Accuracy of verbal autopsy, clinical data and minimally invasive autopsy in the evaluation of malaria-specific mortality: an observational study

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ABSTRACT

Background Global malaria mortality estimates are hindered by the low reliability of the verbal autopsy (VA) and the clinical records, the most common sources of information used to estimate malaria-specific mortality. We aimed to determine the accuracy of these tools, as well as of the minimally invasive autopsy (MIA), a needle-based postmortem sampling method, to identify malaria-specific mortality in a large series of deceased patients from Mozambique, using complete autopsy as the gold standard.

Methods Observational study that included 264 deaths, occurring at a tertiary level hospital in Mozambique, from 1 November 2013 to 31 March 2015 (17 months-long period). Clinical data were abstracted, a computer coded VA was completed using the clinical data as source of information, and an MIA followed by a complete autopsy were performed. Screening for malaria infection was conducted postmortem to all participants using molecular and histological techniques (PCR and immunohistochemistry).

Findings Malaria infection was considered the cause of death in 6/264 (2.3%) cases: 2/54 children (3.7%, both less than 5 years old) and 4/57 (7.0%) maternal deaths. The sensitivity and specificity of the VA, the clinical data and the MIA to identify malaria-specific deaths were 33.3% and 96.1%, 66.7% and 96.1%, and 100% and 100%, respectively. In addition, malaria was identified as a possible contributor in 14 additional patients who died of other diseases. These cases were also accurately identified by the MIA (sensitivity 82.4%, specificity 100%).

Interpretation The high sensitivity and specificity of the MIA in identifying malaria may help to improve current estimates of malaria-specific mortality in endemic areas.

SUMMARY BOX

WHAT IS ALREADY KNOWN?
⇒ Global malaria mortality estimates rely on the verbal autopsy (VA) and the clinical records, both of these methods being prone to misclassification errors.

WHAT ARE THE NEW FINDINGS?
⇒ Malaria was the cause of death (CoD) in 2.3% of fatalities in Maputo (Mozambique).
⇒ Using the complete autopsy as the gold standard for CoD attribution, we found that the sensitivity and specificity of the VA, the clinical data and the minimally invasive autopsy (MIA) to identify malaria-specific deaths were 33.3% and 96.1%, 66.7% and 96.1%, and 100% and 100%, respectively.

WHAT DO THE NEW FINDINGS IMPLY?
⇒ MIA might be a valuable tool to improve estimations of malaria-related deaths in endemic regions.

INTRODUCTION
Cause-specific mortality information is a cornerstone for health policy-making worldwide.1 Unfortunately, civil registration systems and vital statistics are poorly functioning in most low-income and middle-income countries, especially in sub-Saharan Africa, where most countries report only fragmentary and sporadic data and where malaria burden is concentrated.2 3 After the launch of the sustainable development goals and the WHO Global Technical Strategy for malaria 2016–2030,4 5 malaria-endemic countries have set as mid-term objective to move towards low transmission or pre-elimination
status. In this scenario of potentially declining malaria burden, accurate mortality data are crucial to monitor interventions, evaluate progress and adapt strategies. However, obtaining reliable data on malaria-specific mortality, at both national and regional levels, is a major challenge. Indeed, the poor quality and thus reliability of this information seriously hinders the achievement of the Global Technical Strategy goals for malaria 2016–2030.

Most of the available information on malaria-specific mortality is based on data obtained from clinical records or verbal autopsy (VA). However, both methods have consistently shown limitations and are prone to frequent misclassification errors.6–12 Indeed, when clinical diagnoses are contrasted with postmortem findings, clinico-pathological discrepancies are frequently reported, particularly in settings with limited availability of diagnostic techniques,13 and for infectious diseases.6–9 Studies conducted in Malawian children dying with a clinical diagnosis of severe malaria showed significant misdiagnosis in up to a quarter of the cases, where an alternative cause of death (CoD) was identified at autopsy.14

The VA tool was developed as an indirect approach to establish the CoD by interviewing relatives and witnesses of deaths when other registration methods are unavailable.15 However, existing VA methods have also serious limitations in attributing deaths to malaria.10 An example of the weaknesses of VA was the marked differences around the global malaria mortality estimates produced by the WHO and the Institute of Health Metrics and Evaluation (IHME),11 12 with IHME reporting two times as many deaths in the world due to malaria than WHO estimates. More importantly, up to half of the excess malaria deaths were suggested to occur among adults in Africa,12 a finding difficult to credit for most malaria experts. Indeed, it is widely accepted that most malaria-related deaths in stable endemic areas are concentrated in children younger than 5 years,10 16 but the contribution of malaria to mortality in older age groups is still unclear.11 17

In the last few years, our group has developed a minimally invasive autopsy (MIA) approach, designed to determine the CoD mainly in low-resource settings, as a feasible alternative to the complete diagnostic autopsy (CDA).18 19 The procedure leaves hardly any visible trace on the body, and thus, is more acceptable than the CDA.20 Furthermore, the MIA can be performed by trained technicians, and can be conducted close to the place where death occurs.21 Therefore, this approach could be implemented for mortality surveillance, or as a method to complement information gathered through the VA.22 The MIA procedure has been validated in perinatal, paediatric, maternal and other adult deaths in Mozambique and Brazil,23–27 showing a moderate to substantial concordance with the CDA, particularly for infectious diseases even when evaluated blindly to any additional data,23–26 with such concordance increasing to almost perfect when the clinical information is added.28

In the present study, we compare the accuracy of a computerised VA, the clinical records, and the MIA to identify malaria-specific deaths in a large series of in-hospital deaths from Mozambique, comparing the results of these methods against the CDA, the gold standard methodology for CoD attribution.

METHODS

Study area

The study was conducted at the Maputo Central Hospital (Maputo, Mozambique), a government-funded tertiary-level hospital, which serves as the referral centre for other hospitals in Southern Mozambique. Maputo urban area has low malaria transmission, although some of the peripheral suburbs and surrounding peri-urban and rural areas have moderately stable transmission. Malaria incidence is higher during the rainy season (October to May), and lower during the dry season (June to September).29

Patients included in the study

During the study period (November 2013 to March 2015) 10,296 deaths occurred at the Maputo Central Hospital. All patients included in this study fulfilled the following criteria: (1) a complete autopsy requested by the in-charge clinician and (2) informed consent to perform the autopsy given by the relatives. In order to avoid selection bias, the first two bodies among all deaths eligible for complete autopsy, closest in time of occurrence to 08:00 am were recruited each morning for the study. Thus, 264 of these deaths (2.5% of the total deaths) were included in the study to validate the MIA against the CDA. It comprised 41 neonates, 54 children 1 month to 15 years (31 children under 5 years), 57 maternal deaths (fulfilling the standard definition of the WHO, ie, death during pregnancy or within 42 days of termination of pregnancy irrespective of its cause)30 and 112 other adults, including 57 men and 55 non-pregnant women. In all cases an MIA was performed, followed by a CDA on the same body. The general characteristics of each group of patients have been reported elsewhere.23–26

Patient and public involvement

No patients or the public were involved in the study design, recruitment, setting the research questions, interpretation or writing up of results, or reporting of the research.

Review of the clinical charts and clinical diagnosis

The clinical data from all patients were reviewed and abstracted using a standardised questionnaire. A detailed revision of the entire medical record was conducted with abstraction of demographic data, medical history, admission and hospitalisation files, including signs and symptoms, physical examination, laboratory results, imaging reports and treatments received. All clinical diagnoses registered in the medical record by the clinicians in charge prior to death were included in the list
of clinical diagnoses. Although it was assumed that the first diagnosis listed in the clinical record was the principal diagnosis, we considered malaria as a relevant clinical diagnosis, independently of the order in which it was registered.

A specific search for the malaria diagnostic methods conducted during admission (blood smear and/or rapid diagnostic tests) was performed as part of the review of the clinical charts.

**VA diagnosis**
We used the InterVA V.4.04 probabilistic model, one of the most commonly implemented VA tools, which has a good level of agreement with the physician-coded VA, and its interpretation is reproducible and standardised. The InterVA calculates the probability of a set of causes of death given the presence of indicators reported in VA interviews. In this analysis, the information feeding the model was obtained from the clinical record of the patient and from the obstetric record in perinatal deaths, unified into the WHO 2012 VA standard format, converted into the 245 input indicators of the VA model. We set malaria prevalence to ‘low’, and HIV prevalence to ‘high’ using the InterVA4 package V.1.7.5 implemented in R V.3.5.0 software.

**MIA procedure, analysis and diagnosis**
Detailed pathological and microbiological methods of the MIA have been reported elsewhere. The procedure included disinfection of the surface of the body, followed by the collection of blood and cerebrospinal fluid (CSF) and sampling of key solid organs (liver, lungs, central nervous system (CNS), heart, spleen and kidneys), which were analysed through microbiological and pathological techniques. The histological evaluation included H&E stain in all samples and histochemical and/or immunohistochemical stains whenever required to reach a diagnosis. Microbiological methods, which have been reported in detail elsewhere, included investigation for highly incident pathogens. This comprised universal detection of antibodies against HIV-1 and HIV-2, multiplexed PCR analyses for most common respiratory viruses and bacteria, bacterial and fungal cultures using samples of blood, CSF, liver, lungs and CNS; and in some cases, further investigation of bacterial or fungal presence using 16S ribosomal RNA (rRNA) gene PCR or the 18S rDNA-ITS PCR, respectively. In patients confirmed to be HIV-positive, an additional microbiological screening was conducted, for common opportunistic pathogens. Other micro-organisms were further investigated depending on the pathological findings observed in the MIA-obtained tissues. The MIA diagnosis integrated all MIA findings (histology and microbiology), as well as the clinical information.

**CDA procedure, analysis and gold standard diagnosis**
Immediately after the MIA, the CDA was performed by another pathologist not involved in the MIA. Histological and microbiological analyses were conducted in samples from the same viscera collected in the MIA and from any grossly identified lesions. The same analytical methods used for the MIA were used for the histological and microbiological samples of the complete autopsy.

The final diagnosis of the CDA integrated all autopsy findings (macroscopy, histology and microbiology), as well as the clinical information and was considered the gold standard for CoD attribution.

**Diagnosis of malaria in the MIA and the CDA and definitions**
In all 264 cases, Plasmodium falciparum malaria was proactively screened, and parasite density quantified, using a real-time quantitative PCR (qPCR) assay targeting 18S rRNA. Such assay was applied to peripheral blood obtained during the postmortem procedure, and collected as blood spots into Whatman 903 Specimen Collection Paper. Parasitaemia was quantified by extrapolation of cycle thresholds from a standard curve of P. falciparum ring-infected erythrocytes.

We selected all the patients with a diagnosis or suspicion of malaria (either clinical, in the VA or in the post-mortem examination), and a subset of 10 controls with no suspicion of malaria. In these cases, an immunohistochemical stain was performed in all available tissues with the polyclonal rabbit anti-P. falciparum HPRT1/HPRT antibody (LSBio, Seattle, Washington, USA, 30 min incubation, dilution 1/1000), and revealed with Envision FLEX and HRP Magenta as chromogen (Agilent, Santa Clara, California, USA) using the Dako PT Link equipment (Agilent). In all these cases a thorough search of malaria parasites was conducted under immersion oil (1000×) both in the H&E and P. falciparum immunohistochemical stains by two of the investigators (NR and JO). Additionally, a thorough search of haemozoin in macrophages was conducted in all tissues from these patients as evidence of the past malaria.

Malaria was considered the CoD (malaria-specific mortality) based on: (a) presence of cerebral malaria or (b) presence of abundant haemozoin deposition in tissues in the absence of other CoD. Malaria was considered a contributor to death on the basis of: (a) parasitaemia detected by PCR and/or (b) presence of malarial pigment in tissues in the presence of another CoD. In this subset of patients, malaria was considered as a possible contributor to death when haemoglobin levels were below 80 g/L (severe anaemia), due to the possible role of malaria in the chain of events leading to death. Malaria was considered as an associated condition death when the haemoglobin levels were within normal limits or showed only mild to moderate anaemia.

**Statistical analysis**
The diagnostic performance of the MIA, the clinical records and of the VA to identify malaria as the CoD against the CDA (gold standard) was evaluated as sensitivity, specificity, positive predictive value and negative
predictive value, and the 95% CIs for these values were calculated.

Data were analysed with STATA (V.15).

RESULTS
Malaria-specific mortality
Malaria was considered the CoD in 6 out of the 264 autopsies (2.3%). Malaria was attributed as the CoD in 2/54 children (3.7%), both cases being in children under-five (2/31, 6.4% of the deaths in children 1 month to 5 years), and in 4/57 (7.0%) maternal deaths. No death directly attributed to malaria was identified among neonates or other adults.

The demographic, clinical and laboratory characteristics of the six deaths caused by malaria are shown in table 1. Fever and neurological symptoms were documented in four of the six patients. Of the four maternal deaths one was a primigravid woman and three were multigravida (two gravid 2, one gravid 3). Three deaths occurred in the third trimester immediately after delivery (36, 37 and 38 weeks of gestation). All three women delivered a dead fetus, two after vaginal delivery and one after caesarean section. The fourth malaria maternal death led to a first trimester abortion (8 weeks of gestation). In none of the malaria-related maternal deaths there was a history of pre-eclampsia.

Table 2 shows the histological findings of the six malaria-attributable deaths. In five out of the six cases the diagnosis was based on the histological findings (massive sequestration of parasitised erythrocytes in the CNS, consistent with cerebral malaria). In the sixth patient the diagnosis was reached after clinicopathological correlation: identification of isolated CNS parasites and massive pigment deposition in the spleen and liver plus clinical evidence of severe malaria, in the absence of any alternative CoD.

The immunohistochemical staining revealed, in addition to the parasitised erythrocytes in the CNS capillaries, the presence of the parasitised erythrocytes in the splenic sinusoids in five cases, in the capillaries of the septal alveoli of the lung, in the liver sinusoids in four patients and in the capillaries of the heart and kidney in three cases. Lung oedema was identified in two patients. In one case there was massive sequestration of parasitised erythrocytes in the intestinal capillaries. Most parasitised erythrocytes sequestered in the microvasculature of both the CNS and other viscera were mature stages (trophozoites and schizonts), characterised by the presence of pigment dots in the cytoplasm. Several histological examples of severe malaria cases are shown in figure 1. No other plausible CoD was identified in any of the six deaths caused by malaria.

No parasites were identified in the immunohistochemical study in any of the 10 cases selected as controls.

Malaria as contributor to death
Three additional cases tested positive for *P. falciparum* by PCR. One case was a congenital malaria identified in a neonate who showed high blood parasitaemia in the qPCR analysis. Neither parasites, nor malarial pigment were identified in the tissues in the histological and immunohistochemical stains, and a neonatal bacterial sepsis was identified as the CoD. In two cases low blood parasitaemia levels were detected by qPCR (two children aged 7 and 10 years, respectively).

Evidence of past malaria (haemozoin in the liver and the spleen) was identified in further 10 cases (six children and four maternal deaths). No other malaria-related findings were observed, and no parasites were identified in any of these cases through immunohistochemistry. In all these cases a pathologically conclusive CoD different from malaria was identified.

Table 3 shows the demographic, clinical and laboratory characteristics, as well as the evidence of malaria at the CDA and the final CoD of these 13 patients, who died of other diseases but in whom malaria had possibly contributed to death. Seven of these cases had severe anaemia on the basis of haemoglobin levels below 80 g/L, and malaria was considered as a significant contributor to death.

MIA diagnosis of malaria
The histological findings of the MIA of the six deaths caused by malaria are shown in table 2. The MIA identified all six malaria-specific deaths, and correctly diagnosed all cerebral malaria. Finally, 4/7 cases with malaria as a probable contributor to death and all six cases in which malaria was considered a possible contributor to death were diagnosed by the MIA.

Clinical and VA diagnosis of malaria
The demographic characteristics, the clinical features, the laboratory data and the final CDA diagnosis of the patients clinically diagnosed of malaria or classified as malaria-specific deaths by the VA are shown in table 4.

Malaria was clinically diagnosed premortem in 12 patients. In nine of them, malaria was the first clinical diagnosis, whereas in three cases malaria was included in the list of additional diagnoses. Of them, eight patients had received antimalarial drugs. In 11/12 patients the temperature was recorded in the clinical records; 9 of these 11 patients (82%) had fever. One case was a child less than 5 years old, two were children aged 6–15 years, four were maternal deaths and five were other adults. The CDA confirmed a death caused by malaria in four cases whereas in the remaining cases an alternative CoD was identified.

A blood smear for malaria investigation had been performed during admission in 115/264 patients (43.6%) and was positive in four cases. A rapid diagnostic test had been performed in 98/264 patients (37.1%) and was positive in six patients. Two patients had a positive result for both tests (tables 1, 3 and 4).
Table 1  Demographic, clinical and laboratory characteristics of malaria-specific deaths

| Case | Age | Sex | Age group | Origin * | Malaria † seasonality | HIV | Fever | Neurological symptoms | Other symptoms | Haematocrit/haemoglobin | Parasitaemia/rapid test | Clinical diagnosis |
|------|-----|-----|-----------|----------|-----------------------|-----|-------|----------------------|----------------|--------------------------|---------------------|---------------------|
| 1    | 2   | M   | Children u-5 | Urban    | High                  | –   | NA    | NA                   | Dyspnoea       | NA                       | Positive/NA         | Severe malaria, sepsis, anaemia |
| 2    | 3   | M   | Children u-5 | Urban    | High                  | –   | Yes   | Confusion, agitation | Vomiting, diarrhoea, pallor | NA                       | NA/NA              | Acute gastroenteritis, anaemia |
| 3    | 23  | F   | Maternal death | Urban    | High                  | HIV | 40    | No                   | Dyspnoea, headache, leucocytosis, low platelet count | 22/80                     | Positive/positive  | Malaria                     |
| 4    | 28  | F   | Maternal death | Urban    | High                  | HIV | 39    | Lethargic, abnormal behaviour, nuchal rigidity | Dyspnoea, pallor, jaundice, leucocytosis low platelet count, hepatosplenomegaly, hypoglycaemic | 20/70                     | Negative/positive | Malaria                     |
| 5    | 28  | F   | Maternal death | Rural    | Low                   | HIV | No    | Coma                 | Diarrhoea, vomiting, pallor | NA                       | NA/NA              | Haemorrhage postabortion, acute gastroenteritis |
| 6    | 20  | F   | Maternal death | Urban    | High                  | –   | No    | No                   | Pallor, leucocytosis, low platelet count | 24/70                     | NA/positive         | Malaria                     |

*Urban usually corresponds to Maputo city, whereby malaria incidence is generally low. Rural implies other areas where malaria incidence tends to be higher.
†Seasonality for malaria is high during the rainy season (October to May), and low during the dry season (June to September).
F, female; M, male; NA, not available; u-5, under 5 years of age identified by the complete diagnostic autopsy.
### Table 2: Pathological features in the complete diagnostic autopsy (gold standard) and the minimally invasive autopsy of malaria-specific deaths

| Case | Complete diagnostic autopsy | Minimally Invasive autopsy | Diagnosis                        | CNS | Lung | Other | Diagnosis                        |
|------|-----------------------------|-----------------------------|----------------------------------|-----|------|-------|----------------------------------|
| 1    | Negative *                  | Congestion, haemorrhage     | Abundant haemoglobin in spleen and liver | Cerebral malaria (treated) | –    | Abundant haemoglobin in spleen and liver | Severe malaria, pulmonary haemorrhage |
| 2    | Massive parasitaemia         | Massive parasitaemia, haemoglobin | Abundant haemoglobin in spleen, liver, heart, kidney, bowel | Cerebral malaria | Massive parasitaemia | Abundant haemoglobin in spleen, liver, heart, kidney | Cerebral malaria |
| 3    | 2.879                       | Mild parasitaemia, haemoglobin | Abundant haemoglobin in spleen, liver, heart, kidney | Cerebral malaria | Mass haemoglobin in spleen, liver, heart, kidney | Abundant haemoglobin in spleen, liver | Cerebral malaria |
| 4    | 34.958                      | Moderate parasitaemia, oedema | Abundant haemoglobin in spleen, liver | Cerebral malaria | Moderate parasitaemia, oedema | Abundant haemoglobin in spleen, liver | Cerebral malaria |
| 5    | 212.308                     | Moderate parasitaemia, haemoglobin, oedema | Abundant haemoglobin in spleen, liver, heart. Abundant haemoglobin in spleen, liver | Cerebral malaria | Moderate parasitaemia, haemoglobin, oedema | Abundant haemoglobin in spleen, liver | Cerebral malaria |
| 6    | 176.738                     | Massive parasitaemia, haemoglobin | Abundant haemoglobin in spleen, liver, kidney, uterus. Abundant haemoglobin in spleen, liver | Cerebral malaria | Massive parasitaemia, haemoglobin | Abundant haemoglobin in spleen, liver | Cerebral malaria |

*Patient had received antimalarial treatment prior to arrival.
CNS, central nervous system.
Twelve cases were classified as malaria by the VA. In 10 cases, malaria was the only diagnosis provided, whereas in two cases, malaria infection was the second probable CoD. Three cases classified by the VA as malaria-specific deaths were children under-five years of age, four were maternal deaths and five were other adults. The CDA confirmed a death due to malaria in two cases, whereas in the remaining cases an alternative CoD was identified.

Fever was documented in 97 patients (5/41 neonates, 16/31 children under-five, 10/23 children 6–15, 17/57 maternal deaths and 49/112 adults). Nine per cent of the febrile deaths were considered clinically and by the VA as deaths caused by malaria (0% and 13%, respectively of the febrile deaths occurring in children under-five, 20% and 0% of the children 6–15, 12% each or the maternal deaths and 10% each of the adult deaths). The CDA confirmed malaria-related death in only 3% of the cases (6% of the febrile deaths occurring in children under-five and 12% of the maternal deaths).

**DISCUSSION**

To our knowledge, this is the first time that the MIA method has been compared with the currently used methods to ascertain the CoD (the VA and the clinical records) and shown higher sensitivity and specificity (100% and 100%, respectively) in identifying malaria-specific mortality. All patients with massive sequestration of *P. falciparum* parasites in the cerebral capillaries, the pathological hallmark of cerebral malaria and the most conclusive evidence of malaria-specific death both in adults and children, were clearly identified by the MIA. Interestingly, whereas 9% of all febrile deaths were attributed to malaria by the clinicians and the VA, the CDA confirmed malaria-specific death in only 3% of the patients, with marked discrepancies observed particularly in children other than 5 years and adults, which is in keeping with recent data showing a decrease in the malaria test positivity rate in adults.40 Finally, as observed in previous studies,14 41 42 the sequestration of parasitised...
Table 3  Clinical and pathological features of the cases with malaria as a contributor to death, in patients who died of other diseases

| Case | Age | Sex | Age group | Origin* | Malaria † seasonality | HIV | Haematocrit/ Haemoglobin | Parasitaemia/ rapid test | Cause of death | Evidence of malaria at autopsy | Detected by MIA |
|------|-----|-----|-----------|---------|------------------------|-----|--------------------------|-------------------------|----------------|-------------------------------|----------------|
|      |     |     |           |         |                        |     |                          |                         |                |                               |                |
| **Patients with severe anaemia (malaria as a possible contributor to death)** |     |     |           |         |                        |     |                          |                         |                |                               |                |
| 8    | 7   | M   | Children 6—15 | Rural   | High                  | HIV | 23/70                    | Negative/ negative   | Burkitt lymphoma | PCR parasitaemia (low)        | Yes (PCR parasitaemia, low) |
| 9    | 10  | M   | Children 6—15 | Urban   | High                  | –   | 9/30                     | Negative/ negative   | Sepsis due to Gram-negative bacteria | PCR parasitaemia (low)        | Yes (PCR parasitaemia, low) |
| 11   | 10  | M   | Children 6—15 | Rural   | High                  | –   | 9/20                     | Negative/ negative   | Sepsis due to Gram-negative bacteria | Haemozoin in liver, lung and spleen | No               |
| 15   | 2   | M   | Children u-5  | Urban   | High                  | HIV | 21/70                    | NA/negative          | Sepsis (Gram-negative bacteria) | Haemozoin in liver and spleen | Yes (haemozoin in liver and spleen) |
| 16   | 27  | F   | Maternal death | Urban   | High                  | HIV | 18/60                    | Negative/NA          | Genital tract infection following abortion | Haemozoin in lung | No               |
| 17   | 27  | F   | Maternal death | Urban   | High                  | HIV | 14/50                    | Negative/NA          | Liver failure | Haemozoin in spleen | No               |
| 19   | 27  | F   | Maternal death | Urban   | High                  | –   | 18/60                    | NA/negative          | Obstetric haemorrhage | Haemozoin in liver | Yes (haemozoin in liver) |
| **Patients with mild to moderate anaemia or with no available data (malaria as associated condition)** |     |     |           |         |                        |     |                          |                         |                |                               |                |
| 7    | 0   | F   | Neonate    | Urban   | High                  | –   | 41/140                   | NA/NA                 | Neonatal sepsis, congenital malaria | PCR parasitaemia (107,167) | Yes (PCR parasitaemia, 107,167) |
| 10   | 4   | M   | Children u-5 | Urban   | Low                   | –   | 28/90                    | Negative/ negative   | Encephalitis | Haemozoin in liver and spleen | Yes (haemozoin in liver and spleen) |
| 14   | 13  | M   | Children 6—15 | Urban   | High                  | –   | 41/130                   | NA/negative          | Sepsis (Streptococcus pneumoniae) | Haemozoin in liver and spleen | Yes (haemozoin in liver and spleen) |
| 12   | 6   | F   | Children 6—15 | Urban   | High                  | –   | 33/110                   | Negative/NA          | Encephalitis | Haemozoin in liver and spleen | Yes (haemozoin in liver and spleen) |
| 13   | 4   | M   | Children u-5 | Rural   | Low                   | –   | 30/100                   | NA/NA                 | Burkitt lymphoma | Haemozoin in liver | Yes (haemozoin in liver) |
| 18   | 32  | F   | Maternal death | Urban   | High                  | –   | NA                       | NA/NA                 | Obstetric haemorrhage | Haemozoin in liver | Yes (haemozoin in liver) |

*Urban usually corresponds to Maputo city, whereby malaria incidence is generally low. Rural implies other areas where malaria incidence tends to be higher.
†Seasonality for malaria is high during the rainy season (October to May), and low during the dry season (June to September).
F, female; M, male; MIA, minimally invasive autopsy; NA, not available; u-5, under 5 years of age.
Table 4  Demographic characteristics, clinical features, laboratory data and final diagnosis (gold standard) of the patients with clinical diagnosis of malaria or diagnosed of malaria by verbal autopsy

| Case | Age | Sex | Age group | Origin | Malaria * incidence | Previous conditions | Fever | Haematocrit/ Haemoglobin | Parasitaemia/ rapid test | Cause of death |
|------|-----|-----|-----------|--------|---------------------|---------------------|-------|--------------------------|-------------------------|---------------|
| 3    | 23  | F   | Maternal death | Urban | High               | HIV                   | Yes   | 22/80                   | Positive/positive       | Cerebral malaria |
| 4    | 28  | F   | Maternal death | Urban | High               | HIV                   | Yes   | 20/70                   | Negative/positive       | Cerebral malaria |
| 6    | 20  | F   | Maternal death | Urban | High               | –                    | No    | 24/70                   | NA/NA                  | Cerebral malaria |
| 11   | 10  | M   | Children 6–15  | Rural  | High               | –                   | Yes   | 9/20                    | Negative/negative       | Gram-negative sepsis‡ |
| 12   | 6   | F   | Children 6–15  | Urban | High               | –                    | Yes   | 33/110                  | Negative/NA             | Encephalitis‡     |
| 20   | 34  | F   | Maternal death | Urban | High               | –                    | No    | 29/100                  | NA/NA                  | Streptococcal sepsis |
| 21   | 17  | M   | Adult        | Urban | High               | –                    | Yes   | 34/120                  | Negative/NA             | Pneumococcal meningitis |
| 22   | 40  | F   | Adult        | Urban | High HIV           | Yes                  | 22/80 | Negative/NA             | Cytomegalovirus pneumonia |
|      |     |     |             |       |                    |                      |       |                         |                         |               |
|      |     |     |             |       |                    |                      |       |                         |                         |               |
|      |     |     |             |       |                    |                      |       |                         |                         |               |

Clinical diagnosis of malaria and verbal autopsy classified as malaria

| Case | Age | Sex | Age group | Origin | Malaria * incidence | Previous conditions | Fever | Haematocrit/ Haemoglobin | Parasitaemia/ rapid test | Cause of death |
|------|-----|-----|-----------|--------|---------------------|---------------------|-------|--------------------------|-------------------------|---------------|
| 1    | 2   | M   | Children u-5 | Urban  | High               | –                   | NA    | NA/NA                   | Positive/NA             | Severe malaria  |
| 16   | 27  | F   | Maternal death | Urban | High HIV           | Yes                  | 18/60 | NA/NA                   | Negative/NA             | Puerperal sepsis‡ |
| 19   | 27  | F   | Maternal death | Urban | High               | –                    | No    | 18/60                   | NA/negative              | Haemorrhage postabortion‡ |
| 26   | 0   | M   | Children u-5 | Urban  | High               | –                    | Yes   | 18/60                   | Negative/negative       | Pneumococcal meningitis |
| 27   | 28  | F   | Maternal death | Urban | High HIV           | No                  | 14/50 | NA/NA                   | Negative/NA             | Liver failure   |
| 28   | 16  | F   | Maternal death | Urban | High               | –                    | Yes   | 10/30                   | NA/NA                  | Liver failure   |
| 29   | 28  | F   | Adult        | Urban | High               | –                    | Yes   | 12/40                   | Negative/negative       | Bacterial sepsis  |
| 30   | 37  | M   | Adult        | Rural  | High HIV           | Yes                 | NA/NA | NA/NA                   | NA/NA                  | Bacterial sepsis  |

Verbal autopsy classified as malaria

| Case | Age | Sex | Age group | Origin | Malaria * incidence | Previous conditions | Fever | Haematocrit/ Haemoglobin | Parasitaemia/ rapid test | Cause of death |
|------|-----|-----|-----------|--------|---------------------|---------------------|-------|--------------------------|-------------------------|---------------|
| 2    | 3   | M   | Children u-5 | Urban  | High               | –                   | Yes   | NA/NA                   | NA/NA                   | Cerebral malaria |
| 16   | 27  | F   | Maternal death | Urban | High HIV           | Yes                  | 18/60 | NA/NA                   | Negative/NA             | Puerperal sepsis‡ |
| 19   | 27  | F   | Maternal death | Urban | High               | –                    | No    | 18/60                   | NA/negative              | Haemorrhage postabortion‡ |
| 26   | 0   | M   | Children u-5 | Urban  | High               | –                    | Yes   | 18/60                   | Negative/negative       | Pneumococcal meningitis |
| 27   | 28  | F   | Maternal death | Urban | High HIV           | No                  | 14/50 | NA/NA                   | Negative/NA             | Liver failure   |
| 28   | 16  | F   | Maternal death | Urban | High               | –                    | Yes   | 10/30                   | NA/NA                  | Liver failure   |
| 29   | 28  | F   | Adult        | Urban | High               | –                    | Yes   | 12/40                   | Negative/negative       | Bacterial sepsis  |
| 30   | 37  | M   | Adult        | Rural  | High HIV           | Yes                 | NA/NA | NA/NA                   | NA/NA                  | Bacterial sepsis  |

*Urban usually corresponds to Maputo city, whereby malaria incidence is generally low. Rural implies other areas where malaria incidence tends to be higher.
†Seasonality for malaria is high during the rainy season (October to May), and low during the dry season (June to September).
‡Histological evidence of past malaria (haemozoin accumulation in liver and spleen) identified in the autopsy.
F, female; M, male; NA, not available; u-5, under 5 years of age.
In low-income and middle-income countries, including African countries, 50–51 finally, the VA is markedly influenced by the malaria epidemiology, and in endemic areas it tends to assign to malaria any acute febrile illness in the absence of an obvious alternative cause.44

In addition to accurately identifying malaria-specific deaths, the MIA captures with relatively high precision patients who were considered to die of other diseases but who had low-level parasitaemia, or evidence of past malaria infection, and in whom malaria may have contributed to death, including patients with severe anaemia in whom malaria was a probable contributor to death (sensitivity 76.9%, specificity 100%). Most of these cases were either not identified, or wrongly classified as malaria-specific deaths clinically and by the VA. The difficulties of assigning malaria as an underlying, contributing or indirect CoD have been noted,10 and the MIA could provide relevant information in this challenging area. In malaria-endemic areas, the progressive acquisition of natural immunity allows the common occurrence of malarial infection without clinical symptoms. Such malaria ‘tolerance’ is a confounding factor when investigating disease and death, as the detected parasitaemia cannot be ruled out as the aetiology of the disease and death. The MIA may offer an opportunity to improve our knowledge on the indirect contribution of malaria to death.

In this study, malaria-specific deaths occurred only in two groups, namely, children younger than 5 years of age and pregnant women. Indeed, although some reports suggest that cerebral malaria is a common cause of maternal mortality in areas of stable transmission, 42,43 it is generally considered that malaria-specific maternal deaths are largely restricted to low and unstable malaria transmission areas.17 In this regard, studies focusing on determining the real contribution of malaria to maternal mortality are warranted in areas with different malaria transmission. Interestingly, none of the malaria-specific deaths occurred in children aged 6–15 or in adults other than maternal deaths, which is in contrast with some reports suggesting that malaria is responsible for up to 25% of deaths in children 6–15 years old and a significant

### Table 5

|                      | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|----------------------|-------------|-------------|---------------------------|---------------------------|
| **Malaria-specific deaths** |             |             |                           |                           |
| Verbal autopsy        | 33.3% (4.3 to 77.7) | 96.1% (93.0 to 98.1) | 16.7% (2.1 to 48.4) | 98.4% (96.0 to 99.6) |
| Clinical diagnosis    | 66.7% (22.3 to 95.7) | 96.1% (93.0 to 98.1) | 28.6% (8.4 to 58.1) | 99.2% (97.1 to 99.9) |
| Minimally invasive autopsy | 100% (54.1 to 100) | 100% (98.6 to 100) | 100% (54.1 to 100) | 100% (98.6 to 100) |
| **Deaths probably associated with malaria** |             |             |                           |                           |
| Verbal autopsy        | 30.8% (9.1 to 61.4) | 96.8% (93.8 to 98.6) | 33.3% (8.9 to 65.1) | 96.4% (93.3 to 98.4) |
| Clinical diagnosis    | 38.5% (13.9 to 65.4) | 96.4% (93.3 to 98.3) | 35.7% (12.8 to 64.9) | 96.8% (93.8 to 98.6) |
| Minimally invasive autopsy | 76.9% (46.2 to 95.0) | 100% (98.5 to 100) | 100% (69.2 to 100) | 98.8% (96.6 to 99.8) |

The figures in parenthesis indicate 95% CIs.
number of deaths in adults, particularly among elderly people. Moreover, no case of low-level parasitaemia or past malaria was identified among adults, which may be an indirect evidence of the limited contribution of malaria to mortality in this age group.

Our study has several strengths. First, it evaluates the performance of the VA, the clinical records and the MIA against the CDA, the gold standard for CoD attribution. Notably, the CDA included an extensive microbiological testing comprising specific qPCR for malaria infection. Another strength is that all age groups were included in the analysis. The main limitations of the study include a relatively small number of cases, particularly of some age groups, like young children, which may partly explain the small number of malaria-specific deaths. However, the complexity of the CDA and the scarcity of specialised personnel preclude a widespread use of these studies. In addition, the small number of malaria-specific deaths identified in our study may be considered as a limitation. Further studies in high malaria burden settings are warranted to confirm our results. Also, the fact that the study is based in a tertiary hospital might be seen as a limitation to extrapolate findings to deaths occurring in rural health facilities or in the community, since causes of death in rural settings may be different to that of those occurring in a hospital. However, the main objective of this study was evaluating the MIA against a reliable gold standard. Finally, the VA results might have been influenced by the fact that the indicators used to estimate the CoD by the VA model were extracted from medical records, which may have resulted in some missing indicators. Nevertheless, most of these missing indicators were secondary questions related to the duration of the event.

In conclusion, this study provides evidence on the high sensitivity and specificity of the MIA method in identifying malaria-specific deaths. It also shows that data provided by the VA, and ultimately, to improve all-of current VA tools, to mathematically modulate the diagnostic errors, to improve the quality and performance in recognising malaria infection as CoD in an endemic setting. The MIA, implemented in selected surveillance sites, could be used to help clinicians to reduce diagnostic errors, to improve the quality and performance of current VA tools, to mathematically modulate the data provided by the VA, and ultimately, to improve all-cause and disease-specific statistics.

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