at least 14 days after completion of first-line treatment. The same rules applied to all further treatment failures.

In routine practice at our clinic there was no standardised protocol for follow-up of giardia-infected patients, therefore some patients were asked to return for parasitological confirmation of cure, others were asked to present only if symptoms persisted or relapsed following treatment.

Treatment success was therefore defined as either parasitologically "confirmed cure", diagnosed using stool microscopy, PCR or antigen test or a combination of these, or "presumed cure", for cases in which patients did not seek further medical assistance at our clinic.

2.2. Analysis

We performed statistical analysis using MS-Excel 2008 and IBM SPSS-Statistics version 23. An association of the variables that were considered to be potential risk factors for refractory giardiasis (age, sex, destination and duration of travel), with the outcome was assessed in bivariate logistic regression analysis. All variables that showed at least some evidence for an association with the outcome ($p < 0.05$) were thereafter included in a multivariate logistic regression model to adjust for potential confounding.

Interpretation of data in groups of returned travellers is often hampered by the lack of a denominator i.e. a reference group. Therefore, to create a proxy, we compiled a sample of travellers seen at our pre-travel clinic: 1200 questionnaires, routinely filled out at our clinic upon consultation, were included, the first 100 travellers seen at the pre-travel clinic each month from August 2015 until July 2016. Data for age, sex, duration of travel and travel destination were compiled from these questionnaires.

Due to the retrospective design of the study and the anonymisation of patient data our project did not fall under the category of research requiring clearance by the Institutional Review Board.

3. Results

3.1. Description of sample

Between January 2007 and April 2016, 339 returning travellers with parasitologically confirmed *Giardia* infection were treated at our clinic.

180 (53.1%) were female. The mean age was 34 years (range 1–76 years), and the median duration of travel was 6 weeks (range 1–364 weeks).

The relative majority of patients had acquired the infection in South Asia (46.3%, n = 157), followed by Africa (26.8%, n = 91) and South and Central America (13.3%, n = 45). Other travel destinations, including Southeast Asia, accounted for fewer than 10% each (Fig. 1).

Most cases from South Asia were imported from India (87.3%, n = 137), while the other South Asian countries accounted for fewer than 5% each.

Of the 339 patients that received treatment at our clinic for giardiasis, 5 patients suffered from an underlying potentially immunocompromising condition (HIV, CVID, immunglobulin deficiency).

3.2. Outcome of metronidazole first-line treatment

90.9% of the 339 patients received metronidazole as first-line therapy (n = 308). The remaining 9.1% received a different treatment (n = 16), no medication (n = 1) or undocumented medication (n = 9).

Treatment outcome was parasitologically confirmed in 132 cases, using stool microscopy (64.4%, n = 85), PCR (9.8%, n = 13) and antigen-tests (2.3%, n = 3) as well as combinations of microscopy with PCR (21.2%, n = 28) or antigen-test (2.3%, n = 3). In the remaining patients, treatment outcome was only determined clinically (persistence or absence of giardia-specific symptoms).

Metronidazole first-line treatment failed in 93 out of 308 patients (30.2%) (Fig. 2). In patients from South Asia, the failure rate was 47.9% (Fig. 2), whereby India accounted for the vast majority of cases (Table .1).

Dosage and duration of metronidazole therapy varied in this retrospective study. Most patients received 1200 mg daily (400 mg tds) for seven days. 28 patients received less than 750 mg per day. For statistical analysis we compared the results of patients who received more than 1200 mg per day to those who received 1200 mg per day or less. Statistical assessment of longer treatment courses (>7 days) or higher doses (>1200 mg per day) showed no evidence of better efficacy (Table .2).

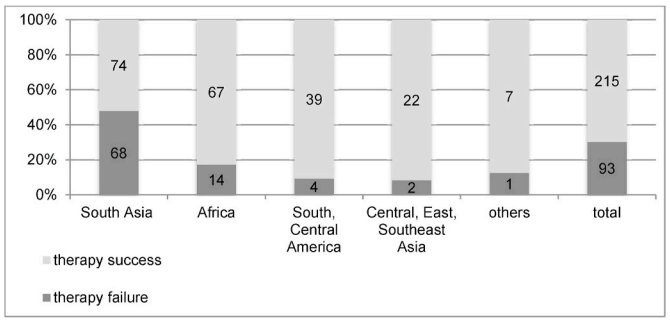

Fig. 2. Outcome of first-line treatment with metronidazole sorted by travel regions (total n = 308, n = 298, no information n = 10).

Table 1
Metronidazole treatment failure in different South Asian countries.

Countries of South Asia	Cases (n = 142)	Treatment failure (n = 68)
Afghanistan	1	1 (100%)
Bangladesh	3	1 (33%)
India	126	62 (49%)
Nepal	5	3 (60%)
Pakistan	5	1 (20%)
Sri Lanka	2	0

3.2.1. Logistic regression analysis of risk factors for metronidazole first-line treatment failure

In the univariate logistic regression model, sex (p = 0.04) and travel destination (p < 0.001) were associated with treatment failure, while no association was found with age and duration of travel.

In the multivariate model (including travel destination and sex), only travel destination remained associated with treatment failure (p < 0.001), suggesting by far the highest risk for travellers returning from South Asia (Fig. 1).

Taking the region of Central and South America as a reference, the odds ratio (OR) for metronidazole first-line therapy failure for South Asia was 8.73 (95% CI 2.96–25.76) (Table .3, Fig. 1).

3.3. Outcome of second-line treatment

After metronidazole first-line failure, second-line treatment was recorded in 93 patients. In total, 14 different regimens were

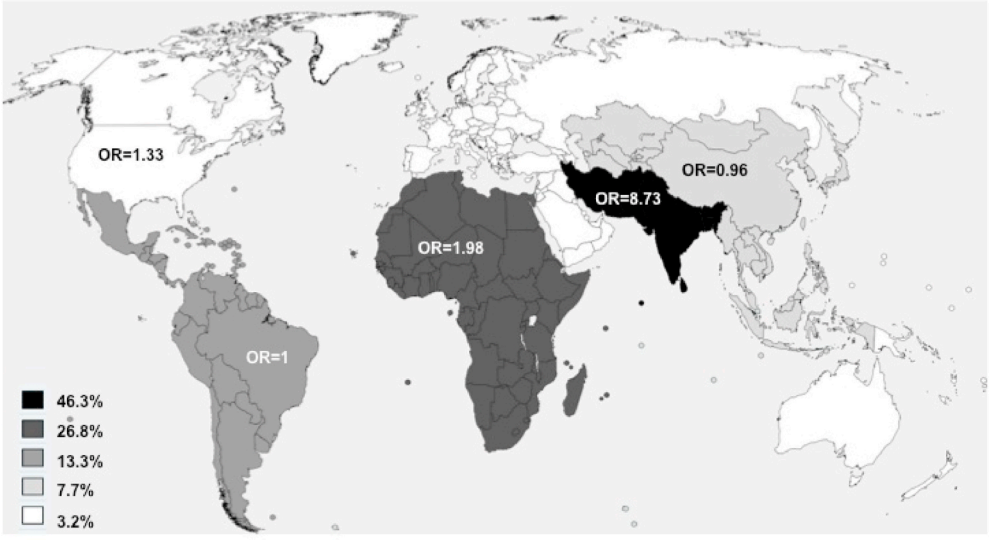

Fig. 1. Distribution of imported *Giardia lamblia* infections by region of acquisition and Odds Ratio of metronidazole first-line treatment failure by region.

Table 2
Statistical assessment of association of daily dosages and duration of treatment with metronidazole treatment failure using univariate logistic regression analysis.

Metronidazole dosage and duration of treatment	Number of patietns n (%)	Treatment failure (n (%)	OR	95% CI	p
Dosage (mg/day) n = 242, n.i. = 66					
≤1200	222 (100)	55 (8,24)	1	0,477–3551	0,607
>1200	20 (100)	6 (30)	1301		
Duration of treatment (days) n = 266, n.i. = 42					
≤7	150 (100)	41 (3,27)	1	0,733–2116	0,418
>7	116 (100)	37 (9,31)	1245		

(OR = odds ratio, 95% CI = 95% confidence interval, p = p-value, n.i. = no information).

implemented in various dosages.

The five most commonly used drugs or drug combinations were paromomycin (n = 27), metronidazole plus paromomycin (n = 17), metronidazole (n = 14), tinidazole (n = 12) or albendazole plus chloroquine (n = 8) (Table 4).

50 patients underwent further testing to confirm the outcome of second-line therapy. The main diagnostic method was stool microscopy, done in 44 cases (88%). In 8 of theses cases additional PCR and in one case additional antigen testing was done. PCR alone was done in 8 cases and antigen testing alone in 2 cases.

Overall, second-line therapy failed in 46.2% of cases. A second course of metronidazole failed in 100% of cases. When tinidazole was used instead of metronidazole, the failure rate was 50% (Table 4).

Combination therapies performed better than single-drug regimens. The most promising second-line regimens were the combinations of metronidazole plus paromomycin (cure rate 88.2%) and albendazole plus chloroquine (87.5%), respectively (Table .4).

For some other regimens cure rates were high, however, since only single cases were treated, no certain conclusion could be drawn.

3.4. Third line and further treatment

43 patients received third-line therapy whereby 26 different regimens were used (data not shown). Case numbers for each treatment combination therefore were too low to allow for any conclusions.

After third-line treatment 24 patients received further work-up to confirm treatment outcome. Stool microscopy was performed in 23 cases, in 4 of these this was combined with the other diagnostic methods.

15 patients required a fourth and two patients a fifth course of antiprotozoal treatment.

3.5. Reference group

The reference group of 1200 pre-travel consultations revealed a similar demographic composition compared to the group of giardiasis patients. 54% (n = 651) were female and mean age was 34 years. The duration of travel was shorter with a median of 3 weeks. Distribution of travel destinations appeared to be different: the percentage of people travelling to South Asia amounted to 8,1% (n = 97) compared to 46,3% (n = 157) among giardiasis patients. In contrast, only 7.7% of giardia patients had chosen other parts of Asia as travel destination, as opposed to 26.7% of travellers in the pre-travel group (Table 5).

Table 4
Outcome of second-line treatment of metronidazole refractory giardiasis by regimen (n = 93).

2nd line treatment regimens in order of frequency prescribed (number of patients treated)	Therapy failure (n = 43)
1. Paromomycin (n = 27)	10/27 (37%)
2. Metronidazole + paromomycin (n = 17)	2/17 (11,8%)
3. Metronidazole (n = 14)	14/14 (100%)
4. Tinidazole (n = 12)	6/12 (50%)
5. albendazole + chloroquine (n = 8)	1/8 (12,5%)
6. albendazole (n = 3)	2/3 (66,6%)
7. Metronidazole + albendazole (n = 3)	3/3 (100%)
8. Paromomycin + albendazole (n = 2)	1/2 (50%)
9. Paromomycin + tinidazole (n = 2)	1/2 (50%)
10. Metronidazole + cefazolin (n = 1)	1/1 (100%)
11. Metronidazole + ciprofloxacin (n = 1)	1/1 (100%)
vancomycin (n = 1)	1/1 (100%)
metronidazole + paromomycin + albendazole (n = 1)	0/1 (0%)
paromomycin + albendazole + ciprofloxacin (n = 1)	0/1 (0%)

Table 3
Risk factors for metronidazole first-line therapy failure.

Risk factor	n	n (%) 1st-line MTZ failure	univariate			multivariate		
			OR	95% CI	p	OR	95% CI	p
Sex (n=308)					0.04			
male	147	36 (24.5)	1			1		0.26
female	161	57 (35.4)	1.69	1.03-2.77		1.37	0.79-2.37	
age (years) (n=308)					0.26	-	-	-
≤ 25	111	32 (28.8)	1					
26-40	91	23 (25.3)	0.84	0.45-1.56				
> 40	106	38 (35.9)	1.38	0.78-2.44				
duration of travel (weeks) (n=290, n.i.=18)					0.31	-	-	-
≤ 4	132	46 (34.8)	1					
5-12	54	15 (27.8)	0.72	0.36-1.44				
≥ 13	104	27 (26.0)	0.66	0.37-1.16				
travel destination (n=298, n.i.=10)					<0.001			
South & Central America	43	4 (9,3)	1			1		<0.001
South Asia	142	68 (47.9)	8.96	3.04-26.39		8.73	2.96.-25.76	
Central, East, Southeast Asia	24	2 (8.3)	0.89	0.15-5.24		0.96	0.61-6.46	
Africa	81	14 (17.3)	2.03	0.63-6.63		1.98	0.16-5.74	
Others	8	1 (12.5)	1.39	0.13-14.38		1.33	0.13-13.75	

(MTZ = metronidazole, OR = odds ratio, 95% CI = 95% confidence interval, p = p-value, n.i. = no information).

Table 5
Comparison of Travel destinations: Giardia Patients vs pre-travel consultations.

Travel Destinations	Giardia patients (n = 308) % (n)	Travellers pre travel (n = 1200) % (n)
South Asia	46.3% (142)	8.1% (97)
Africa	26.8% (81)	34.3% (412)
South & Central America	13.3% (43)	21.7% (260)
Central, East, South-East Asia	7.7% (24)	26.7% (320)
Others	3.2% (8)	2.2% (26)
More than one destination/undecided, no information	3,2% (10)	7.1% (85)

4. Discussion

4.1. Main findings

Almost half of all imported *Giardia* cases came from South Asia. In contrast, only around 8% of a representative sample of travellers in our travel clinic went to that region, allowing the conclusion, that the risk of acquiring giardiasis in South Asia is likely higher than in other parts of the world.

In addition, South Asia was the region with the highest rates of metronidazole first-line treatment failure: almost every other patient required second-line treatment (Fig. 2). Overall, one third of cases were metronidazole-refractory.

The only identifiable risk factor for metronidazole-failure was the region the infection was acquired: the risk of metronidazole treatment failure was eight times higher for travellers from South Asia compared to the reference region of South America (Fig. 1), (Table 3).

Even though causes of treatment failure could not be systematically assessed in this retrospective study, the strong geographical preponderance of South Asia as a source region of metronidazole-refratory cases makes it highly likely that true drug resistance was causing it, rather than host-factors. South Asia is also a region known for rapid development of antimicrobial resistance, e.g. ESBL-strains and multi-resistant *Salmonella* Typhi, which in a globalised world easily spread by international travel [37–44].

This phenomenon is facilitated in the South Asian region by high population density, poor hygiene and sanitation, close contact with animals and overuse or unregulated use, respectively, of antimicrobials which are commonly available over-the-counter [45,46]. Antimicrobial drugs sold may be of poor quality, falsified of fake [47]. It seems likely that *Giardia* in the presence of subtherapeutic drug concentrations or insufficient treatment duration may develop resistance in analogy to bacterial pathogens.

Even though this study was done at a referral center for tropical medicine with great expertice in the treatment of parasitic diseases, drug regimens prescribed after first-line metronidazole failure varied greatly, revealing the fact that even expert doctors lack clarity as to the choice of the second-line regimen (Table 4).

Second-line treatment failed even more commonly than first-line treatment, and nearly half of cases required further, third line therapy.

Repetition of metronidazole treatment after first-line failure does not seem promising as it failed in all our cases. Combination therapies yielded higher cure rates than single drug regimens in second line treatment (Table 4).

4.2. Comparison to other studies

Even though nitroimidazole-refractory giardiasis in travellers is a challenge well known to clinicians, the number of larger studies looking into this, is very limited [31,48–50]. Like ours, most studies are observational and none of the studies on travellers looks into possible mechanisms of drug-resistance.

5-Nitroimidazoles (5-NI), particularly metronidazole, are commonly used for first-line treatment of giardiasis. Metronidazole has been shown to be effective in 60–90% of cases with a large variety between studies [28,49,51]. The results for our total giardiasis collective are well inside the reported range. Still, the sub-group of travellers returning from South Asia showed a distinct deviation with a metronidazole failure rate of nearly 50%.

In a study done at the London Hospital for Tropical Diseases (HTD) tinidazole was used as first-line therapy and showed insufficient efficacy in cases from South Asia - especially from India - comparable to metronidazole in our study [31]. Cross-resistance within the group of 5-Nitroimidazoles seems likely. In one recent meta-analysis though, tinidazole was deemed the best available treatment for giardiasis, with the best efficacy and the lowest side effects [30].

In addition, the study from HTD found an increasing incidence of nitroimidazole-refractory giardiasis over time from 15% in 2008 to 45% in 2013 [31]. In our own analysis, this trend could not be detected (2007–2015).

Second-line treatment failed in nearly half of patients, whereby combination therapies seemed to have a better outcome compared to monotherapies (Table 4). A second course of metronidazole failed in all cases.

Few prospective studies look at second-line treatment of giardiasis. Existing studies are mostly retrospective or hampered by low case numbers. Nevertheless, it is possible when accumulating the results, to see a general trend that has been replicated in our study: uncertainty among clinicians as to the choice of the right treatment, the benefits of combination therapy, and the futility of repeating the failed first-line treatment [31,33,36,48,49].

4.3. Limitations and strengths of this study

This is one of the largest studies assessing the challenge of 5-nitroimidazole-refractory giardiasis in travellers observing data from more than 9 years. In addition, the study follows patients with treatment failure past first- and second line failure.

Even though *Giardia* in low-prevalence settings is commonly reported to be travel-related, one epidemiological study from Germany found that in fact almost half (48%) of Giardia patients did not have a history of recent travel [9]. Since the referral mechanisms of our institution did not allow us to tend to patients without a travel history, a comparison with domestically acquired cases was not possible.

Due to the retrospective study design patients were not subjected to standardised questionnaires, diagnostic protocols or routine follow-up after treatment including education on post-giardiasis lactose intolerance. Patients who did not return to our clinic were considered cured. This might lead to underestimation of treatment failure, with the possibility of patients having received further treatment at another clinic. At the same time we have no data on patients with parasitological cure and persistent symptoms caused e.g. by infection-induced lactose-intolerance.

Treatment was not standardised at our center during the study period, and over the years, many different physicians were involved in the treatment of giardia cases. We therefore arrived at a broad spectrum of treatment regimens leading to small patient numbers in the respective groups. We are therefore unable to give clear recommendations as to the preferred second line treatment, and treatment regimens beyond second line failure.

None of our patients were treated with nitazoxanide or quinacrine (mepacrine) for which other studies show promising results:

Nitazoxanide, approved in the US for treatment of giardiasis in 2004, is usually well tolerated. With a 3-day regimen, cure-rate was ranging between 71% and 85% when given as a first-line therapy [52–55].

In one randomized, open-label trial in Cuban children, a 3-day regimen of nitazoxanide was however inferior to tinidazole single dose treatment (78.4% vs 90.5% parasitological cure) [52]. In

NI-refractory giardiasis, nitazoxanide only achieved parasitological cure in 50% of cases [31].

Quinacrine (mepacrine) appears to be the most efficacious single drug treatment for nitroimidazole-refractory giardiasis. Treatment courses of 5–7 days achieved cure rates up to 100% [31,48–50].

A recent European open label, multicentre study assessed clinical and parasitological efficacy of combination chloroquine–albendazole vs. quinacrine monotherapy in patients with nitroimidazole treatment failure and evaluated adverse effects. Their findings show quinacrine as a highly effective treatment in nitromidazole-refractory giardiasis whereas the combination of albendazole and chloroquin achieved parasitological cure in less than half of cases [58].

However, treatment success in quinacrine has to be weighed against rare but potentially serious neuropsychiatric side-effects [36,56,57].

Practically, neither nitazoxanide nor quinacrine are licensed in Germany (Table 6), nor in most European countries. Both drugs have to be imported and costs for a single treatment course are at several hundred Euros, which may explain why none of the patients in this study had received it.

Persistance of *Giardia* infection has been linked to a variety of causes including suboptimal drug concentrations which may favour the development of resistance mechanisms. Particularly in less-developed countries, poor quality drugs, counterfeit or expired drugs may play a role in therapy-refractory disease.

Host factors include immunocompromised states, eg. Common variable immunodeficiency (CVID), IgA deficiency, HIV-infection and lymphoproliferative disorders. Also, reinfection may occur [16,32,34]. Neither cause could be systematically assessed in this retrospective study.

However, immunocompromise or lack of adherence fail to explain the excess risk of metronidazole-refractory *Giardia* seen in patients from South Asia so that primary parasitic drug resistance seems most likely.

Knowledge about resistance mechanisms in Giardia is limited: Most research into potential resistance mechanisms has been performed in cell lines in which resistance can be induced over time through exposure to progressively increasing drug concentrations [59,60].

Susceptibility testing for *Giardia* is not available in most laboratories since culture is difficult and time consuming [22,61]. Only a small fraction of Giardia isolates can at all be successfully grown in vitro which may create a bias and challenges the generalisability of the results gained from cell cultures [22].

In addition, there are currently no molecular markers of drug resistance for *Giardia* [34,35].

Mechanisms involved in 5-nitroimidazole resistance are reduced uptake, up- and down regulation, respectively, of different nitroreductase enzymes, and down regulation of the pyrovate ferredoxin oxidoreductase pathway (PFOR) [22,34].

Table 6
Recommended second-line therapy for giardia and drug license status in Germany [23].

recommended second-line therapy	licensed in Germany	licensed in Germany for treatment of giardiasis
tinidazole 2 g STAT	No	No
ornidazole 2 g od for 3 days	No	No
secnidazole 2 g od for 3 days	No	No
nitazoxanide 500 mg bd for 3 days	No	No
albendazole 400 mg od for 5 days	Yes (*for treatment of hydatid disease*)	No

STAT = single dose treatment, od = once daily, bd = twice daily.

4.4. Implications for clinical practice, future research and policy

4.4.1. Clinical practice

Given the high rates of metronidazole-failure it has to be discussed, if metronidazole is still an acceptable first-line treatment at all.

For patients from South Asia with a failure rate of nearly 50%, it may be worth considering the use of an alternative therapy in the first place. When choosing an alternative drug or drug combination, accessibility, cost and side-effects of treatment need to be considered as giardiasis is usually a fairly mild and not life-threatening infection. Patients need to be informed, that there is a high chance that their treatment might fail and a follow-up plan should be made.

4.5. Future research

A lot about refractory giardiasis and possible mechanisms of drug resistance is unknown. Parasitological and pharmacological research progress is hampered by the fact that *Giardia* is still difficult to grow and there are currently no molecular markers indicating drug resistance. In addition, in vitro data for giardiasis do not easily translate into clinical outcome [34]. These challenges need to be overcome to generate useful information to clinicians. Accurate and effective therapeutic management is hampered by the lack of opportunity to perform pre-therapy testing of antimicrobial resistance as done in bacterial infections [31,34].

Large and robust trials on treatment of refractory giardiasis are still lacking. Multi-center studies of clinics seeing larger numbers of returned travellers are urgently needed. However, clinical research on its own is not sufficient either and translational projects should be created to link the understanding of resistance mechanisms with epidemiological data and clinical outcome.

Most drugs used in giardiasis are ancient and either lack effectiveness, or have potential side-effects. Research into novel treatment options therefore should not seize.

4.6. Policy

None of the drugs or drug combinations recommended in Germany for treatment of metronidazole-refractory giardiasis (Table 6) [23] is currently licensed for this use.

Prescribing off-label drugs is a tedious process since insurance companies are not obliged to cover the costs, and even if costs are covered or patients are willing to pay for their treatment out of pocket it may take several weeks to import a drug from abroad exposing patients to prolonged suffering. This problem is not limited to Germany but exists in many other countries as well [36].

However, the challenge of inaccessibility of drugs and forced off-label use reaches far beyond the problem of *Giardia* infection, it is true for the treatment of most tropical diseases in non-endemic countries. A recent GeoSentinel study on *P. vivax* malaria in Eritrean refugees to Europe and Israel highlighted the fact that primaquine, the long-time mainstay of hypnozoite eradication in tertian malaria, was not licensed for this use in most European countries and extremely difficult to procure, even for expert centres [62].

This calls for action of tropical medicine advocacy groups: specialists should link up, as can be seen in networks such as GeoSentinel or the European network TropNet. Network strategists need to inform political leaders, researchers, non-profit research consortia like the Foundation for Innovative New Diagnostics (FIND) and the pharmaceutical industry about research gaps and gaps in drug supply for neglected tropical diseases.

5. Conclusions

The high rate of 5-nitroimidazole refractory *Giardia* seen in returning travellers is alarming as it prolongs clinical illness, and increases

treatmentcosts.

Means to explain its mechanisms, to diagnose and treat it are currently unsatisfactory. Translational research efforts are urgently required. Procurement for nonlicensed antiparasitic drugs has to be made easy.

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CRediT authorship contribution statement

Tanja E. Peters: Investigation, Formal analysis, Data curation, Visualization, writing (original draft). **Benno Kreuels:** Formal analysis, Validation. **Marylyn M. Addo:** Conceptualization, Supervision. **Egbert Tannich:** Conceptualization, Methodology, Resources, Supervision. **Camilla Rothe:** Conceptualization, Methodology, Project administration, Supervision, writing (reviewing and editing).

Declaration of competing interest

All authors declare that there is no conflict of interest.

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MAJOR ARTICLE

Efficacy and Tolerability of Quinacrine Monotherapy and Albendazole Plus Chloroquine Combination Therapy in Nitroimidazole-Refractory Giardiasis: A TropNet Study

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Background. Giardiasis failing nitroimidazole first-line treatment is an emerging problem in returning European travelers. We present data on the efficacy and tolerability of 2 second-line treatment regimens.

Methods. This prospective, open-label, multicenter study assessed the efficacy and tolerability of quinacrine monotherapy (100 mg 3 times per day for 5 days) and albendazole plus chloroquine combination therapy (400 mg twice daily plus 155 mg twice daily for 5 days) in nitroimidazole-refractory giardiasis. The defined end points were the clinical outcome, assessed at week 5 after treatment and the parasitological outcome, assessed using microscopy of 2 stool samples, ≥2 to ≤5 weeks after treatment.

Results. A total of 106 patients were included in the study. Quinacrine achieved clinical and parasitological cure in 81% (59/73) and 100% (56/56), respectively. Albendazole plus chloroquine achieved clinical and parasitological cure in 36% (12/33) and 48% (12/25), respectively. All patients (9/9) who clinically and parasitologically failed albendazole plus chloroquine treatment and opted for retreatment with quinacrine achieved clinical cure. Mild to moderate treatment-related adverse events were reported by 45% and 30% of patients treated with quinacrine and albendazole plus chloroquine, respectively. One patient treated with quinacrine developed severe neuropsychiatric side effects. The majority of nitroimidazole-refractory *Giardia* infections (57%) were acquired in India.

Conclusions. Quinacrine was a highly effective treatment in nitroimidazole-refractory giardiasis, but patients should be cautioned on the low risk of severe neuropsychiatric adverse event. Albendazole plus chloroquine had a low cure rate in nitroimidazole-refractory giardiasis. Nitroimidazole-refractory giardiasis was primarily seen in travelers returning from India.

Keywords. albendazole; chloroquine; *Giardia*; quinacrine; treatment.

Giardia duodenalis (syn. *G. lamblia*, *G. intestinalis*) is a flagellated protozoan that parasitizes the upper part of the small intestine of mammals, including humans. Transmission occurs via the fecal–oral route, either directly from person to person or indirectly through contaminated water or food. *Giardia duodenalis* is considered the most common parasite causing diarrheal disease worldwide and is a frequently identified pathogen in travelers returning from tropical and subtropical regions [1]. The clinical picture ranges from asymptomatic colonization to acute or chronic presentations with watery diarrhea, steatorrhea, nausea, abdominal cramps, vomiting, and malabsorption-related weight loss [2].

Before the late 1930s, treatment of giardiasis was largely empirical and included arsenicals, mercury, naphthalene, pyretines, bismuth sublimate, and other drugs [3]. Then, quinacrine (syn. mepacrine, atabrine), the leading antimalarial agent used during World War II, became the first systematically studied drug for the treatment of giardiasis (cure rate, 95%–100%) [4, 5]. Despite its side effects (primarily neuropsychiatric), quinacrine was the only available drug for giardiasis until metronidazole (the first 5-nitroimidazole compound) became commercially available in 1957 [6-8]. Similar to quinacrine's replacement as an antimalarial drug by the better tolerated and more effective chloroquine in the late 1940s, metronidazole progressively replaced quinacrine for giardiasis

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treatment in the 1960s due to its favorable side-effect profile and high efficacy (cure rate approximately 90%) [5, 9]. Consequently, metronidazole and the later introduced other 5-nitroimidazole compounds (tinidazole, ornidazole, secnidazole) became the first-line drugs for giardiasis [10].

Although the effectiveness of the 5-nitroimidazoles remained high over decades, an increase of nitroimidazole-refractory cases, defined as cases with persisting or relapsing infection despite single or repeated courses of nitroimidazole treatment, has been reported in recent years [11-13]. At the Hospital for Tropical Diseases in London, the rate of nitroimidazole-refractory giardiasis increased from 15% in 2008 to 40% in 2013 [12]. Although the reason for this increase is not fully understood, emerging drug resistance is considered very likely, with the vast majority of nitroimidazole-refractory cases being observed in travelers returning from South Asia, primarily India [12, 14, 15]. However, proving drug resistance in giardiasis is difficult because the organism is difficult to culture (success rate, 21%–44%) [16], no antimicrobial break points are established, and in vitro susceptibility or resistance does not always correlate with clinical outcome [17, 18]. Despite being a common clinical problem, data on how to manage giardiasis failing nitroimidazole first-line therapy are scarce. Retreatment with a second course of nitroimidazole is often offered, but efficacy is as low as 17% [19]. Most experts opt for a nonnitroimidazole compound (such as albendazole, mebendazole, paromomycin, nitazoxanide, furazolidone, chloroquine, quinacrine) as second-line treatment, either alone or in combination with a nitroimidazole.

Since data on the effectiveness of these drugs in second-line treatment are scarce, second-line regimens are chosen based on studies in which these drugs have been evaluated as first-line treatment, on the drugs' local availability, on the drugs' prize, and on physicians' preferences. This is well reflected in a survey conducted among members of the European Network for Tropical and Travel Medicine (www.tropnet.eu) in 2012. Fifty-three TropNet centers reported the use of 24 second-line treatment regimens, consisting of 7 drugs given as monotherapy or combination therapy in various dosages and durations (Neumayr A., unpublished data). Selecting a second-line treatment regimen according to its performance as first-line treatment is particularly problematic since efficacy data may not be transposable as such. In 2014, Meltzer et al published a retrospective analysis of 110 nitroimidazole-refractory giardia infections (12 of their own cases and 98 cases identified by reviewing the literature published between 1962 and 2013) that revealed the following treatment success rates: albendazole, 19%; paromomycin, 29%; nitazoxanide, 40%; albendazole plus a nitroimidazole, 79%; quinacrine, 91%; quinacrine plus a nitroimidazole, 100%; and quinacrine plus paromomycin, 100% [14]. Of note, the low effectiveness of monotherapy with albendazole, paromomycin, and nitazoxanide in second-line treatment contrasts with their reported efficacy in first-line treatment [20-23]. These observations suggest that nitroimidazole failure in first-line treatment may be a predictor of reduced effectiveness of most of the drugs evaluated in second-line treatment. Consequently, many experts advocate combination therapy in refractory giardiasis, although evidence from well-designed and adequately powered studies is lacking [24].

The most effective second-line drug (re-)emerging in recent years is quinacrine. Several small retrospective studies (number of patients, 2–14) confirmed the high effectiveness of quinacrine as second-line treatment for refractory giardiasis [12, 13, 19]. Although quinacrine is highly effective, its availability has decreased and its price has increased. This is problematic. Because quinacrine is not being licensed for the treatment of giardiasis in any country we know of, insurance companies may not reimburse the costs. Finally, there are concerns about the drug's neuropsychiatric side effects, especially toxic psychosis, which has been reported in 0.4% (35 of 7604) of soldiers who received quinacrine as malaria treatment (cumulative dose, 2.1 g) during World War II [25]. The risk of developing toxic psychosis is low with conventional antimalarial doses of 2.1–2.8 g, which are higher than the dosage usually used for giardiasis (1.5 g). It substantially increased when higher doses (1.4 g/day or cumulative doses of 3.8–4.8 g) were administered [26, 27].

In order to identify the best second-line regimen for nitroimidazole-refractory giardiasis, we conducted a prospective study to evaluate 2 treatment options selected after reviewing the literature: quinacrine in monotherapy (Q-MT) and albendazole plus chloroquine in combination therapy (AC-CT). Q-MT was chosen because of the promising data accumulated from smaller studies in recent years, and AC-CT was chosen because this regimen has not yet been evaluated and the wide availability of the 2 drugs would allow for the regimen's eventual broad use.

METHODS

We conducted a prospective, open-label, multicenter study at 4 travel clinics (Munich, Germany; Antwerp, Belgium; Bordeaux, France; Basel, Switzerland) within the *TropNet* network (www.tropnet.eu).

Any person having failed *G. duodenalis* first-line treatment with a single or repeated 5-nitroimidazole course, defined as having persisting or relapsing symptoms together with a stool microscopy positive for *G. duodenalis* at ≥2 weeks after completing therapy, was eligible for study inclusion. To exclude cases of reinfection, the upper time limit for study inclusion was set at 3 months after completing first-line treatment. Exclusion criteria for study participation were having received a non–5-nitroimidazole first-line treatment regimen; concomitant bacterial, helminthic, or protozoal gastrointestinal infection; and contraindications for the selected drug regimen according to the manufacturers' specifications.

After enrollment, the patients' basic demographic data, their travel history, relevant chronic medical conditions, and data on the failed nitroimidazole regimen were collected. The patients received either quinacrine (100 mg 3 times per day for 5 days) monotherapy or albendazole (400 mg twice daily for 5 days) plus chloroquine (155 mg base equivalent twice daily for 5 days) combination therapy (concomitant intake). Treatment allocation was based on local availability of drugs.

All study participants were counseled on temporary *Giardia*-related post-infectious lactose intolerance and instructed to avoid lactose-containing products until all gastrointestinal symptoms completely subsided in order to minimize the potential impact of lactose intake on the clinical outcome assessment.

The clinical outcome and the tolerability of the assigned second-line treatment regimen were assessed at week 5 by telephone using a standardized questionnaire after treatment was finished (see Supplementary Materials). If adverse events (AEs) were reported, study participants were asked to grade their severity on a visual analogue scale (VAS) from 1 to 10. The parasitological outcome was assessed by microscopy of at least 2 stool samples in all patients not reporting clinical cure at follow-up. Stool testing in patients who reported clinical cure was offered but not systematically requested.

The clinical outcome, evaluated at ≥4 to ≤5 weeks after completing treatment, was graded as follows: clinical cure: absence of gastrointestinal symptoms; clinical improvement: persisting gastrointestinal symptoms but improvement; the patients were asked to quantify persisting symptoms as a percentage of their maximum experienced symptoms; and clinical failure: persisting gastrointestinal symptoms without improvement or relapse of the initial/similar symptoms following transient resolution.

The parasitological outcome, evaluated at ≥2 to ≤5 weeks after finishing treatment, was graded as follows: parasitological cure: absence of *G. duodenalis* by microscopy in at least 2 stool samples and parasitological failure: detection of *G. duodenalis* by microscopy in a stool sample. In the case of clinical and parasitological failure of the assigned second-line regimen, treatment with the other optional second-line regimen was offered ("crossover").

The study results were summarized descriptively. A statistical comparison of the 2 study groups was omitted due to the nonrandomized design of the study. The study was approved by the responsible ethics committees at the participating study sites.

RESULTS

From 2014 to 2020, 106 patients with *G. duodenalis* infection who had clinically and parasitologically failed first-line treatment with a 5-nitroimidazole regimen were enrolled in the study (Table 1). Fifty study participants were female with a median age of 33 years (range, 21–68), and 56 patients were male with a median age of 34 years (range, 8–59). Fifty-seven percent of the study participants (60 of 106) acquired their infection in India (Figure 1).

After enrollment, 73 study participants received Q-MT and 33 received AC-CT. Tables 2 and 3 summarize the clinical and parasitological outcomes of the 2 regimens. An additional 9 participants who failed AC-CT were given Q-MT in crossover.

Treatment-related AEs were reported in 41% (47 of 115) of all treatment courses by 45% (37 of 82) of the study participants who received Q-MT and by 30% (10 of 33) of those who received AC-CT. Table 4 shows the frequency and severity of the reported AEs. Plausibly, quinacrine-specific AEs (urine discoloration, scleral discoloration) were only reported by study participants who received quinacrine.

All but 2 study participants completed their treatment. These 2 patients were both treated with Q-MT. One patient did not take the last quinacrine dose because of severe neuropsychiatric symptoms (sleep disturbance [VAS 9], nightmares [VAS 9], psychotic symptoms [VAS 9], and hallucinations [VAS 8]) that required a short hospitalization. After discontinuing Q-MT, the symptoms subsided

Table 1. Details of the Failed First-Line 5-Nitroimidazole Treatment Regimens

		Daily NI Dose	Number of Days of NI Treatment	Cumulative NI Dose	Assigned Second-Line Treatment Regimen	
Failed First-Line NI	Number of Patients	Median (Range), g	Median (Range)	Median (Range), g	Quinacrine	Albendazole + Chloroquine
Metronidazole	65	1.5 (1.2–2)	7 (7–30)	10.5 (8.4–45)	33	32
Ornidazole	12	1	5 (5–5)	5 (5–5)	11	1
Tinidazole	29	2	1 (1–7)	2 (2–14)	29	0

Abbreviation: NI, nitroimidazole.

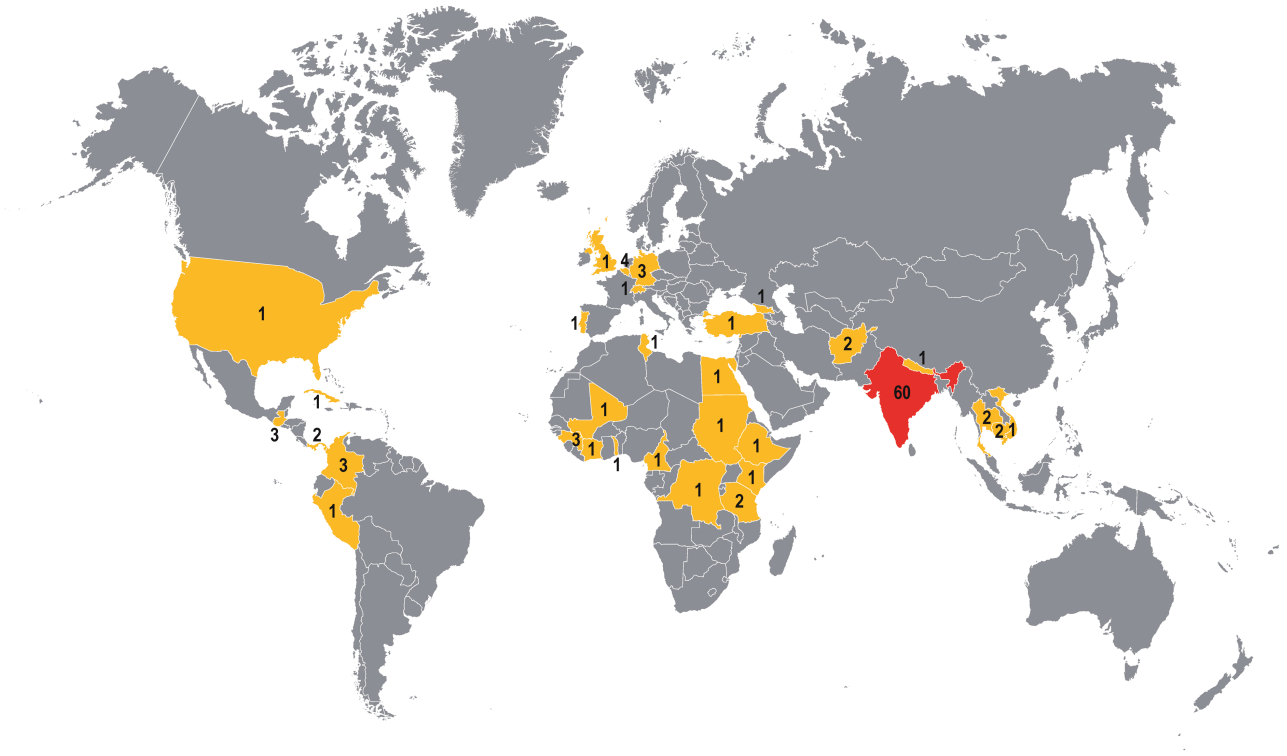

Figure 1. Countries where the 106 study participants acquired their nitroimidazole-refractory *Giardia* infections and the number of nitroimidazole-refractory *Giardia* infections from each country.

completely. Despite the incomplete treatment, the patient showed clinical and parasitological cure. In the other patient, Q-MT was discontinued by the study physician because of observed acute kidney injury. Although the observed renal impairment was judged to be most likely attributable to *Giardia*-related diarrhea, Q-MT was discontinued for safety reasons. The impaired kidney function normalized after rehydration. In this case, the clinical assessment was delayed much beyond the scheduled time but was favorable as well.

Table 2. Clinical Outcome of Second-Line and Crossover Treatment

Second-Line Treatment	Number of Patients	Clinical Outcome		
		Cure	Improvement	Failure
		% (n)	% (n)	% (n)
Quinacrine	73	81% (59/73)	19%[a] (14/73)	0% (0/0)
Albendazole + chloroquine	33	36% (12/33)	55%[b] (18/33)	9% (3/33)
Quinacrine crossover [c]	9	100% (9/9)	0% (0/0)	0% (0/0)

[a]Median rate of persisting symptoms 20% (range, 10%–80%).

[b]Median rate of persisting symptoms 50% (range, 10%–80%).

[c]Nine patients who did not achieve clinical cure and parasitologically failed albendazole + chloroquine treatment opted for retreatment with quinacrine, which led to clinical cure in all 9 patients.

DISCUSSION

Our study is the first and largest prospective investigation on the efficacy and tolerability of Q-MT and AC-CT as treatment for nitroimidazole-refractory giardiasis in international travelers. Overall, clinical and parasitological cure was very high in patients treated with Q-MT and rather disappointing in patients who received AC-CT. In general, AEs were mild to moderate, except in 1 case of severe neuropsychiatric decompensation undoubtedly related to quinacrine.

The clinical cure rate, the proportion of clinical cure vs clinical improvement, the median rate of persisting symptoms, and the parasitological cure rate were all very high in the Q-MT group, confirming previous results of smaller retrospective studies [12, 13, 19, 28]. Of note, despite parasitological cure, 19% of the quinacrine-treated patients still reported gastrointestinal symptoms at follow-up. This highlights that patients need to be counseled on the delayed resolution of symptoms, especially on the possibility of post-giardiasis lactose intolerance (affecting 20%–40% of patients [29]) in order to avoid unnecessary retreatment.

Although the clinical cure rate, the proportion of clinical cure vs clinical improvement, the median rate of persisting symptoms, and the parasitological cure rate in the AC-CT group were all inferior to those for Q-MT, AC-CT is still very likely superior to retreatment with a nitroimidazole, which has a reported effectiveness of as low as 17% [19] to 27% [30].

Table 3. Parasitological Outcome of Second-Line and Crossover Treatment

Second-Line Treatment	Number of Patients	Parasitological Cure			Parasitological Failure		
		Patients With Clinical Cure, % (n)	Patients With Clinical Improvement, % (n)	Patients With Clinical Failure, % (n)	Patients With Clinical Cure, % (n)	Patients With Clinical Improvement, % (n)	Patients With Clinical Failure, % (n)
Quinacrine	73	100% (42/42[a])	100% (14/14[a])	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)
Albendazole + chloroquine	33	86% (6/7[a])	33% (6/18[a])	0% (0/0) 0% (0/0)	14% (1/7[a])	67% (12/18[a])	100% (3/3[a])
Quinacrine crossover[b]	9	100% (9/9[a])	0% (0/0)		0% (0/0)	0% (0/0)	0% (0/0)

[a]In patients with clinical cure, parasitological retesting was optional. Patients with clinical failure or clinical improvement routinely underwent parasitological follow-up.
[b]Nine patients who did not achieve clinical cure and parasitologically failed albendazole + chloroquine treatment opted for retreatment with quinacrine, which led to clinical cure in all 9 patients.

Among the 82 cases treated with quinacrine, treatment was discontinued in 2. In 1 patient the occurrence of acute kidney injury was plausibly explained by giardia-related symptoms, and a causal relation with quinacrine was unlikely. The neuropsychiatric adverse events observed in the other patient are a well-known problem, extensively studied when the drug was used as an antimalarial compound in the 1940s. Among 5 recent studies that investigated quinacrine as a second- or third-line drug in treatment-refractory giardiasis [12, 13, 19, 28, 31], data on treatment-related adverse effects were reported in

Table 4. Frequency and Severity of Treatment-Related Adverse Effects

Treatment-Related Adverse Effect	Quinacrine (n = 73 + 9)		Albendazole + Chloroquine (n = 33)	
	Frequency	Severity	Frequency	Severity
	n (%)	VAS Median (Range)	n (%)	VAS Median (Range)
Fever	4 (5)	3.5 (2–10)	1 (3)	4
Dysgeusia/change of taste	10 (12)	3 (1–7)	1 (3)	3
Nausea	7 (9)	3 (1–10)	4 (12)	4 (1–8)
Vomiting	2 (2)	6.5 (3–10)	2 (6)	5 (2–8)
Abdominal discomfort	11 (13)	4 (2–8)	4 (12)	6 (5–8)
Diarrhea	7 (9)	3 (1–10)	3 (9)	5 (1–8)
Headache	10 (12)	5 (1–5)	3 (9)	3 (2–5)
Vertigo/dizziness	11 (13)	2 (1–10)	3 (9)	2 (1–9)
Nightmares	5 (6)	8 (1–9)	1 (3)	7
Psychotic symptoms	2 (2)	8.5 (8–9)	0 (0)	-
Hallucinations	1 (1)	8	2 (6)	3.5 (1–6)
Vision disturbance	1 (1)	1	3 (9)	3 (1–3)
Paresthesia[a]	2 (2)	3.5 (2–5)	1 (3)	3
Pruritus	3 (4)	2 (1–4)	0 (0)	-
Skin rash	1 (1)	5	1 (3)	2
Urticaria	0 (0)	-	0 (0)	-
Urine discoloration	6 (7)	2 (1–6)	0 (0)	-
Scleral discoloration	1 (1)	2	0 (0)	-
Skin discoloration	0 (0)	-	0 (0)	-
Other symptoms/complications[b]	5[c]	n. a.	2[d]	n. a.
Other central nervous system symptoms[b]	3[e]	n. a.	1[f]	n. a.

Abbreviations: n. a., not assessed; VAS, visual analogue scale (patients' subjective grading of the severity of symptoms from 1 to 10 points).
[a]Includes tickling, tingling, burning, pricking, chilling, or numbness of the skin.
[b]In addition to the above-mentioned prespecified symptoms (assessed using a fixed questionnaire), these symptoms were additionally reported/observed by the patients/treating physicians.
[c]Tiredness, n = 1; dyspepsia, n = 1; borborygmi, n = 1; oversensitive eyes, n = 1; acute kidney failure, n = 1.
[d]Back pain, n = 1; localized pain in right upper quadrant, n = 1.
[e]Tremor, n = 1; increased nervousness, n = 1; increased frequency of epileptic seizures (absences), n = 1.
[f]Emotional lability, n = 1.

4. In the study by Nash et al (n = 6; quinacrine plus metronidazole or tinidazole; cumulative quinacrine dose, 4.2–10.5 g), 2 patients suffered from toxic psychosis, 1 patient from seizure, and 1 patient from significant liver function abnormalities [28]. In the study by Mørch et al (n = 3; quinacrine plus metronidazole combination treatment for 2–3 weeks; cumulative quinacrine dose, 5.1–6.3 g), confusion was reported by 2 patients, nightmares by 1 patient, dizziness by 1 patient, and discoloration of skin or urine by 2 patients. In 1 case, the duration of treatment was limited to 2 weeks instead of 3 because of side effects [31]. In the study by Muñoz-Gutiérrez et al (n = 14; cumulative quinacrine dose, 1.5 g), adverse effects were not systematically assessed, but the authors stated that "none of the patients had to discontinue quinacrine therapy because of major adverse effects" [19]. In the study by Requena-Méndez et al (n = 13; cumulative quinacrine dose, 1.5 g), treatment-related irritability and somnolence were reported in 1 child [13]. Of note, neuropsychiatric adverse effects and even toxic psychosis have also been reported with chloroquine [32, 33], and mild disorders were reported by our patients treated with AC-CT as well.

In general, these reports and our observations indicate that quinacrine-related neuropsychiatric adverse effects do occur but are very infrequent at the usual cumulative dose of 1.5 g. Patients should be warned of this risk, and those with underlying neuropsychiatric disorders should not be offered this treatment.

Most patients acquired nitroimidazole-refractory giardiasis in India. Although our data are observational and lack a denominator, identical observations have been reported in the United Kingdom and Israel, where the majority of travel-related cases of nitroimidazole-refractory giardiasis also were acquired in India (United Kingdom: 69.9%, 51 of 73 [12]; Israel: 50%, 6 of 12 [14]). To date, data linking the observed nitroimidazole failure in giardiasis acquired in India to a specific molecular resistance mechanism are lacking. Subsequently, postulated drug resistance of *G. duodenalis* currently remains a clinical observation in patients who received an appropriately dosed treatment regimen. Since the patients in our study all received appropriately dosed nitroimidazole treatment courses (Table 1) according to current treatment recommendations [34-36], our data support previous observations that suggested that some nitroimidazole-tolerant or -resistant *G. duodenalis* strains are emerging in India.

Our study has several limitations. First, we chose an open-label, nonrandomized design, mainly because quinacrine was not always continuously available at all study sites, and also because in situations where treatment costs were not reimbursed by the patient's health insurance, the choice of regimen was influenced by its price. Second, inclusion in the study was based on stool microscopy and not on the more sensitive antigen-based assays or polymerase chain reaction methods. Thus, cases of treatment-refractory giardiasis may have been missed. However, robust longitudinal studies are lacking for both latter methods, and interpretation of post-treatment parasitic outcome is challenging [11]. For the purpose of this comparative study, it was considered preferable to follow up treatment response with microscopy as a more consistent assessment tool. Third, we limited parasitological follow-up to patients who had not achieved clinical cure at follow-up. This decision was intentionally made as there is no evidence for retreatment of a parasitologically persisting infection after clinical cure. However, by not systematically testing stool of clinically cured patients for persisting infection, some cases of parasitological failure may have been missed. Finally, no systematic testing for possible underlying immunological causes predisposing to treatment failure (eg, immunoglobulin [Ig] A deficiency, hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency) was conducted. This is in contrast to a previous study where IgA deficiency was quite frequently found [14]. However, in the absence of a suggestive medical history, such investigations are rarely performed after a single treatment failure in clinical practice. The single patient in our study with known hereditary hypogammaglobulinemia was treated with Q-MT and achieved clinical and parasitological cure.

The question arises of how is nitroimidazole-refractory giardiasis treated if quinacrine is unavailable or contraindicated? Smaller studies (n = 10–53) suggest that the combination of albendazole plus a 5-nitroimidazole is effective in 60%–90% of nitroimidazole-refractory cases [12, 14, 37, 38], which may be superior to albendazole plus chloroquine. This has recently been confirmed in a Cuban study (n = 456) in which a treatment ladder of metronidazole (first-line), secnidazole (second-line), tinidazole (third-line), secnidazole plus mebendazole (fourth-line), and quinacrine (fifth-line) was investigated. While low cure rates were observed in patients retreated with a 5-nitroimidazole (secnidazole, 24%; tinidazole, 27%) and quinacrine cured all patients (100%, n = 15), secnidazole (-g single dose) plus mebendazole (200 mg 3 times per day for 3 days) achieved parasitological cure (confirmed by 3 negative fecal samples) in 87% (100 of 115) of the cases [39].

Further research on this neglected topic should ideally be designed as a multicenter, randomized, controlled trial among networks of specialized travel clinics. However, country-specific availability of orphan drugs remains a challenging issue.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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throughout Europe. One of its goals is to provide rational assessments and recommendations in disputed management issues of infections in returned travelers.

Potential conflict of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Our study has some potential limitations. We could not determine whether the infection in the nursery worker was the real index case because one of the children's parents had tested positive within the previous 2 weeks and that child could also have been the primary case. Moreover, we could not verify the information we obtained from the nursery about the facility's prevention methods.

Our report questions the role of young children in driving the COVID-19 pandemic. Of note, most children in our study were asymptomatic, and this cluster would likely not have been detected without subsequent testing of persons who had direct contact with the index case-patient. We believe further studies are needed to clarify young children's role in the transmission of SARS-CoV-2.

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Developing Endemicity of Schistosomiasis, Corsica, France

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Urogenital schistosomiasis was diagnosed in a man from Germany who had never traveled outside Europe. He likely acquired the infection in Corsica, France, but did not swim in the Cavu River, which was linked to a previous outbreak. This case highlights that transmission of schistosomiasis in Corsica is ongoing.

A 49-year-old man from Germany experienced macrohematuria in June 2020 and underwent cystoscopy in August 2020. Histologic analysis of a bladder biopsy specimen showed ova of *Schistosoma* spp. He was referred to the outpatient department for tropical medicine at LMU Hospital Munich.

The patient had never traveled outside Europe. He had, however, traveled twice to Corsica, France, in 2013 and 2019. He never swam in the Cavu River, which has been associated with cases of schistosomiasis in recent years (*1*–*3*).

The patient visited Corsica during August 22–September 4, 2019. By using GPS data from his smartphone and camera, he reconstructed his bathing sites precisely. During August 22–24, he swam

several times in the Solenzara River in the southeastern part of the island, near a busy campsite (Figure). Further minor water contacts might have occurred at the Gravona River in western Corsica near Ajaccio, at a turtle park and near a campsite, and at the Tavignano River (Figure). He did not bathe in either of Corsica's other rivers but could not rule out contact with water for cooling purposes. The patient reported swimming in the Restonica River in 2013 (Figure), but he did not recall any itchy rash suggestive of cercarial dermatitis.

Figure. Locations of rivers referenced in investigation of developing endemicity of schistosomiasis, Corsica, France. Map adapted by using UBehrje (https://www.demis.nl); in public domain (https://upload.wikimedia.org/wikipedia/commons/3/3e/Corsica_Map.png).

Physical examination was unremarkable. Full blood count did not show any eosinophilia. Urine sediment microscopy showed terminal-spined ova resembling those of *S. hematobium* parasites. Urine tested in-house by quantitative PCR for *S. hematobium* parasites was positive. A schistosoma serologic test was positive by enzyme immunoassay (64 U/L; reference <10 U/L); results of a blood immunochromatographic rapid diagnostic test were positive, but Western blot results were negative. Results of a urine circulating cathodic antigen point-of-care test was weakly positive. His son, who traveled with him in 2013, and his wife, who had joined him on both trips, had negative serologic test results and were negative for urine schistosoma by PCR.

DNA extracted from 7 ova was successfully amplified on nuclear internal transcribed spacer and mitochondrial cytochrome oxidase I markers. For the nuclear marker, DNA (995 bp) of all eggs showed a typical signature of *S. hematobium* parasite. The mitochondrial marker (873 bp) of all eggs had a typical signature of *S. bovis* parasite and displayed the same haplotype. In summary, all eggs were identified as hybrids of *S. hematobium* and *S. bovis* (ShxSb) parasites. This type of hybrid (nuclear Sh and mitochondrial Sb) was the most frequently observed type during the 2013 outbreak (*4*). The mitochondrial cytochrome oxidase I sequences of all eggs had 100% homology with haplotype Sb2, the most frequent haplotype observed during the 2013 outbreak (*4*). Presence of this haplotype was also documented in subsequent reports on the first outbreak in Corsica (*2*,*3*).

Transmission of schistosomiasis in Corsica has been documented since 2013 (*3*,*5*). Cases have been detected in residents and in tourists (*5*,*6*). In spring 2014, after the first outbreak, intense screening and public health efforts led to the identification of 106 cases linked to the Cavu River (*7*). During 2015–2018, sporadic cases were detected and linked to the Cavu (*8*). The cases reported in 2018 involved 2 patients who had bathed in the Cavu and Solenzara Rivers (*3*).

The patient we describe reported having no contact with the Cavu River. Instead, the infection was most likely acquired in the Solenzara River, near a busy campsite. The Solenzara neighbors the Cavu, but their waters do not intermingle. No confirmed cases acquired in the Solenzara River have been documented, but *Bulinus truncatus* snails or their DNA have been found along the river during environmental surveys (*1*,*9*). Snail density close to the main bathing site of our patient appears to be high (*9*). Neither of the other rivers mentioned was ever proven to be a source of schistosomiasis; in addition, they are all relatively far away from the Cavu region.

Genotyping revealed the *S. hematobium–S.bovis* hybrid parasite described in previous outbreaks, suggesting ongoing transmission rather than reintroduction. The parasite's emergence in another river cannot be explained by the persistence of infected snails (*9*) but could be explained by reseeding of the river by a mammalian host.

Animal reservoirs have been discussed as a possible explanation for ongoing transmission; however, evidence of a major role is lacking. No infection has been detected in livestock in the region, and the only infected animals found were 2 rats (*10*). Even if we cannot rule out the influence of an undetected animal reservoir (e.g., *Ovis aries musimon,* wild sheep native to Corsica, have never been tested), the most likely explanation is that 1 or several infected persons continue to infest the water.

In summary, this case highlights that transmission of schistosomiasis in Corsica is ongoing and is no longer restricted to the Cavu River. The parasite appears to be of the same strain detected previously on the island. The infection was acquired at a frequented tourist site, suggesting that more persons might have been infected. Further screening of residents and tourists is urgently needed.

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Relapsing Fever Group Borreliae in Human-Biting Soft Ticks, Brazil

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Retrospective clinical case series study in 2017 identifies *Plasmodiumknowlesi* as most frequent *Plasmodium* species in returning travellers from Thailand to Germany

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Febrile illnesses are common in travellers returning from south-east Asia. However, malaria is a rare diagnosis in this population. A series of *Plasmodium knowlesi* infections was noted in German travellers returning from Thailand since 2012. Infectious disease and tropical medicine facilities registered by the German Society for Tropical Medicine and International Health were contacted in March 2017, and asked to report previous *P. knowlesi* cases. In addition, surveillance data from the Robert Koch-Institute were analysed. The facilities reported a total of six *P. knowlesi*-positive cases, all were returning travellers from Thailand. The *P. knowlesi*-positive cases made up 6/9 of all diagnosed malaria cases imported from Thailand in the time period 2012 to 2017. In 4/5 of cases where a malaria rapid diagnostic test had been applied it revealed a negative result. *P. knowlesi* is an important differential diagnosis in travellers returning from south-east Asia with itineraries that include Thailand. This study highlights the importance of this *Plasmodium* species in this patient subgroup. Whenever malaria is suspected in a returning traveller from Thailand, *P. knowlesi* should be taken into consideration and a differential PCR be executed as currently the unequivocal diagnosis of *P. knowlesi* is based on nuclear amplification techniques.

Background

Germany is currently the second leading country worldwide in numbers of international travels with about 84 million international travel departures in 2015, most of them for leisure purposes [1]. About one in seven international travellers to overseas from Germany suffers from a medical condition during their travel, and among those affected, about one quarter require some kind of medical assistance [2]. Malaria remains a relevant differential diagnosis in febrile patients arriving from tropical and subtropical areas, with 1,068 imported malaria cases reported in Germany in 2015 [3]. Thailand is a favourite travel destination for German travellers with 761,000 arrivals from Germany in 2015. In the same year, Thailand reported almost 30 million international arrivals in total [4].

Malaria is considered hypoendemic in Thailand with a reported total of 14,755 confirmed cases and 33 deaths in a total population of about 68 million in 2015 (incidence: 21 cases/100,000). However, the estimated true incidence of malaria infections is expected to be much higher at an annual incidence rate of 176 cases per 100,000 population [5].

The German guidelines for malaria prevention in travellers are issued annually by the German Society for Tropical Medicine and International Health (DTG). DTG recommends carrying an anti-malarial standby

Table

Cases of *Plasmodium knowlesi* infections reported in Germany, 2012–2017

Case ID	Date of admission	Age in years	Sex	Level of care	RDT	Smear parasitaemia[a]	Nuclear amplification techniques	Treatment
1	January 2012	54	Male	IPD	Not done	0.01%	Sequencing positive[b]	Atovaquone/proguanil
2	January 2013	55	Female	IPD	BinaxNOW, pan-aldolase (T2 band) positive	0.2%	PCR positive[c]	Artesunate followed by Artemether/lumefantrine
3	December 2013	73	Male	ICU	BinaxNOW negative	3%	PCR positive[c]	Quinine/doxycycline
4	December 2014	52	Female	IPD	BinaxNOW negative	1%	PCR positive[c]	Artemether/lumefantrine
5	February 2015	42	Male	IPD	BinaxNOW negative	0.02%	PCR positive[c]	Atovaquone/proguanil
6	January 2017	45	Male	OPD	BinaxNOW negative	0.0002%	PCR positive[c]	Atovaquone/proguanil

ICU: intensive care unit; IPD: in-patient department; OPD: out-patient department; RDT: rapid diagnostic test.

[a] Percentages of infected erythrocytes are presented.

[b] Sequencing of small subunit ribosomal RNA, Basic Local Alignment Search Tool analysis of the obtained sequence, yields a 96% match with the respective *P. knowlesi* sequence.

[c] A PCR with primers specific for *P. knowlesi* was conducted.

medication when travelling to Thai regions bordering Cambodia, Laos and Myanmar only, and only if urban settings with a presumably adequate medical infrastructure are left behind [6].

The *Plasmodium* species mentioned in the latest 2016 World Malaria Report as being prevalent in Thailand are *P. falciparum* (41.8% of all specified cases) and *P. vivax* (58.2%) [5]. Previous publications in 2011 and 2015 have indicated occasional cases of *P. knowlesi* infections in the resident population of Thailand [7,8]. In addition, on rare occasions, *P. knowlesi* infections in German travellers returning from Thailand have been reported [9-13]. Published studies, mostly on the basis of individual case reports, from other European countries, such as Finland, France, Spain, Sweden and the Netherlands, have also reported on importations of *P. knowlesi* infections from south-east Asia in returning travellers, indicating the diagnosis of a *P. knowlesi* infection as a generally rare event. The advent of occasional *P. knowlesi* identifications in returning European travellers seems to have started about a decade ago [14-20]. Of note, a recent analysis by the GeoSentinel Network, a global network for surveillance of travel-related illnesses, which included data from 29 countries, only reported three cases between 2003 and 2016, all imported from south-east Asia, whereby the explicit countries of acquisition were not indicated [21].

Malaria caused by *P. knowlesi* is a zoonotic infection endemic to south-east Asia, with a primary reservoir in macaques. The geographical distribution of *P. knowlesi* is therefore linked to the presence of its primary hosts. Humans can be infected occasionally, especially in areas where human settlements are advancing into habitats of the reservoir host, namely forested areas. Investigations on archived blood samples in Malaysia have revealed that the large majority of cases previously classified by microscopy as *P. malariae* infections were in fact *P. knowlesi* infections [22]. The parasite species has a short replication cycle of only 24 hours and can thus rapidly generate high parasite loads [23]. More frequently than in infections with *P. malariae*, *P. vivax* and *P. ovale*, *P. knowlesi* causes severe courses of disease that resemble *P. falciparum* malaria. In particular, respiratory and renal complications have been reported [16].

Upon noticing a series of *P. knowlesi* cases in people returning from Thailand to Germany, this study was conceived in order to investigate the relevance of this parasite as a causative agent for febrile conditions in such travellers.

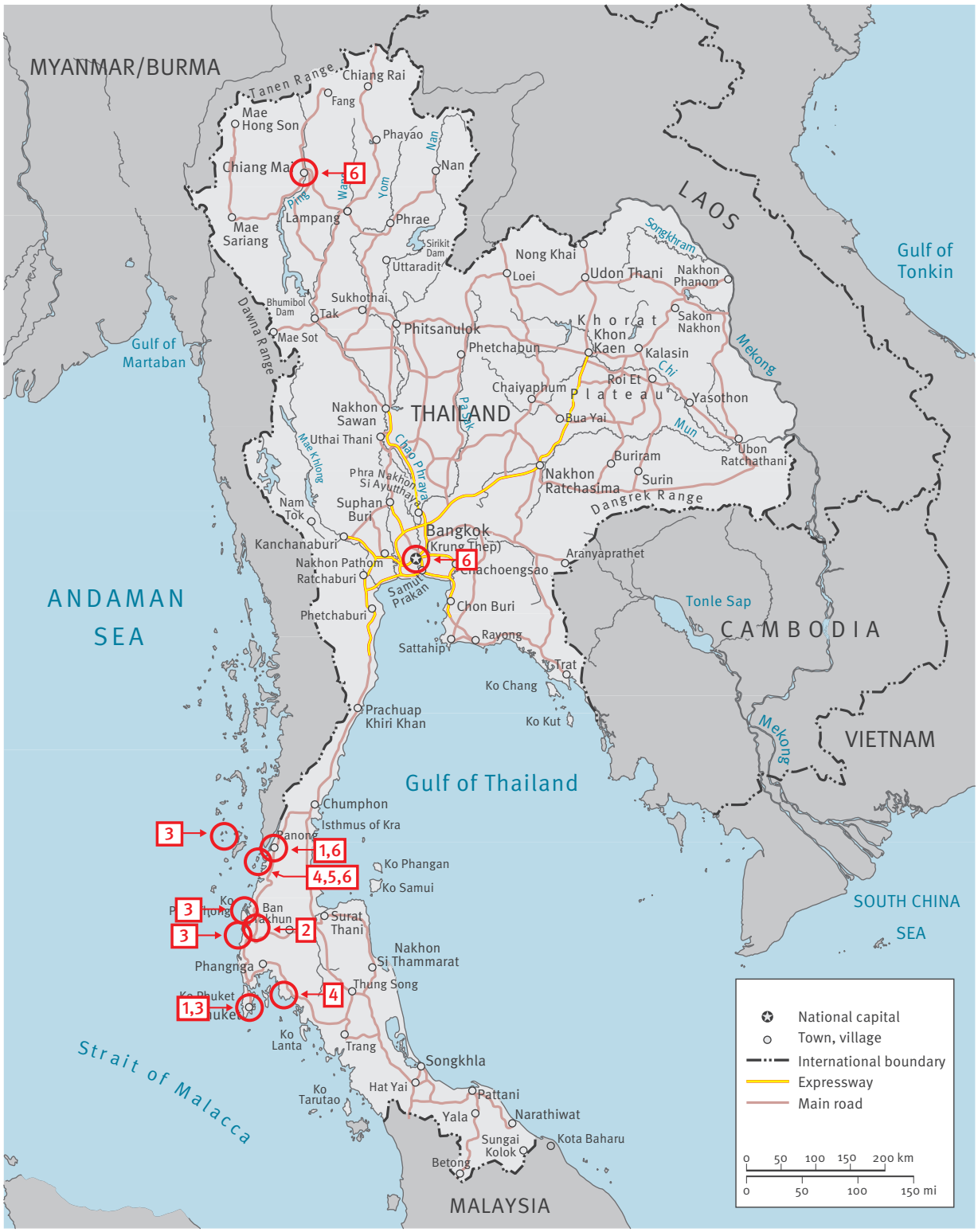
Methods

Questionnaire

A digital questionnaire was compiled in the format of a case report file (Microsoft Word). For each reported *P. knowlesi*case, one questionnaire was to be completed by the facility (as described below) that identified the case. Questions included age, sex, purpose of travel, travel itinerary, date of return, date of presentation, clinical presentation, means of diagnosis employed, highest level of care, treatment regimen and clinical course. In addition, details were requested on the total number of malaria cases in the respective facility for the respective reporting year in patients returning from the same country from which the reported *P. knowlesi* case had returned.

Figure

Map of Thailand with travel destinations of cases of *Plasmodium knowlesi* infections, 2012–2017



This map is adapted from a map by the Department of Field Support, Cartographic Section of the United Nations [32]. Encircled in red are locations of stay as indicated by the reported cases. Numbers in arrowed boxes are indicating case IDs as outlined in the Table.

Data sources

The DTG is a reference institution in the field of travel medicine in Germany with annual publications on malaria risk profiles for worldwide travel destinations and guidelines on prevention and treatment of malaria. The society lists all major infectious diseases and tropical medicine facilities in Germany. The complete list of 17 facilities was contacted on 16 March 2017 with an invitation to participate in the study by reporting whether *P. knowlesi* cases had been diagnosed previously at each respective facility, and if so, to complete one questionnaire per *P. knowlesi* case. Furthermore, corresponding authors of already published cases (who were found as further described) were directly contacted. The last completed form was returned on 23 May 2017.

In addition, a literature search of articles in English language in PubMed using the keywords 'knowlesi' AND 'Germany' was conducted. Malaria in Germany is mandatorily reported to the national public health institute (the Robert Koch-Institute; RKI). The archive and surveillance data of the RKI were searched to potentially identify additional cases [24].

Results

Case finding

In total 17 facilities were contacted, including the facility of the study coordinators. Sixteen of the 17 facilities replied and provided completed questionnaires if cases had been observed. Eleven facilities indicated never having diagnosed any case of *P. knowlesi* infections. Four facilities reported one case each and one facility reported two respective cases, resulting in a total of six *P. knowlesi* cases. The earliest case ever reported in Germany was in 2012 and all six cases had travelled to Thailand. A literature search revealed only cases who were also reported directly by the participating facilities [9-13].

An investigation of the archive and the surveillance data of the RKI provided a total of nine notified malaria cases across all *Plasmodium* species that were imported from Thailand to Germany in the time period 2012 to 2017 as denominator. As part of these nine, all six *P. knowlesi* cases who were reported by the specialised facilities through this study could also be retrieved in the RKI surveillance data. In addition, one *P. knowlesi* case with missing information on travel purpose, destination and probable country of infection was revealed by the surveillance data; this case could not be included in the analysis.

Epidemiological and demographic description of cases

The reported six cases comprised four males and two females, the age range was 42 to 73 years and all cases were German citizens. The earliest case was from 2012 and had travelled to Thailand. Six further cases of *P. knowlesi*infections subsequently occurred, five of which had also travelled to Thailand and one with travel information missing, as mentioned above. Cases are hereafter referred to by case identification numbers, as outlined in the Table.

Tourism was the main purpose of travel in five of six cases, one case reported to have visited friends and relatives (case 1). All travels took place in the months of November to February.

From a more detailed investigation of the respective travel itineraries, it was noted that all cases had travelled to the southern Thai provinces of Ranong, Phang-Nga and Surat Thani (Figure).

It has to be pointed out that the island of (Little) Koh Chang in the Andaman Sea, which should not be confused with the larger island of Koh Chang in the Gulf of Thailand, was mentioned as a destination by three of the travellers (cases 4,5,6), and for all three this island was the location with a considerably longer duration of stay. Only one patient (case 3) also travelled outside Thailand during the itinerary; however, his destination in neighbouring Myanmar was the island of Macleod, which lies in the immediate vicinity of Ranong Province. In addition to the above named provinces, patients mentioned Bangkok as city of entry including just a brief stop-over, and one patient (case 6) also travelled to the northern city of Chiang Mai before passing the largest part of the stay in the southern province of Ranong (Figure).

Case clinical presentation, diagnostics and management

All cases presented with fever (temperature ≥ 38.0 °C) as the leading symptom. The lag-time between return from travel and presentation at the facilities was between 1 and 23 days. In all cases a positive blood smear led to the diagnosis of a *Plasmodium* infection. In 5/6 cases, the microscopy result was initially reported as presence of *P. falciparum* or *P. malariae*, but also morphological resemblance to *P. vivax* and *P. ovale* was reported in two respective cases. Co-infections by multiple species were reported in the initial smear results in three of the six cases. The parasite load was in the range between 0.0002% and 3%. The parasite load was particularly low in case 6, where the microscopy result that led to further investigations was based on a single gametocyte in a total of 100 investigated fields at 400x magnification. In five of the six cases (cases 2–6) a rapid diagnostic test (RDT) for malaria was conducted. All facilities used the BinaxNOW Malaria assay (Alere, Scarborough, Maine, USA). In four of five RDTs conducted the test revealed a negative result. Only one facility reported a positive T2 band (case 2), corresponding to the pan-plasmodial aldolase (Table). According to the BinaxNOW Malaria product specifications a positive T2 band with a negative T1 band is interpreted as a mono- or mixed infection by *P. vivax*, *P. ovale* or *P. malariae*, in the absence of *P. falciparum*. All six reported cases were confirmed

to be *P. knowlesi* by nuclear amplification techniques. For case 1 the small subunit ribosomal RNA sequence was amplified and the sequence was further analysed by Basic Local Alignment Search Tool (BLAST). This yielded 96% identitity with sequencing data from *P. knowlesi* [13]. For the other cases a differential PCR with primers for *P. knowlesi* was conducted. Five of the six cases, were hospitalised. In two cases the World Health Organization (WHO) criteria for severe malaria were fulfilled [25]. In one case admission to an intensive care unit was necessary due to severe presentation with renal and respiratory insufficiency, acidosis and reduced vigilance (case 3). This case was at the same time the one with the highest parasitaemia of 3% in this case series. A second case of complicated *P. knowlesi* malaria was treated with renal impairment; however, in this case intensive care was not pursued (case 2). Treatment was in all cases initiated prior to the conclusive diagnosis of *P. knowlesi* infection, and corresponded with the results of initial microscopy. As the applied regimens were assumed to be effective also in *P. knowlesi* infections, no change in treatment regimen had to be executed in any of the cases (Table).

The treatment outcome was considered successful in all cases. The time until improvement of symptoms was indicated as 1, 2 and 3 days after commencement of anti-malarial treatment, for three of the reporting facilities where this information was retrievable from files. No recrudescence was reported in any of the cases.

Proportion of *Plasmodium knowlesi* cases among malaria cases imported from Thailand to Germany

The five facilities reporting *P. knowlesi* cases were asked to indicate the total number of malaria cases for the same travel destination from which the index case had imported the *P. knowlesi* infection (in all cases Thailand) for the respective year of diagnosis of the *P. knowlesi* case. In total, seven cases of malaria were reported by the facilities in returning travellers from Thailand in the respective years, including the six cases of *P. knowlesi* infections. Only one facility indicated one additional case other than *P. knowlesi* in a returning traveller from Thailand for the year 2014. The proportion of *P. knowlesi* infections among all malaria cases from Thailand in the reporting facilities for the reported years is therefore amounting to 6 of 7. When the total number of nine malaria cases who were reported to the RKI as imported from Thailand to Germany in the time period 2012 to 2017 is taken as denominator, the proportion of *P. knowlesi* infections amounts to 6 of 9 of all imported cases from Thailand. The remaining cases were two *P. vivax* and one *P. falciparum* infection.

Discussion and conclusions

Malaria remains a very low risk for German travellers to the favourite travel destination Thailand. In 2015, a total of 761,000 travellers from Germany departed for this country. The RKI reported a total number of 1,068 imported malaria cases to Germany for 2015. Only two of these were imported from Thailand [3]. In the entire time period from 2012 to 2017, only a total of nine malaria cases were reported to the national public health institute as imported from Thailand. The facilities participating in this study, which are among the largest facilities in Germany specialised in the field of infectious diseases and tropical medicine, identified six infections that were due to *P. knowlesi*. This species is not reported as prevalent in Thailand in the latest World Malaria Report of 2016 [5]. Given the finding, that in most cases a *Plasmodium* species other than *P. knowlesi* was initially morphologically indicated, and that the common range of anti-malarials applied are also active against *P. knowlesi*, we assume that imported *P. knowlesi* infections may frequently remain undetected. It is of note that resemblance in *P. knowlesi* morphology may be indicative of the stage in replication cycle, as early stages have been reported to rather resemble *P. falciparum*, whereas later stages more frequently show features of *P. malariae* [26].

In addition, more and more non-specialist institutions rely for their primary diagnosis of malaria on RDTs, which are highly sensitive for *P. falciparum* malaria but frequently fail to detect non-falciparum species [27]. In this study, the specialised facilities executed an RDT in five of the six *P. knowlesi* cases. In all cases the RDT used was the BinaxNOW Malaria. Only one test revealed a positive result for the aldolase antigen only. The BinaxNOW has been designed and validated by the manufacturer for *P. falciparum* and *P. vivax* infections only. The T1 band represents the histidine-rich-protein II, which is a specific antigen for *P. falciparum*, whereas the T2 band represents the pan-plasmodial aldolase antigen. For *P. falciparum* the manufacturer indicates an overall sensitivity of 95.3% and a specificity of 94.2%; the test performance is lower in *P. vivax* infections, with an overall sensitivity of 68.9% (specificity 99.8%). The product information by the manufacturer indicates that the RDT is also able to detect *P. ovale* and *P. malariae* but for these species the clinical performance has not been adequately established. *P. knowlesi* is not mentioned in the test specifications [28]. A study conducted in Malaysia reported low sensitivity of RDTs that are based on the pan-plasmodial aldolase for all *Plasmodium* species, with the lowest sensitivity in *P. knowlesi* infections at 23%. Although sensitivity generally increased with parasite load, only 45% of highly parasitaemic patients with a *P. knowlesi* infection (›10,000 parasites/µL) revealed a positive result [27]. This finding is corroborated by the results of this study where one positive pan-plasmodial aldolase test was found in five patients. RDTs with a pan-plasmodial lactate dehydrogenase component generally seem to perform with a higher sensitivity for all species including *P. knowlesi*, but even there one in four *P. knowlesi* infections remains undetected by the assay [27]. Of note, three of the six cases reported in this study had a low parasitaemia of ‹0.1% infected

erythrocytes, which contributes to impaired test performance even more.

In settings where malaria detection is depending on RDT performance, a possible case of *P. knowlesi* malaria is likely to remain undetected or to be complicated by late diagnosis. Severe courses of infection in immunologically naive individuals are assumed to be less frequent in *P. knowlesi* than in *P. falciparum* infections, but more frequent than in *P. malariae*, *P. vivax* or *P. ovale*, which corroborates the importance of a valid species differentiation. In this study, two of six cases fulfilled criteria for severe malaria.

Since malaria still is a possible aetiology for febrile conditions in returning travellers with a potentially severe clinical course, the findings of this study suggest that *P. knowlesi* may need to be added to the risk profile of Thailand. The high proportion of *P. knowlesi* infections in the investigated population of this study (6/9) demands that once the diagnosis of a malaria infection is established in a traveller returning from Thailand (or indeed elsewhere from south-east Asia), a differential malaria PCR that comprises *P. knowlesi* should be executed, irrespective of the species differentiation through microscopy, and irrespective of an RDT result. In addition, in cases with a persistent suspicion of a malaria infection but with negative smear and RDT results, a differential PCR comprising *P. knowlesi* should be considered as a means of diagnosis of a possible *P. knowlesi* infection presenting with a low parasitaemia. However, the extremely low incidence of only two cases of malaria across all species in an annual 761,000 travels from Germany to Thailand may provoke a reconsideration of current travel recommendations with regard to malaria prevention, especially in light of reports of inadequate use of anti-malarial standby medication [29].

The findings of this study are expected to be of equal relevance to other European countries with comparable tourism profiles of their citizens. As we previously highlighted, identification of *P. knowlesi* remains currently a rare event for countries such as Finland, Sweden, Spain and France. However, as the overall incidence of *Plasmodium* infections across all species in European travellers returning from south-east Asia is largely declining [30] the consideration of *P. knowlesi* infections becomes important based on our and earlier findings.

There are some limitations to this study. A selection bias could have occurred, as only infectious disease and tropical medicine facilities registered by the DTG were contacted. A relevant number of cases of malaria are detected outside these facilities in Germany. In addition, the authors suspect a number of unrecognised — or more specifically misclassified — cases of *P. knowlesi* infections, as in many cases of febrile illnesses in returning travellers from Thailand the necessary differential PCR will not have been executed. The authors are convinced that a number of the cases, who were previously classified as infections by especially *P. malariae* and *P. falciparum* from south-east Asia, were actually infected by *P. knowlesi*.

The importance of considering *P. knowlesi* in malaria control efforts in south-east Asia has been pointed out in a recent publication by Barber et al. [31]. Taking travellers as sentinels, our cases series may highlight an, as yet, under-addressed public health problem in some parts of Thailand. Further PCR-based studies in targeted areas of Thailand should be of public health interest to find out which share *P. knowlesi* infection takes in human as well as in simian malaria.

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Conflict of interest

None declared.

Authors' contributions

GF and CR conceived the study, were responsible for data collection and wrote the manuscript; GF, HDN, FvS, GB, MP, MH and CR conducted the data analysis; RP, IK, MS, HMO, SJ, PK and SVB collected data and filled case report forms. All authors critically revised and approved the final version of the manuscript.

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RESEARCH

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Response to fever and utilization of standby emergency treatment (SBET) for malaria in travellers to Southeast Asia: a questionnaire-based cohort study

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Abstract

Background: Guidelines in several European countries recommend standby emergency treatment (SBET) for travellers to regions with low or medium malaria transmission instead of continuous chemoprophylaxis: travellers are advised to seek medical assistance within 24 h in case of fever and to self-administer SBET only if they are not able to consult a doctor within the time period specified. Data on healthcare-seeking behaviour of febrile travellers and utilization of SBET is however scarce as only two studies were performed in the mid-1990s. Since tourism is constantly increasing and malaria epidemiology has dramatically changed in the meantime more knowledge is urgently needed.

Methods: Some 876 travellers to destinations in South and Southeast Asia with low or medium malaria transmission were recruited in the travel clinic of the University Medical Center Hamburg-Eppendorf. Demographic and travel-related data were collected by using questionnaires. Pre-travel advice was carried out and SBET was prescribed in accordance to national guidelines. Post-travel phone interviews were performed to assess health incidents during travel and individual responses of travellers to febrile illness.

Results: Out of 714 patients who were monitored, 130 (18%) reported onset of fever during travel or 14 days after return. Of those travellers who reported fever, 100 (80%) carried SBET during travel. The vast majority of 79 (79%) febrile travellers who carried SBET did not seek medical assistance. Overall, 14 (14%) febrile patients who carried SBET and six (20%) patients who did not carry SBET took the correct measure (doctor visit or timely SBET administration) as a response to febrile illness, respectively. Only two travellers self-administered SBET, but both of them applied the wrong regimen.

Conclusions: In view of declining malaria transmission and improving medical infrastructure in most countries of Southeast Asia and obvious obstacles concerning SBET as shown in this study the current strategy should be re-evaluated. Pre-travel advice concerning malaria in SEA should focus on appropriate mosquito bite protection and clearly emphasize the need to see a doctor within 24 h after onset of fever.

Keywords: Malaria, Standby emergency treatment, SBET, Southeast Asia, Travellers, Healthcare-seeking behaviour, Malaria guidelines, Pre-travel advice

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Background

National recommendations for malaria prophylaxis for travellers to low- or intermediate-risk areas vary. Depending on seasonality, mode of travel, length of stay, travellers are advised to perform mosquito protection and chemoprophylaxis or carry standby emergency treatment (SBET). SBET is a concept according to which travellers carry anti-malarials (e.g., atovaquone/proguanil or artemether/lumefantrine), which have to be self-administered in case of fever and the inability to rule out malaria as the cause of fever within 24 h. The rationale behind the strategy of SBET is that the potential occurrence of side effects during continuous chemoprophylaxis might outweigh the relatively small risk of developing malaria in areas of low malaria transmission. The continuous decrease in malaria incidence in many low-transmission areas, e.g., in South- and Southeast Asia (SEA), as well as increasing drug resistance have also served as arguments in favour of SBET. The Societies for Tropical Medicine of Germany and Switzerland, for example, promote SBET for all areas of low and medium malaria transmission along with protection against mosquito bites instead of chemoprophylaxis [1]. The World Health Organization (WHO) recommends this approach to travellers "in some occupational groups who make frequent short stops in countries or areas with malaria risk over a prolonged period of time" and short-term travellers to "remote rural areas where there is very low risk of infection" [2]. The Centers for Disease Control and Prevention (CDC) in the USA, in contrast, recommend to carry SBET to ensure high-quality treatment in case of a diagnosis of malaria, accounting for the increasing numbers of fake anti-malarials in some countries of SEA [3].

While the concept of SBET appears straightforward in theory, it is unclear whether or not travellers are willing or able to apply SBET correctly while being abroad. In fact, there has been repeated criticism that travellers may be unable to make correct decisions in case of onset of fever during travel and tend to apply medication incorrectly [4, 5]. To date only two publications from 1995 are available concerning these issues. Both studies demonstrated that only a small fraction of travellers had to self-administer SBET. Additionally, when *Plasmodium falciparum* antibody levels were assessed in all travellers after return it turned out that even this low number of self-administrations proved to be a massive overuse of SBET, since most SBET users had no antibodies [4, 6].

Moreover, the SBET concept is based on the assumption that reliable medical care is generally not available at malaria-endemic tourist destinations. Yet, infrastructure for tourism, as well as medical emergency care has improved significantly in the past decades in many countries of SEA. At the same time, numbers of travellers to those regions have increased substantially due to a major surge in the availability of flight connections as well as a dramatic decrease in airline fares [7]. Concurrently to increased travel, malaria transmission in most parts of SEA has decreased, resulting in smaller numbers of imported malaria from the region [8, 9]. India also recorded a decline in malaria cases, but predominantly through control of falciparum malaria. In contrast, the relative proportion of *Plasmodium vivax* cases was increasing on the Indian sub-continent [10].

The aim of the present study was to assess utilization of SBET in the face of changing background conditions. Therefore, a cohort study in a population travelling to areas of low and medium malaria transmission in South Asia and SEA was performed.

Methods

Study design and recruitment

Between October 2013 and November 2014, a prospective, questionnaire-based cohort study was conducted at the travel clinic of the University Medical Center Hamburg-Eppendorf. Travellers aged ≥18 years with intention to travel to South Asia and SEA were screened for eligibility in the waiting area prior to seeing a trained physician for travel medicine advice. The following destination countries were defined for participation: India, Thailand, Laos, Cambodia, Vietnam, Sri Lanka, Myanmar, Indonesia, Bangladesh, the Philippines, or Malaysia. Additional inclusion criteria were travel duration between 7 days and 12 weeks and willingness to provide contact data for a post-travel interview as well as signed informed consent to participate. Study procedures comprised completion of a pre- and post-travel questionnaire.

Pre-travel interview

The pre-travel questionnaire had to be completed before travel medicine consultation with a physician. The pre-travel questionnaire comprised questions concerning the travel destination, mode of travel and basic health data of the participant, such as pre-existing illness and regular medication. To avoid a possible behavioural bias of study participants regarding the awareness of fever or application of SBET, participants were informed that a post-travel telephone interview would be conducted concerning health incidents during and after travel without specifying precise question items or topics.

Standby emergency treatment was prescribed according to the current recommendations of the German Society of Tropical Medicine (DTG) for the years 2013/2014 [1]. In brief, the DTG advised travellers to all countries, except certain regions in Indonesia, to carry out mosquito bite prevention at all times and to carry SBET, such as atovaquone/proguanil or artemether/lumefantrine.

For regions in Indonesia located east of Bali, travellers were advised to take continuous chemoprophylaxis either with atovaquone/proguanil or mefloquine.

Use of SBET was thoroughly explained, travellers were advised to take a thermometer with them and administer SBET only in case of fever with temperatures of >38 °C and if they were unable to seek medical advice within 24 h. Fever, chills, myalgia, and symptoms suggestive of a common cold were mentioned as key symptoms to prompt travellers to seek medical advice. Additionally, a leaflet with all instructions was handed out to all participants. Participation in the study did not influence recommendations of preventive measures against mosquito bites, vaccinations or prescription of SBET.

Post-travel interview

Between 4 and 6 weeks after travellers had returned home, all participants were contacted by a trained member of the study team to be screened for participation in the post-travel interview by asking the following four questions:

1. Did you experience fever, chills or flu-like symptoms during your travelling abroad or within 14 days after returning home?
2. Did you consult a doctor during your travelling abroad or within 14 days after returning home?
3. Did you or somebody from your travel group carry any anti-malarial medication (e.g., mefloquine, atovaquone/proguanil, doxycycline, artemether/lumefantrine) or buy one in the destination country?
4. Did you self-administer any anti-malarial medication?

If one of these questions was answered with "Yes", travellers qualified for participation in the detailed post-travel questionnaire concerning symptoms, date and place of onset of symptoms, doctor visit, diagnosis and medication.

Data analysis

Data analysis was carried out by using Stata v11.1 (StataCorp, College Station, TX, USA). Data analysis was descriptive and no statistical hypothesis was tested.

Notification data on malaria

To define the context in which standby emergency treatment is currently used in Germany, notification data of malaria cases from the destination countries covered by the study were analysed. In Germany, notification of malaria cases by laboratories is mandatory and based on the direct detection of the malaria parasite in the human blood. The diagnosing laboratory reports directly to the Robert Koch Institute (RKI). A second data form with information on travel destinations and purposes, clinical findings, prophylaxis, and treatment is completed by the attending physician. The department of infectious disease epidemiology of the RKI joins the information of both data forms in a unique database and analyse the data on an annual basis. Despite the mandatory nature, notification data are incomplete: not all cases are reported; for some cases the RKI receives only one of the two data forms; some of the data forms are only partially filled out.

Ethical considerations

The study protocol was approved by the ethics committee of the Medical Council in Hamburg, Germany. Prior to recruitment and pre-travel advice, eligible travellers were informed about the study by a member of the study team. They were included only after providing written informed consent.

Results

Characteristics of travellers and itineraries

Out of 1671 travellers screened for eligibility, pre-travel questionnaires were completed by 876 travellers with a median age of 32 years [interquartile range (IQR) 17–45]. Gender distribution was about equal (52.7% women). The majority of participants were well educated, more than half (53.9%) of them held a university degree. Pre-existing illness was reported by only a minor proportion of 14.6% of study participants. Thyroid disease and chronic respiratory diseases accounted for the most frequent disorders with 2.5 and 2.1%, respectively. Cardiac disease and diabetes were stated by eight (0.9%) and five (0.6%) of travellers. More than a quarter of travellers reported carriage of regular medication such as contraceptives (8.6%), drugs related to thyroid disease (4.9%) or hypertension (2.8%) (Table 1).

Thailand (35.6%), Vietnam (25.5%) and Cambodia (20.8%) were the most popular destinations. The median duration of travel was 21 days (IQR: 21–28). Top reason for travel was tourism, almost three-quarters of travellers intended to travel independently, i.e., without any kind of guidance. Only 32.4% of study participants had never travelled to (sub-) tropical regions before (Table 2).

Post-travel

Of the 876 travellers recruited before travel, 714 could be contacted via telephone for a post-travel interview. Amongst these, 130 (18.2%) reported onset of fever during travel or within 14 days after return. Of these, 31.5% reported concomitant diarrhoea, 14.6% reported vomiting. Myalgia, chills and other flu-like symptoms were reported by 47.7% (Table 3).

Table 1 Characteristics of travellers to Southeast Asia (n = 876)

Travellers' characteristics		IQR
Age (median)	32	18 (27–45)
Travellers' characteristics	**n**	**%**
Sex		
Male	414	47.3
Female	462	52.7
Education		
Tertiary degree	515	58.8
Upper secondary degree	212	24.2
Lower secondary degree	133	15.2
Primary	16	1.8
Pre-existing illness		
Any	128	14.6
Thyroid disease	22	2.5
Chronic respiratory diseases	18	2.1
Allergies	13	1.5
Hypertension	11	1.3
Neurologic disorders	11	1.3
Cardiac diseases	8	0.9
Diabetes mellitus	5	0.6
Other	61	6.9
Medication		
Any	260	26.3
Other	95	10.8
Contraceptive	75	8.6
Thyroid medication	43	4.9
Antihypertensives	25	2.8
Antiplatelet drugs	12	1.4
Drugs for respiratory diseases	10	1.1

IQR interquartile range

SBET utilization

Overall, 511 (71.6%) travellers carried SBET during travel. Out of 130 febrile travellers, 100 (76.9%) carried SBET during travel. Amongst those febrile travellers who carried SBET, 21 (21%) attended a local medical care facility because of the fever, only 14 (14%) of those within 24 h after onset of fever. Only 2 out of 714 travellers self-administered SBET in terms of emergency medication, but did not apply the correct scheme (Fig. 1). A 25-year old male traveller to Malaysia and Indonesia stated to have mistakenly applied one tablet of atovaquone/proguanil during his stay in Malaysia due to "lack of knowledge". After his return to Germany he experienced fever, chills and diarrhoea and consulted a doctor who diagnosed an infection with *Salmonella* spp.

Another 45-year old traveller to India experienced fever, diarrhoea and flu-like symptoms on his 21st day of travel. He did not attend a medical facility, but self-administered one tablet of atovaquone/proguanil seven days after onset of the symptoms, while he was staying in Kolkata/India (Table 4).

Out of 30 febrile travellers not carrying SBET during travel, nine (30%) visited a healthcare facility after the onset of fever, six of those within 24 h. Overall, 14 travellers (14%) experiencing fever during travel followed instructions as recommended by pre-travel advice by carrying SBET and visiting a health care facility within 24 h. None of the 14 travellers visiting a clinic for fever, took SBET, since malaria was ruled out at the health facility. Among travellers who did not experience fever during travel, no one self-administered SBET as emergency medication.

Recorded cases of imported malaria from South Asia and SEA

Table 5 shows the distribution of notified imported malaria cases from South Asia and SEA to Germany between 2011 and 2015. Throughout this period, most travellers with malaria returned from India and Indonesia, while no malaria was imported from Myanmar and Laos. The majority of notified cases was attributable to *P. vivax* infections (46.6%), while only 13 (17%) cases of *P. falciparum* were notified.

Discussion

A substantial proportion of 18% of travellers to regions of South Asia and SEA with medium and low risk for malaria transmission reported febrile illness during travel. Only very few travellers adhered to the pre-travel advice to seek medical support within 24 h in case of fever. Only 2 out of 714 travellers self-administered SBET during travel, but both of them applied an incorrect regimen and took a single tablet only, which would not have any therapeutic effect in case of true malaria. The study team is not aware of any case of malaria in the study population.

In assessing the concept of SBET, three main problems were identified in travellers with fever during travel: (i) a major proportion of travellers did not carry SBET although it was prescribed; (ii) non-adherence to pre-travel advice while being abroad; and, (iii) incorrect self-administration of SBET.

Travellers recruited for this study were young with a median age of 32 years, healthy, disproportionally well educated and the majority was proficient with travelling to (sub-) tropical countries. In this respect characteristics of this population are comparable to those from other studies and it seems justifiable to assume, that the current results can be generalized to other traveller populations attending pre-travel clinics [11, 12].

Table 2 Itinerary characteristics (n = 876)

Itinerary characteristics		IQR
Duration of travel (days)	21	7 (21–28)
Itinerary characteristics	**n**	**%**
Destination		
Thailand	312	35.6
Vietnam	223	25.5
Cambodia	182	20.8
India	166	18.9
Indonesia	150	17.1
Malaysia	93	10.6
Laos	87	9.9
Sri Lanka	74	8.4
Myanmar	53	6.1
Philippines	48	5.5
Reason for travel		
Tourism	796	91.5
Business	29	3.3
Volunteering/education	30	3.4
Visiting friends and relatives (VFR)	15	1.7
Other	6	0.7
Type of travel		
Backpacking	340	38.8
Individual travelling	306	34.9
Organized round trip	120	13.7
Package holiday	26	3.0
Ship cruise	14	1.6
No answer	70	8.0
Previous travel experience		
1–2 times	285	32.5
3 to −5 times	169	19.3
>5 times	134	15.3
None	284	32.4
No answer	4	0.5

IQR interquartile range

Despite the changed landscape of malaria since 1995 when the last data on SBET utilization was published, the findings are in line with the prior studies. In a Swiss study from 1995, only six (0.5%) out of 123 febrile travellers applied SBET and only four of them applied the correct regimen [4]. In a German multi-centre study, 1.4% (40/2867) of travellers reported SBET use. Malaria antibody levels were later demonstrated in four participants who applied SBET [6]. Increasing evidence therefore indicates that only a small proportion of travellers to low risk malaria areas adheres to pre-travel advice based on the SBET concept.

The low acceptance and carriage rate of SBET in this study population is however surprising. Only 16% of travellers carrying SBET took any correct measure and 20% of travellers not carrying SBET sought medical assistance after the onset of fever which is a strikingly low figure regarding the standardized pre-travel advice and the educational level of the study population. Explanations for this result may be various: available data suggest that the recall rate of information after medical consultations showed overall good results suggesting that key messages seem to be well captured [13–15]. Since most consultations incorporate one or more vaccinations, injection anxiety, which has been shown quite common in some populations could be a potential distractor [16, 17]. However, the only available study in this context showed no association between recall of information and injection anxiety [18]. In any case, provision of simplified key messages after vaccination may facilitate better recognition of information. In general, studies assessing a traveller's knowledge about travel-related health issues underlined a general increase of knowledge after pre-travel advice, so that other factors are likely to have contributed to non-adherence to emergency measures in case of fever in the current study [19–21]. In particular, in areas where population density and accessibility to medical facilities is good, tourists might tend to delay the decision to consult a doctor in favour of waiting for spontaneous recovery. Carriage of SBET could further encourage travellers to defer a medical consultation thereby waiting for the fever to drop. Challenges in adherence are not limited

Table 3 Symptoms of travellers reporting fever during travel or 14 days after return (n = 130)

Fever	n	%
Yes	130	18.2
Uring their travel	89	68.5
Ithin 14 days after return	41	31.5
No	559	78.3
No answer	25	3.5
Additional symptoms in febrile patients (n = 130)	**n**	**%[a]**
Diarrhoea	41	31.5
Myalgia	31	23.8
Chills	27	20.8
Headache	21	16.1
Vomiting	19	14.6
Sore throat	17	13.1
Abdominal pain	15	11.5
Tiredness	12	9.2
Stomache ache	8	6.2
Flu-like symptoms	4	3.1
Vertigo	3	2.3
Symptoms of sinusitis	3	2.3

[a] May not sum up to 100% since some patients recalled multiple symptoms

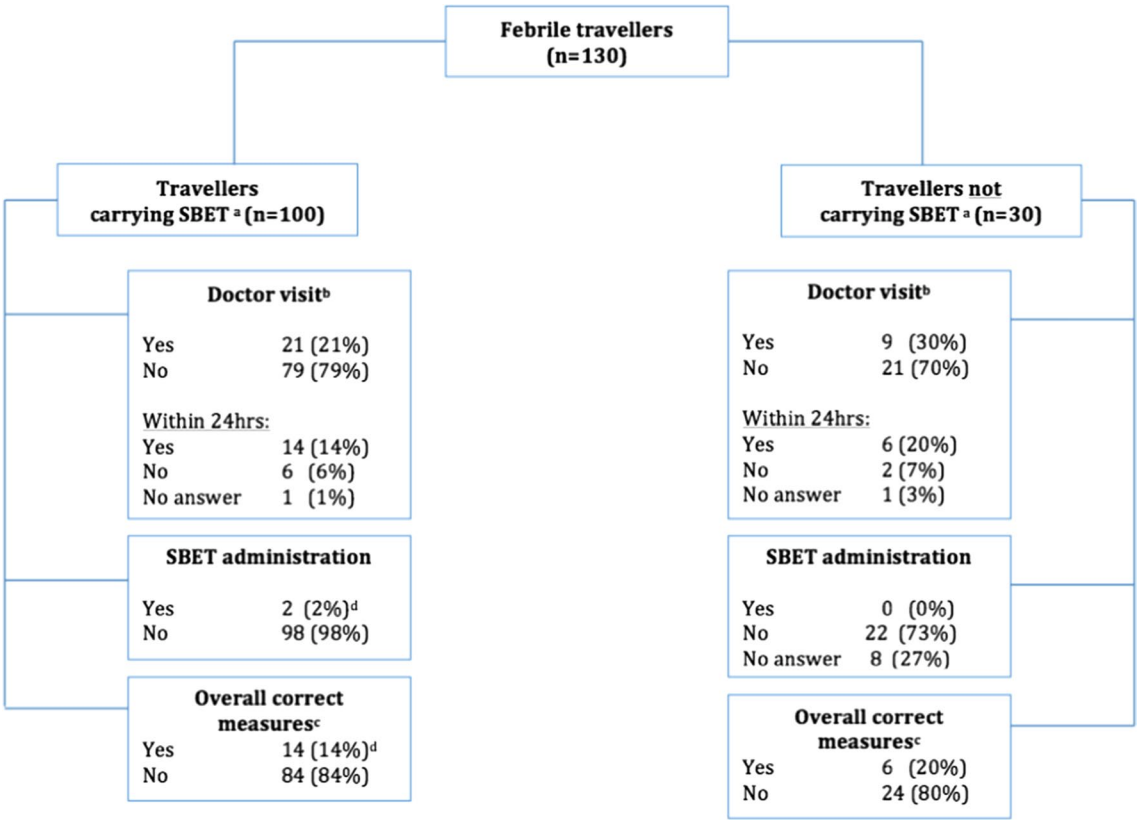

Fig. 1 Responses of travellers to febrile illness. [a]Stand-by emergency treatment. [b]Doctor visit in country of travel. [c]Correct measures: febrile travellers took correct measures if they sought for medical assistance within 24 h or self-administered SBET. [d]Since both travellers applied an incorrect scheme of SBET, administration of the medication was not counted as "correct measure"

Table 4 Febrile travellers self-administering SBET (n = 2)

Sex, age (years)	Destination	Complaints	Consultation of doctor	Place of SBET administration	Correct regimen
Male, 25	Malaysia, Indonesia	Fever, chills, diarrhoea; onset after return to Germany	In Germany	Malaysia	No
Male, 45	India	Fever, diarrhoea, flu-like symptoms; onset travel day 21	No	Kolkata, India	No

to the concept of SBET but are also seen for continuous malaria chemoprophylaxis for travellers to high-risk countries [22–26]. Several studies prove that the majority of returning travellers with malaria did not take malaria chemoprophylaxis or applied an incorrect regimen, the majority being travellers visiting friends or relatives (VFR) abroad [22, 27–31].

In consequence of an expanding tourism industry, travel to SEA is steadily increasing. For 2014, international tourist arrivals in the region rose to 96.7 million, which is a nearly fivefold increase since 1990 [7]. Concurrently to increasing travel, malaria transmission in South Asia and SEA has decreased over the past years. Between 2000 and 2015 the estimated number of malaria cases and deaths for the whole WHO region Southeast Asia declined by 39 and 37%, respectively [32]. It has been argued before that the malaria risk for travellers is not correlated with infection rates in the local population and attack rates in visitors may likely be higher because of the absence of partial immunity in contrast to the local populations [33]. Recent studies confirmed the trend of decreasing cases of imported malaria cases from SEA [9, 34]. According to data from malaria surveillance reports from the USA and 12 European countries, malaria cases imported from countries of SEA declined by 47% between 2003 and 2008 [8]. Another assessment of 320 imported cases of malaria between 1994 and 2012 from Denmark showed an annual decline of 6.5% [35].

Table 5 Reported cases of malaria imported into Germany from SEA between 2011 and 2014

	2011	2012	2013	2014	2015	Total
Country of travel						
Myanmar	0	0	0	0	0	0
India	17	17	1	5	5	45
Indonesia	4	1	1	1	2	9
Cambodia	1	0	0	1	2	4
Laos	0	0	0	0	0	0
Malaysia	0	0	0	0	1	1
Philippines	0	0	0	0	1	1
Sri Lanka	0	0	1	0	0	1
Thailand	0	1	3	1	2	7
Vietnam	0	0	0	1	0	1
Not specified	2	5	1	0	0	8
Plasmodium species						
Total (all species)	24	24	7	9	13	77
Plasmodium falciparum	0	5	1	3	4	13
Plasmodium vivax	18	15	4	4	5	46
Other and non-specified	1	1	2	1	1	6

Cases reported to the Robert Koch Institute Berlin, Germany

However, India and Indonesia, as it is the case with notification data in Table 5, constitute two of the main source countries for imported malaria. In a retrospective analysis of national notification data from Canada, 9.2% of imported malaria cases originated from India. India displayed the most common source country for vivax malaria cases [36]. Whilst this should be taken into account during pre-travel advice, the notification data to the RKI presented in this study show low and declining numbers of malaria cases imported from South and SEA to Germany. Indeed, these data underline the economical impact of over-prescription of SBET, if compared to the annual numbers of travellers to SEA. Using figures provided by the German Travel Association (DRV), 1,769,825 individuals travelled to countries considered in this study in 2015 [37]. Considering a price of 42 Euro per unit, this would translate to 71.4 million Euros spent for SBET in 2015 in Germany alone. Facing the low number of imported falciparum malaria (13 cases during the past 5 years), these expenses hardly pass any cost-benefit analysis. In the light of the decreased numbers of imported malaria and the fact that the majority of reported cases are *P. vivax* cases, strategies for travellers to these regions have to be reassessed, in particular, because relapse of *P. vivax* is not adequately prevented by regular chemoprophylaxis or treated by a standard regimen of standby emergency treatment.

Even though atovaquone/proguanil is generally considered safe, up to 82% of patients reported an adverse event [38, 39]. The benefit-risk ratio for continuous malaria chemoprophylaxis is consecutively very low for most regions in SEA, and does not pose a viable alternative [40]. However, data from the current study reinforce the assumption that SBET has to be critically assessed. Equipping travellers with an anti-malarial has the potential to lead to a false sense of security and an uncritical perception of the risk of malaria during travel, favouring short-sleeved clothes and avoidance of repellents. Unattended administration of SBET harbours the risk of missing other medical conditions. The vast majority of travellers sojourn on popular tourist routes and visit similar places at their destinations. During the past two decades healthcare systems in most countries of South Asia and SEA have improved, although they remain at a low standard. However, from the main tourist tracks medical facilities can usually be reached within 24 h, particularly in metropolitan areas. One of the male travellers in this study who used SBET started intake of medication in Kolkata, where it would easily have been possible to find a hospital to rule out malaria [21]. There are however arguments in favour of SBET. First, travellers have advanced to more remote areas worldwide and carriage of SBET can be life-saving in cases when travellers are unable to reach a health facility due to missing infrastructure or external factors, e.g., severe weather conditions. Secondly, carriage of high-quality medication ensures safe treatment in view of increasing numbers of fake anti-malarials, especially in Asia [41–43]. Third, studies have shown that long-term travel can result in a higher cumulative risk for malaria [44, 45]. Those travellers may benefit from provision of SBET

given the assumption that they tend to visit more rural regions. However, it is not possible to support this recommendation with the current study data, since only individuals travelling for no longer than 12 weeks were recruited.

This study has some limitations to be taken into account. As described above, a selective population of travellers visiting a health facility for pre-travel advice was studied. This population may not be comparable to the overall population travelling to South Asia and SEA. Earlier data demonstrated that travellers who attended a travel clinic have better knowledge about potential risks and protective measures [46]. Yet unpublished data from the Hamburg Airport Survey also suggest that travellers in the overall population have less frequently attended a travel clinic and are less frequently carrying SBET. Finally, it was not possible to completely rule out malaria in returned travellers, since no serology for *Plasmodium* spp. was performed. However, conduct of phone interviews 4–6 weeks after return makes unrecognized episodes of clinically relevant malaria unlikely.

Conclusions

Only a very small proportion of travellers to low-risk malaria areas experiencing fever while abroad adhered to pre-travel advice related to the concept of SBET. Travel advice concerning malaria in South Asia and SEA should focus on appropriate mosquito bite protection and clearly emphasize the need to see a doctor within 24 h after onset of fever. Travellers with need of SBET should be carefully selected. Recommendations related to SBET should be revisited and limited to selected situations only, e.g., long-term travel or travel to remote rural areas.

Authors' contributions

CV, TR and JPC designed the study. CV and TR carried out statistical analyses and wrote the first draft of the manuscript. SVB contributed notification data from the Robert-Koch-Institute. CV, MMA, CR, JPC, TR, and BK recruited subjects to the study. All authors participated in writing the final draft. All authors read and approved the final manuscript.

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Competing interests

JPC is an employee of Takeda Pharmaceuticals. Other authors declare that they have no competing interests.

Availability of data and materials

The datasets analysed during the current study are not publicly available due to participants' privacy issues. The datasets are available from the corresponding author on reasonable request.

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