Etiology of Severe Non-malaria Febrile Illness in Northern Tanzania: A Prospective Cohort Study

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Abstract

Introduction: The syndrome of fever is a commonly presenting complaint among persons seeking healthcare in low-resource areas, yet the public health community has not approached fever in a comprehensive manner. In many areas, malaria is over-diagnosed, and patients without malaria have poor outcomes.

Methods and Findings: We prospectively studied a cohort of 870 pediatric and adult febrile admissions to two hospitals in northern Tanzania over the period of one year using conventional standard diagnostic tests to establish fever etiology. Malaria was the clinical diagnosis for 528 (60.7%), but was the actual cause of fever in only 14 (1.6%). By contrast, bacterial, mycobacterial, and fungal bloodstream infections accounted for 85 (9.8%), 14 (1.6%), and 25 (2.9%) febrile admissions, respectively. Acute bacterial zoonoses were identified among 118 (26.2%) of febrile admissions; 16 (13.6%) had brucellosis, 40 (33.9%) leptospirosis, 24 (20.3%) had Q fever, 36 (30.5%) had spotted fever group rickettsioses, and 2 (1.8%) had typhus group rickettsioses. In addition, 55 (7.9%) participants had a confirmed acute arbovirus infection, all due to chikungunya. No patient had a bacterial zoonosis or an arbovirus infection included in the admission differential diagnosis.

Conclusions: Malaria was uncommon and over-diagnosed, whereas invasive infections were underappreciated. Bacterial zoonoses and arbovirus infections were highly prevalent yet overlooked. An integrated approach to the syndrome of fever in resource-limited areas is needed to improve patient outcomes and to rationally target disease control efforts.

Introduction

Fever without a localized cause is one of the most common presenting complaints among persons seeking healthcare in many low- and middle-income countries [1,2]. However, unlike the syndromes of pneumonia and diarrhea that feature in global disease burden estimates and have well coordinated programs integrating efforts across the range of responsible pathogens to avert morbidity and mortality, there has been a lack of a coordinated approach for febrile illness. While illness and death due to some specific infections causing fever, such as malaria [3] and increasingly bacterial sepsis are well quantified [4–6], others such as a range of zoonoses and viral infections are uncounted and consequently may be underappreciated.

The various etiologies of febrile illnesses are difficult to distinguish from one another clinically [7,8]. As clinical laboratory services are often limited in areas where febrile conditions are particularly common [9,10], clinicians may have few diagnostic tools to establish an etiologic diagnosis. Therefore, clinical management is often driven by syndrome-based guidelines employing empiric treatment [11–13]. In the absence of systematically collected data on fever etiology, considerable mismatch between clinical diagnosis, clinical management, and actual etiology may occur resulting in poor patient outcomes [14]. It is


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increasingly recognized that malaria is over-diagnosed in many areas [14,15]. To address this problem, the World Health Organization (WHO) malaria treatment guidelines moved away from clinical diagnosis of malaria to treatment based on the results of a malaria diagnostic test such as a blood smear or a malaria rapid diagnostic test. With more widespread availability of diagnostic tests to exclude malaria and apparent declines in malaria worldwide [3], clinicians in resource-limited areas are faced with a growing proportion of febrile patients who do not have malaria and few tools to guide subsequent management.

We sought to describe comprehensively the causes of febrile illness in northern Tanzania among patients sufficiently ill to require hospitalization. Febrile patients admitted to two hospitals were evaluated for a wide range of infectious etiologies using conventional standard diagnostic techniques.

Methods

Ethics statement

This study was approved by the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and Institutional Review Boards of Duke University Medical Center and the CDC. All minors had written informed consent given from a parent or guardian and all adult participants provided their own written informed consent.

Setting

Moshi (population, >144,000) is the administrative center of the Kilimanjaro Region (population, >1.4 million) in northern Tanzania and is situated at an elevation of 890 m above mean sea level. The climate is characterized by a long rainy period (March–May) and a short rainy period (October–December) [16]. Malaria transmission intensity is low [17]. KCMC is a consultant referral hospital with 458 inpatient beds serving several regions in northern Tanzania, and Mawenzi Regional Hospital (MRH), with 300 beds, is the Kilimanjaro Regional hospital. Together KCMC and MRH serve as the main providers of hospital care in the Moshi area. In 2008, KCMC admitted 22,099 patients and MRH admitted 21,763 patients.

Laboratory evaluations

Laboratory evaluations were selected to reflect a range of infectious diseases that might occur in northern Tanzania. Priority was given to laboratory evaluations for infectious diseases that might require specific management.

Malaria. Thick and thin blood films stained with Giemsa were examined for blood parasites by oil immersion microscopy. Parasite density was determined by standard methods [18].
**Bacteria and fungal bloodstream infections.** Blood culture bottles were assessed for volume adequacy comparing the weight before and after inoculation with blood. Adequate volume was defined as the recommended volume ± 20%. BacT/ALERT standard aerobic and mycobacterial bottles were loaded into the BacT/ALERT 3D Microbial Detection system (BioMérieux), where they were incubated for 5 and 42 days, respectively. Standard methods were used for identifying bloodstream isolates [7,8].

**Serum antigen testing.** Cryptococcal antigen level was measured using the Latex Cryptococcal Antigen Detection System assay (Immu-No-Mycologics).

**Urine antigen testing.** Urine was tested for all participants for *Legionella pneumophila* serogroup 1 antigen using the Binax NOW Legionella urinary antigen test, and for adolescents and adults using the *S. pneumoniae* antigen using the Binax NOW S. pneumoniae antigen test (Binax). Urine was tested for *Histoplasma capsulatum* antigen using the MVista *H. capsulatum* quantitative antigen enzyme immunoassay (Miravista Diagnostics) [19,20].

**Leptospirosis.** Leptospirosis laboratory diagnosis was made using the standard microagglutination test (MAT) performed at the CDC. Live leptospiral cell suspensions representing 20 serovars and 17 serogroups described elsewhere [21] were incubated with serially diluted serum specimens. Resulting agglutination titers were read using darkfield microscopy. The reported titer was the highest dilution of serum that agglutinated at least 50% of the cells for each serovar tested [22]. Confirmed leptospirosis was defined as a ≥4-fold rise in the agglutination titer between acute and convalescent serum samples [23].

**Brucellosis.** Brucellosis serology was performed using the standard microagglutination test (MAT) performed at the CDC. Standardized *Brucella abortus* strain 1119-5 killed antigen (National Veterinary Services Laboratory, Ames, IA) was used for MAT at 1:25 working dilution described elsewhere [24]. Results were read on a Scienceware Plate Reader (Bel-Art Products, Wayne, NJ). Minor modifications were made to the CDC’s standard MAT, including the use of U-bottom plates, incubation at 26°C, and discontinued use of staining techniques [25]. Confirmed brucellosis was defined as a ≥4-fold rise in the agglutination titer between acute and convalescent serum samples.

**Q fever.** Convalescent-phase serum samples were screened using *C. burnetii* immunoglobulin (Ig) G enzyme-linked immunosorbent assay (ELISA) against Phase II antigen (Inverness Medical Innovations). For samples that were either positive or equivocal by ELISA, paired serum samples were tested by indirect immunofluorescence antibody (IFA) IgG assay to *C. burnetii* (Nine Mile strain) Phase I and Phase II antigens. A fourfold or greater increase in IFA reciprocal titer to Phase II antigen defined acute Q fever [26].

**Spotted fever group and typhus group rickettsioses.** Serum samples were tested for SFGR and TGR by IgG IFA to *R. conorii* (Moroccan strain) and to *R. typhi* (Wilmington strain), respectively. Among paired serum samples, a fourfold or greater increase in IFA titer to *R. conorii* and *R. typhi* defined acute SFGR and TGR infections, respectively [26].

**Arboviruses.** RNA was extracted from serum samples using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany). Reverse transcription was performed using Invitrogen Superscript III First Strand Synthesis System (Life Technologies, Carlsbad, CA). Real-time PCRs for flavivirus, DENV, and CHIKV were carried out with the LightCycler 480 SYBR Green I Master kit (Roche Diagnostics, Penzberg, Germany) in a total reaction volume of 20 μL containing 2 μL of cDNA using primers published elsewhere [27–29]. Confirmed acute CHIKV, DENV, and flavivirus infections were defined as a positive PCR result for CHIKV, DENV, and flavivirus viral RNA, respectively [30].

**HIV.** HIV-1 antibody testing was done on whole blood using both the Capillus HIV-1/HIV-2 (Trinity Biotech) and Determine HIV-1/HIV-2 (Abbott Laboratories) rapid HIV antibody tests. The Capillus test was replaced with the SD Bioline HIV-1/HIV-2 test (version 3.0; Standard Diagnostics) on 4 March 2008 after a change in Tanzania Ministry of Health HIV testing guidelines. If rapid tests were discordant, the sample was tested using enzyme-linked immunosorbent assay (ELISA; Vironostika Uni-Form II plus O Ab; bioMe’rieux). If the ELISA was negative, no further testing was done. If the ELISA was positive, a Western blot (Genetic Systems HIV-1 Western blot kit; Bio-Rad) was done to confirm the result [31]. HIV-1 RNA PCR was done using the Abbott m2000 system RealTime HIV-1 assay (Abbott Laboratories) [32,33].

**Statistical analysis**

Data were entered using the Cardiff Teleform system (Cardiff Inc., Vista, CA, USA) into an Access database (Microsoft Corp., Va., USA). When a diagnostic test was not applied to the whole cohort due lack of availability of an acute or convalescent sample, the proportion of confirmed cases in the tested group was extrapolated to the untested group by assuming that prevalence was the same in the tested group as in the untested group. Statistical analyses were performed with SAS version 9.1 software (SAS Inc, Cary, NC).

**Results**

**Participant characteristics**

Figure 1 summarizes participant screening, enrollment, and diagnostic testing. Of 870 febrile admissions to two hospitals in northern Tanzania enrolled in the study 484 (55.6%) were female. Of participants, 467 (53.7%) were infants and children with a median (range) age of 2 years (2 months - 13 years); the remainder adolescents and adults with a median (range) age of 38 (14–96) years. Fifty seven (12.2%) infants and children were HIV-infected compared with 157 (39.0%) adolescents and adults. Among infants and children 34 (7.3%) of 464 with hospital outcome data died; 2 (5.9%) of those who died had invasive infections. Among adolescents and adults, 41 (10.3%) of 398 with hospital outcome data died; 11 (26.8%) of those who died had invasive infections. In hospital deaths could not be attributed to etiologies requiring serologic diagnosis due to the requirement for testing a convalescent serum sample.

**Proportions of febrile admissions attributed to specific etiologies**

Table 1 shows the number of patients with acute and convalescent samples available for testing for each etiologic agent or group of etiologic agents. Not all tests could be applied to all participants because of limited volumes of sample for some participants, and by the lack of availability of convalescent serum for participants who died before the follow up visit or who did not return. The number of confirmed cases in each group is also shown. The proportion of febrile admissions attributed to each etiology is calculated. A complete sample set was available for 243–467 (52.0–100.0%) infants and children and for 207–403 (51.4–100.0%) adolescents and adults.

**Etiology of fever among infants and children**

Of 467 infants and children enrolled, malaria was the clinical diagnosis for 282 (60.4%), but was the actual cause of fever in
(1.3%). Bacterial and fungal bloodstream infections described in detail elsewhere [8] accounted for 16 (3.4%) and 4 (0.9%) febrile admissions, respectively, and were underrepresented on admission differential diagnoses. Bacterial zoonoses were identified among 49 (20.2%) of febrile admissions; 5 (2.0%) had brucellosis, 19 (7.7%) leptospirosis, 7 (2.6%) had Q fever, 18 (7.4%) had spotted fever group rickettsioses, and none had typhus group rickettsioses. In addition, 34 (10.2%) of participants had a confirmed acute arbovirus infection, all due to chikungunya (Table 1). No patient had a bacterial zoonosis or an arbovirus infection included in the admission differential diagnosis.

Etiology of fever among adolescents and adults

Of 403 adolescents and adults enrolled, malaria was the clinical diagnosis for 254 (63.0%), but was the actual cause of fever in 8 (2.0%). Bacterial, mycobacterial, and fungal bloodstream infections described in detail elsewhere [7] accounted for 69 (17.1%), 14 (3.5%), and 21 (5.2%) febrile admissions, respectively, and were underrepresented on admission differential diagnoses. Bacterial zoonoses were identified among 69 (33.3%) of febrile admissions; 11 (5.3%) had brucellosis, 21 (10.1%) leptospirosis, 17 (7.9%) had Q fever, 18 (8.7%) had spotted fever group rickettsioses, and 2 (1.0%) had typhus group rickettsioses. In addition, 21 (5.7%) of participants had a confirmed acute arbovirus infection, all due to chikungunya (Table 1). No patient had a bacterial zoonosis or an arbovirus infection included in the admission differential diagnosis.

Etiology of fever overall

Among all 870 participants, malaria was the clinical diagnosis for 528 (60.7%), but was the actual cause of fever in 14 (1.6%). By contrast, bacterial, mycobacterial, and fungal bloodstream infections accounted for 85 (9.8%), 14 (1.6%), and 25 (2.9%) febrile admissions, respectively, and were underrepresented on admission differential diagnoses. Bacterial zoonoses were identified among 118 (26.2%) of febrile admissions; 16 (13.6%) had brucellosis, 40 (33.9%) leptospirosis, 24 (20.3%) had Q fever, 36 (30.5%) had spotted fever group rickettsioses, and 2 (1.8%) had typhus group rickettsioses. In addition, 55 (7.9%) of participants had a confirmed acute arbovirus infection, all due to chikungunya (Table 1). No patient had a bacterial zoonosis or an arbovirus infection included in the admission differential diagnosis. The proportional etiology of febrile illness among study participants after extrapolating to the untested group is summarized in Figure 2.

Discussion

We demonstrate among hospitalized febrile patients in northern Tanzania that malaria is uncommon and over-diagnosed, while invasive bacterial, mycobacterial, and fungal infections are underappreciated. At the same time, the bacterial zoonoses leptospirosis, Q fever, and spotted fever rickettsioses, and to a lesser extent brucellosis, and the arbovirus infection chikungunya are common yet unrecognized causes of fever. Our findings point to important mismatches between clinical diagnosis and management with actual diagnoses that have major implications for patient care, disease control and prevention, and for judicious use of antimalarial medications. While the problem of malaria over-diagnosis has been appreciated for some time [14,15], studies that comprehensively describe the causes of severe non-malaria fever requiring hospital admission beyond bloodstream infections have been lacking. The
over-diagnosis of malaria results in inappropriate use of antimalarial medications and may be associated with higher case fatality rates among patients treated for malaria who do not have the infection [14,15,34]. While the underlying causes of the over-diagnosis of malaria are complex [35], the lack of epidemiologic information about the importance of alternative infections and guidance on their management is likely to play a role. Our findings confirm the potential benefits of making reliable malaria diagnostic tests available at healthcare facilities and using the results as the basis for prescription of antimalarial medications and antibacterials, patients with brucellosis, Q fever, and rickettsioses were diagnosed predominantly by serology, based on a 4-fold or greater rise in antibody titer between an acute and convalescent sample.

Due to changing denominators for individual diagnostic tests, the proportion with no diagnosis is calculated as the proportion without a positive result from any test. Bloodstream infections are those diagnosed predominantly by blood culture, including organisms such as *Salmonella enterica*, *Streptococcus pneumoniae*, *Cryptococcus neoformans*, and *Mycobacterium tuberculosis*. Bacterial zoonoses, including brucellosis, leptospirosis, Q fever, and rickettsioses were diagnosed predominantly by blood culture and, with subsequent collection of acute serum along with the blood culture to inform vaccine policy could collect acute serum for targeted studies that could provide much more comprehensive diagnostic information to clinicians [37]. Unfortunately, many rapid diagnostic tests for infections related to fever management for febrile patients are likely to require the development and incorporation of reliable diagnostic tests that provide timely diagnostic information to clinicians [37]. Unfortunately, many rapid diagnostic tests for infections related to fever management other than malaria and HIV suffer from poor performance characteristics [38,39].

Lack of coordination among groups working on the various etiologies of febrile illness in low-resource areas has meant that sentinel studies that could provide much more comprehensive information on a wide range of responsible organisms instead have focused on only one or a small handful of etiologies. For example, a clinical trial evaluating the impact of pneumococcal conjugate vaccine on rates of *Streptococcus pneumoniae* bacteremia in a community has the potential to identify and report all bloodstream infections. Similarly, a study designed to estimate the incidence of typhoid fever to inform vaccine policy could collect acute serum along with the blood culture and, with subsequent collection of convalescent serum, would have the ability to estimate the

### Table 1. Calculation of the proportion of hospitalized infants and children, and adolescents and adults, with specific etiologies of febrile illness, northern Tanzania, 2007–8.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Infants and children</th>
<th>Adults and adolescents</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n confirmed cases</td>
<td>n tested (%)</td>
<td>n confirmed cases</td>
</tr>
<tr>
<td><strong>Bloodstream infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial</td>
<td>16 467 (3.4)</td>
<td>69 403 (17.1)</td>
<td>85 870 (9.8)</td>
</tr>
<tr>
<td>Mycobacterial</td>
<td>0 467 (0.0)</td>
<td>14 403 (3.5)</td>
<td>14 870 (1.6)</td>
</tr>
<tr>
<td>Fungal</td>
<td>4 467 (0.9)</td>
<td>21 403 (5.2)</td>
<td>25 870 (2.9)</td>
</tr>
<tr>
<td>Malaria</td>
<td>6 467 (1.3)</td>
<td>8 403 (2.0)</td>
<td>14 870 (1.6)</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>26 467 (5.6)</td>
<td>112 403 (27.8)</td>
<td>138 870 (15.9)</td>
</tr>
<tr>
<td><strong>Bacterial zoonoses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucellosis</td>
<td>5 246 (2.0)</td>
<td>11 207 (5.3)</td>
<td>16 453 (3.5)</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>19 246 (7.7)</td>
<td>21 207 (10.1)</td>
<td>40 453 (8.8)</td>
</tr>
<tr>
<td>Q fever</td>
<td>7 268 (2.6)</td>
<td>17 215 (7.9)</td>
<td>24 482 (5.0)</td>
</tr>
<tr>
<td>Spotted fever group rickettsioses</td>
<td>18 243 (7.4)</td>
<td>18 207 (8.7)</td>
<td>36 450 (8.0)</td>
</tr>
<tr>
<td>Typhus group rickettsioses</td>
<td>0 243 (0.0)</td>
<td>2 207 (1.0)</td>
<td>2 450 (0.4)</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>49 243 (20.2)</td>
<td>69 207 (33.3)</td>
<td>118 450 (26.2)</td>
</tr>
<tr>
<td><strong>Arboviruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chikungunya</td>
<td>34 332 (10.2)</td>
<td>21 368 (5.7)</td>
<td>55 700 (7.9)</td>
</tr>
<tr>
<td>Flaviviruses</td>
<td>0 332 (0.0)</td>
<td>0 368 (0.0)</td>
<td>0 700 (0.0)</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>34 332 (10.2)</td>
<td>21 368 (5.7)</td>
<td>55 700 (7.9)</td>
</tr>
<tr>
<td><strong>No diagnosis</strong></td>
<td>(64.0)</td>
<td>(33.2)</td>
<td>(50.1)</td>
</tr>
</tbody>
</table>

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incidence of leptospirosis and a range of other etiologic agents using conventional serologic methods [40]. However, resources for research have tended to be targeted to specific pathogens and researchers have struggled to leverage additional resources to address a broader range of organisms. Sentinel site studies seeking to understand the infectious causes of febrile illness in low-resource settings have utilized blood culture to highlight the importance of invasive bacterial and fungal infections [4,41]. Expanding laboratory evaluations to include serologic and molecular approaches to diagnosing infections requiring specific antimicrobial management such as the bacterial zoonoses brucellosis, leptospirosis, Q fever, and the rickettsioses adds considerable value [40]. Detection of infections of public health importance such as those caused by the arboviruses dengue, Rift Valley fever, and yellow fever can inform national control programs. Since considerable etiologic overlap exists between the syndromes of fever, acute respiratory tract infection, and diarrhea [12,43], addressing these simultaneously in integrated sentinel studies would inform enhancements in empiric treatment guidelines and improvements in the accuracy of syndrome-based disease burden estimates.

Our study had a number of limitations. While we examined a wide range of etiologies of fever, a large proportion of patients were undiagnosed suggesting that we failed to identify potentially important infections. The undiagnosed group is being investigated further using pathogen discovery approaches. Some of the diagnostic tests used in our study are less than 100% sensitive and specific and we did not test for every known pathogen. As a consequence, we probably underestimated the prevalence of some infections while misclassifying others that were falsely positive. Because a number of our diagnostic tests relied on the demonstration of a four-fold rise in antibody titer between the acute and convalescent serum sample, not all enrolled patients returned for collection of convalescent serum to have diagnoses confirmed. It follows that calculation and comparison of case fatality rate was not possible since those who died before the convalescent visit could not be confirmed cases. Incomplete diagnostic information meant that we had to extrapolate prevalence from the tested population to the untested population, potentially introducing bias. Similarly, instances of apparent infection with multiple agents were not accounted for in presentation of pie graphs. Inclusion of a well control group would have allowed the calculation of attributable fractions for individual pathogens, something that should be considered for future febrile illness research, especially in areas where malaria is endemic. Since considerable geographic variation in fever etiology is known to occur, the generalizability of our findings is uncertain.

What is needed to support an integrated approach to the syndrome of fever in resource-limited areas? First, fever should be recognized alongside pneumonia and diarrhea as a major clinical syndrome of public health importance. Achieving this is likely to require leadership from international institutions of public health. Second, efforts are needed to bring together the diverse groups and disciplines currently working on the febrile illnesses to quantify the morbidity and mortality attributable to each major etiologic agent. Such integration could be facilitated by support for research efforts that study the syndrome of fever comprehensively as well as its etiologies individually, an approach that has been modeled by studies addressing the syndromes of pediatric pneumonia and diarrhea in developing countries [46,47].


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Supporting Information

Text S1 STROBE checklist. (DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: JAC JAB. Performed the experiments: JAC ABM WLN RFM RLG LEO VPM WS GDK JAB. Analyzed the data: JAC. Contributed reagents/materials/analysis tools: WLN RFM RLG EEO. Wrote the paper: JAC ABM WLN RFM RLG EEO VPM WS GDK CM JAB. Managed the research program: CM. Sought and obtained funding: JAB.

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