The effect of multi-vitamin/mineral supplementation on mortality during treatment of pulmonary tuberculosis: a randomised two-by-two factorial trial in Mwanza, Tanzania

Nyagosya Range, John Changalucha, Henrik Krarup, Pascal Magnussen, Ase B. Andersen and Henrik Friis*

1National Institute for Medical Research, Muhimbili Research Station, PO Box 3436, Dar es Salaam, Tanzania
2National Institute for Medical Research, Mwanza Medical Research Centre, PO Box 1462, Mwanza, Tanzania
3Department of Clinical Biochemistry, Aalborg University Hospital, PO Box 561, 9100 Aalborg, Denmark
4DBL Institute for Health Research and Development, Jaegersborg Allé 1D, Charlottenlund, Denmark
5Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
6Department of Epidemiology, Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5B, DK-1014 Copenhagen K, Denmark

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Malnutrition is common in pulmonary tuberculosis (TB), and may impair survival. The objective of this study was to assess effects of multi-vitamin/mineral (MVM) and zinc (Zn) supplementation during TB treatment on mortality. Patients diagnosed with sputum-positive pulmonary TB in Mwanza, Tanzania, were randomised, using a two-by-two factorial design, to Zn (45 mg) or placebo, and MVM (vitamins A, B, C, D, E, and selenium and copper) or placebo. Survival status was ascertained at the end of the 8-month TB treatment and supplementation period. Of 499 TB patients, 213 (43%) had HIV. The mean weight gain at 7 months was 6·88 kg (95% CI 6·36, 7·41). Zn and MVM combined, but neither alone (interaction, \( P = 0.03 \)), increased weight gain by 2·37 kg (95% CI 0·91, 3·83), irrespective of HIV status. Survival status at 8 months was determined for 422 patients (84·6 %), of which fifty-two (12·3 %) had died. Among fifty-two deaths, there were no effects of MVM (relative risk (RR) 0·73; 95% CI 0·43, 1·23) and Zn (RR 0·76; 95% CI 0·46, 1·28). However, among HIV co-infected patients, marginally significant effects of both MVM (RR 0·60; 95% CI 0·34, 1·05) and Zn (RR 0·63, 95% CI 0·37, 1·08) were seen, and MVM and Zn combined reduced mortality (RR 0·29; 95% CI 0·10, 0·80; interaction ratio 0·52). In conclusion, supplementation with MVM, including Zn, during treatment of pulmonary TB may reduce mortality in those co-infected with HIV. A randomised trial of the effect of the combined intervention used in this study should be conducted in a different setting to confirm the finding.


The risk of active tuberculosis (TB) disease depends on exposure to *Mycobacterium tuberculosis* and reduced host defence. One-third of the world’s population is infected, nine million develop clinical disease each year, and two million die due to the infection (Frieden et al. 2003). HIV infection is considered the strongest single risk factor of developing TB disease after primary infection, and of death during treatment.

Deficiencies of vitamins and minerals, leading to nutritionally acquired immune deficiency syndrome (Beisel, 2001) may be another important determinant of TB infection and disease, as well as poor treatment outcomes (Cegielski & McMurray, 2004). For example, it is well-documented that zinc (Zn) supplementation reduces risk and severity of diarrhoea and pneumonia in children (Bhutta et al. 1999, 2000), and it has been shown that micronutrient supplementation reduces progression of HIV to AIDS and death in adults (Fawzi et al. 2004; Jiamton et al. 2003).

Despite appropriate anti-TB chemotherapy, the usual cereal-based diet will not ensure an adequate micronutrient intake during convalescence. Micronutrient deficiencies not only impair host immune functions, but may also affect efficacy of TB drugs (Thurnham, 2005), and impair weight gain which may be important to survival (Paton et al. 2004). Although nutritional supplementation during the short-course TB treatment seems logistically feasible and affordable, and may improve treatment outcomes, nutritional assessment and support is currently not part of the case-management.

We conducted a randomised, placebo-controlled, double-blind, two-by-two factorial trial to assess the effect of daily Zn and multi-vitamin/mineral (MVM) supplementation on treatment outcomes. Although we found no beneficial effect

Abbreviations: MVM, multi-vitamin/mineral; RR, relative risk; TB, tuberculosis.

* Corresponding author: Dr Henrik Friis, fax +45 35 32 73 83, email h.friis@pubhealth.ku.dk
on sputum conversion (Range et al. 2005), the effects on weight gain and mortality over the full duration of TB treatment were also assessed and are reported here.

Subjects and methods

New or relapse sputum microscopy-positive TB patients aged 15 years or above and resident in the area were consecutively recruited from five health facilities in Mwanza and Magu districts, Mwanza Region, Tanzania, between August 2001 and July 2002. Patients were given information about the study and offered inclusion after informed consent. TB patients returning to treatment after defaulting or smear-negative failure cases, or those with serious TB or other disease unlikely to survive, and pregnant or lactating women were excluded. The study was conducted within the framework of the National TB and Leprosy Programme (Ministry of Health, 1991), with diagnosis, classification, registration and treatment of TB patients done in accordance with recommended standard procedures (World Health Organization, 2003). In brief, new TB cases were given a combination of four drugs daily under supervision of a health worker during the initial 2-month intensive phase, while two drugs were self-administered daily by the patient, during the 6-month continuation phase.

TB suspects attending in- and out-patient clinics at any of the recruitment centres were asked to submit three sputum specimens (spot, early morning, spot) for examination of acid-fast bacilli using the Zielh-Neelsen staining technique. Those found sputum microscopy-positive were requested to submit a morning sputum specimen in a sterile universal bottle for confirmation at the Zonal TB Reference Laboratory at Bugando Medical Centre, based on microscopy after fluorochrome (Auramine O) staining, and culture on Lowenstein Jensen solid media (Githui et al. 1993). Only those confirmed to be sputum microscopy- or culture-positive at the Zonal TB Reference Laboratory were included in the trial (Fig. 1).

All patients found sputum microscopy-positive at the recruitment centres were started on TB treatment, and given a study number and supplement according to the randomisation, as described later. Prior to that, the TB Clinical Officers collected demographic, socio-economic and medical history data from all patients, examined the patients anthropometrically, and collected stool, urine and blood samples for later diagnosis of parasitic infections (World Health Organization, 1991), and determination of Hb concentration and leucocyte counts. Heights were measured to the nearest 0.1 cm and weights to the nearest 0.1 kg, with the patient barefoot and wearing light clothing. The same staff member measured the patient at baseline and follow-up. Patients found anaemic or infected with Schistosoma spp., intestinal helminths or malaria were treated accordingly (World Health Organization, 1995). HIV testing was done using two different ELISA. In samples found positive, HIV-1 load was determined using a modification of an in-house developed RT-PCR (Krarup et al. 1998). CD4 cell count was determined by manual immuno-cytochemistry (Gomo et al. 2004).

Study design and intervention

The study was a randomised, placebo-controlled trial, using a two-by-two factorial design, to assess the effects of daily supplementation with a MVM and a Zn tablet. Although sputum conversion and weight gain during the 2-month intensive phase were the main outcomes on which sample size considerations were based as previously reported (Range et al. 2005), daily Zn and MVM supplementation was continued throughout the 8 months of short-course TB treatment when survival status was assessed. The MVM tablets contained vitamin A (1.5mg), vitamin B1 (20 mg), vitamin B2 (20 mg), vitamin B3 (25 mg), vitamin B12 (50 μg), folic acid (0.8 mg), niacin (40 mg), vitamin C (200 mg), vitamin E (60 mg), vitamin D3 (5 μg), selenium (0.2 mg) and copper (5 mg), and Zn tablets contained 45 mg elemental Zn (Table 1). The rationale for our interventions was the high requirements during TB treatment, when considerable lean body mass is being synthesised. As for the vitamins, the doses were based on the multivitamin supplement proven beneficial among HIV-infected women (Fawzi et al. 1999). However, we included more vitamin E (60 instead of 30 mg), since higher doses have been shown to improve immune functions (Food and Nutrition Board, 2000, pp. 186–283), and less vitamin C (200 instead of 1000 mg), since there has been concern that high doses may cause oxidative damage in individuals with high iron stores (Food and Nutrition Board, 2000, pp. 95–185). However, we included preformed vitamin A only, since it has been

Supplementation and tuberculosis mortality

530 sputum-positive at health facility randomised to zinc or placebo and MVM or placebo

123 placebo/placebo

133 zinc/placebo

133 MVM/placebo

132 zinc/MMV

7 sputum-negative at reference laboratory

50 sputum-negative at reference laboratory

20 sputum-negative at reference laboratory

5 sputum-negative at reference laboratory

10 sputum-negative at reference laboratory

5 sputum-negative at reference laboratory

10 sputum-negative at reference laboratory

97 weighed at 7 months

96 weighed at 7 months

95 weighed at 7 months

94 weighed at 7 months

93 weighed at 7 months

92 weighed at 7 months

91 weighed at 7 months

90 weighed at 7 months

89 weighed at 7 months

88 weighed at 7 months

87 weighed at 7 months

86 weighed at 7 months

85 weighed at 7 months

84 weighed at 7 months

83 weighed at 7 months

82 weighed at 7 months

81 weighed at 7 months

80 weighed at 7 months

79 weighed at 7 months

78 weighed at 7 months

77 weighed at 7 months

76 weighed at 7 months

75 weighed at 7 months

74 weighed at 7 months

73 weighed at 7 months

72 weighed at 7 months

71 weighed at 7 months

70 weighed at 7 months

69 weighed at 7 months

68 weighed at 7 months

67 weighed at 7 months

66 weighed at 7 months

65 weighed at 7 months

64 weighed at 7 months

63 weighed at 7 months

62 weighed at 7 months

61 weighed at 7 months

60 weighed at 7 months

59 weighed at 7 months

58 weighed at 7 months

57 weighed at 7 months

56 weighed at 7 months

55 weighed at 7 months

54 weighed at 7 months

53 weighed at 7 months

52 weighed at 7 months

51 weighed at 7 months

50 weighed at 7 months

49 weighed at 7 months

48 weighed at 7 months

47 weighed at 7 months

46 weighed at 7 months

45 weighed at 7 months

44 weighed at 7 months

43 weighed at 7 months

42 weighed at 7 months

41 weighed at 7 months

40 weighed at 7 months

39 weighed at 7 months

38 weighed at 7 months

37 weighed at 7 months

36 weighed at 7 months

35 weighed at 7 months

34 weighed at 7 months

33 weighed at 7 months

32 weighed at 7 months

31 weighed at 7 months

30 weighed at 7 months

29 weighed at 7 months

28 weighed at 7 months

27 weighed at 7 months

26 weighed at 7 months

25 weighed at 7 months

24 weighed at 7 months

23 weighed at 7 months

22 weighed at 7 months

21 weighed at 7 months

20 weighed at 7 months

19 weighed at 7 months

18 weighed at 7 months

17 weighed at 7 months

16 weighed at 7 months

15 weighed at 7 months

14 weighed at 7 months

13 weighed at 7 months

12 weighed at 7 months

11 weighed at 7 months

10 weighed at 7 months

9 weighed at 7 months

8 weighed at 7 months

7 weighed at 7 months

6 weighed at 7 months

5 weighed at 7 months

4 weighed at 7 months

3 weighed at 7 months

2 weighed at 7 months

1 weighed at 7 months

0 never seen

10 sputum-negative at reference laboratory

6 lost before day 210

11 lost before day 244

12 lost before day 210

13 lost before day 244

14 lost before day 210

15 lost before day 244

16 lost before day 210

17 lost before day 244

18 lost before day 210

19 lost before day 244

20 lost before day 244

Fig. 1. Trial profile. Sputum-positive pulmonary tuberculosis patients were randomised to daily supplementation with zinc or placebo and, independently, to multi-vitamin/mineral (MVM) or placebo. Those found sputum-negative at the reference laboratory after randomisation were excluded.
The profile for recruitment, randomisation and trial participants is shown in Fig. 1. Trial participants were asked to come to the recruitment centre after 2, 5 and 7 months. Collection of blood was repeated at the 2-month visit, and anthropometric assessment and collection of sputum were repeated at all follow-up visits. Those not seen at the clinic during scheduled follow-up visits were followed up by the study team using addresses given during registration. In cases where the patient had died, the date of death was obtained from relatives. In the analysis of the effect of supplementation on mortality, a person was considered to have died if death occurred within the 8-month supplementation, i.e. not later than 244 d after start of treatment and supplementation, and to have survived if examined at the 7-month visit or known to be alive later. Cause of death was not ascertained.

The sample size of 500 was initially based on sputum culture conversion and weight gain as the primary outcomes, as previously reported (Range et al. 2005). However, assuming a 10 % cumulative mortality during the 8 months of treatment we would be able to detect a reduction to 5 % or less with 80 % power and 95 % confidence, while allowing for 10 % loss to follow-up.

Permission to conduct the study was granted by the Ethics Committee of the National Institute for Medical Research in Tanzania, and the Danish Central Medical Ethics Committee also approved the study. Permission was also obtained from the Regional Medical Officer and the Council Medical Officers in Mwanza Region. Informed oral consent was obtained from all study participants. Pre-test HIV counselling was given to all, and post-test counselling was given to those who wanted to know their HIV results. Antiretroviral treatment was not available.

Statistical analysis

Two-sample t test and one-way ANOVA were used to test for differences in means, and \( \chi^2 \) test to test for differences in proportions between groups. Assessment of the effect of the study interventions on mortality was done prior to breaking the code. The analysis was based on intention-to-treat, and done with and without stratification for HIV status. Multiple logistic regression analysis was used to estimate the effects of the study interventions while controlling for potential baseline differences, and to assess for interactions. Binreg was used to compute relative risks (RR), rather than odds ratios, using Stata version 8.2 (StataCorp LP, College Station TX, USA). Survival analysis using Cox regressions was also done, with follow-up time to either the date of death, the date last to follow-up or end of supplementation period. \( P \) values below 0.05 were considered significant.

Results

Of 530 patients found positive based on detection of tubercle bacilli in sputum on microscopy and randomised to the two study interventions, thirty-one could not be confirmed positive for the full 8-month supplementation. Of 530 patients found positive based on detection of tubercle bacilli in sputum on microscopy and randomised to the two study interventions while controlling for potential baseline differences, and to assess for interactions. Binreg was used to compute relative risks (RR), rather than odds ratios, using Stata version 8.2 (StataCorp LP, College Station TX, USA). Survival analysis using Cox regressions was also done, with follow-up time to either the date of death, the date last to follow-up or end of supplementation period. \( P \) values below 0.05 were considered significant.

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Results

Of 530 patients found positive based on detection of tubercle bacilli in sputum on microscopy and randomised to the two study interventions, thirty-one could not be confirmed positive based on re-examination at the reference laboratory and were excluded from the study (Fig. 1). Thus, 499 confirmed sputum-positive cases were included in the trial. The mean age was 35·4 (range 15–85) years, and 41·1 % were women. Most of the participants were recruited at Sekou Toure
Hospital (47.7%), and the rest from Bugando Medical Centre (16.6%), Magu Hospital (18.0%), and Buzuruga (10.2%) and Butimba (7.4%) Health Centres. The prevalence of HIV infection was 42.7% (213), and 34.7% (173) were found to excrete *Schistosoma mansoni* and 20.3% (101) hookworm eggs, and 4.6% had malaria parasitaemia. As seen in Table 2, baseline equivalence was achieved with respect to age, BMI, Hb, and prevalence of heavy culture intensity and HIV, although there seemed to be more women in the placebo/placebo group.

As seen from the trial profile (Fig. 1), viral load and CD4 counts were determined after 2 months, weight at 7 months, and survival up to the end of treatment and supplementation at 8 months.

**Viral load and CD4 count**

At the 2-month follow-up examination, viral load and CD4 count data were determined in 184 (86.4%) of the 213 HIV-infected participants (Table 3). The mean viral load increase was 0.23 log(10)(geq/l) (95% CI 0.01, 0.46). There was no significant effect of MVM (0.22 log(10)(geq/l); 95% CI 0.67, 0.23; P = 0.35) or Zn supplementation (0.27 log(10)(geq/l), 95% CI −0.18, 0.72; P = 0.24) (interaction, P = 0.37), and no effect of any combination of the two interventions. Zn supplementation alone was associated with a 0.54 log(10)(geq/l) (95% CI 0.08, 1.15; P = 0.08) higher increase. Similarly, there were neither effects of Zn (18 cells/m(3); 95% CI 101, 137) nor MVM (46 cells/m(3); 95% CI 72, 165) on CD4 counts (interaction, P = 0.22), and no effect of any combination of the two interventions (Table 3).

**Weight gain**

At the 7-month follow-up examination, 389 (80.0%) of the 499 patients were weighed. The mean weight gain was 6.88 kg (95% CI 6.36, 7.41). However, an interaction was found between Zn and MVM supplementation (P = 0.03). The effect of each combination of Zn and MVM was therefore compared to placebo, based on the use of dummy variables in multiple linear regression analysis (Table 4). As seen, those receiving both Zn and MVM supplementation had a 2.37 kg (95% CI 0.91, 3.83; P = 0.002) greater weight gain than those receiving placebo and placebo, whereas neither Zn nor MVM alone had any effect. After adjustment for sex, age, HIV status and heavy culture intensity, the estimated effects of Zn and MVM supplementation combined was 2.63 kg (95% CI 1.18, 4.09; P = 0.001), whereas there were no effects of Zn or MVM alone.

**Survival status**

As seen in the trial profile (Fig. 1), survival status could not be ascertained at any time-point after commencement of treatment and supplementation for ten (2.0%) of the 499 study participants. Forty-nine (9.8%) were lost to follow-up before 210 d post-treatment, and 77 (15.4%) before 244 d post-treatment. The median observation time among those lost to full follow-up was 197 d (interquartile range 121–221 d). Hence, 422 (84.6%) of the 499 study participants were known either to have died within 244 d post-treatment or to be alive after that. There were no differences between the 422 followed...
Table 3. Effects of zinc and multi-vitamin/mineral (MVM) supplementation on HIV viral load (log_{10}(geq/l)) and CD4 count (cells/μl) during first 2 months of treatment of pulmonary tuberculosis patients among 213 HIV co-infected patients†

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>Placebo + placebo (n 48)</th>
<th>Placebo + Zn (n 58)</th>
<th>MVM + placebo (n 59)</th>
<th>MVM + Zn (n 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral load</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3·90 (3·53, 4·27)</td>
<td>3·83 (3·52, 4·15)</td>
<td>4·02 (3·76, 4·27)</td>
<td>3·72 (3·38, 4·05)</td>
</tr>
<tr>
<td>2 months</td>
<td>4·10 (3·67, 4·54)</td>
<td>4·28 (3·86, 4·71)</td>
<td>4·14 (3·72, 4·55)</td>
<td>3·85 (3·46, 4·25)</td>
</tr>
<tr>
<td>Change‡</td>
<td>0·08 (−0·40, 0·55)</td>
<td>0·62 (0·20, 1·03)</td>
<td>0·16 (−0·22, 0·56)</td>
<td>−0·01 (−0·47, 0·45)</td>
</tr>
<tr>
<td>Difference§</td>
<td>−0·54 (−0·08, 1·15)</td>
<td>0·09 (−0·27, 0·55)</td>
<td>−0·09 (−0·71, 0·54)</td>
<td></td>
</tr>
<tr>
<td>CD4 count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>460 (351, 569)</td>
<td>406 (327, 485)</td>
<td>365 (289, 442)</td>
<td>460 (349, 570)</td>
</tr>
<tr>
<td>2 months</td>
<td>403 (308, 569)</td>
<td>422 (331, 512)</td>
<td>363 (281, 444)</td>
<td>423 (318, 529)</td>
</tr>
<tr>
<td>Change‡</td>
<td>−104 (−243, 34)</td>
<td>−9 (−113, 96)</td>
<td>19 (89, 127)</td>
<td>−48 (−175, 85)</td>
</tr>
<tr>
<td>Difference§</td>
<td>96 (−67, 259)</td>
<td>124 (−45, 292)</td>
<td>60 (−113, 233)</td>
<td></td>
</tr>
</tbody>
</table>

† Data at follow-up available for 184 participants. For details of procedures, see pp. 763–764.
‡ Change (2 months – baseline); t test.
§ Difference (each active group – placebo + placebo). Linear regression analysis, interaction between interventions, \(P = 0.10\) (viral load) and \(P = 0.18\) (CD4 counts). None were significant.

Table 4. Effects of zinc and multi-vitamin/mineral (MVM) supplementation on weight gain during treatment of pulmonary tuberculosis among 389 patients weighed at 7 months

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>Placebo + placebo (n 97)</th>
<th>Placebo + Zn (n 96)</th>
<th>MVM + placebo (n 101)</th>
<th>MVM + Zn (n 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>50·2 (48·5, 51·9)</td>
<td>51·6 (49·6, 53·6)</td>
<td>50·6 (49·1, 52·2)</td>
<td>50·7 (49·3, 52·2)</td>
</tr>
<tr>
<td>7 months</td>
<td>57·9 (55·8, 60·0)</td>
<td>56·2 (54·6, 58·0)</td>
<td>57·2 (55·6, 58·7)</td>
<td>59·4 (57·8, 61·0)</td>
</tr>
<tr>
<td>Change‡</td>
<td>6·28 (5·26, 7·30)</td>
<td>6·07 (5·01, 7·13)</td>
<td>6·58 (5·76, 7·39)</td>
<td>8·65 (7·42, 9·88)</td>
</tr>
<tr>
<td>Difference§</td>
<td>−0·21 (−1·66, 1·29)</td>
<td>0·30 (−1·14, 1·73)</td>
<td>2·37* (0·91, 3·83)</td>
<td></td>
</tr>
</tbody>
</table>

Mean value was statistically different from the placebo + placebo group: *\(P = 0.002\).
† For details of procedures, see pp. 763–764.
‡ Change (7 months – baseline); t test.
§ Difference (each active group – placebo + placebo). Linear regression analysis, interaction between interventions, \(P = 0.03\).
The deteriorating TB treatment outcome in the wake of the HIV pandemic is a major concern (UNAIDS, 2004; World Health Organization, 2004), and new adjunctive interventions are needed (Harries et al. 2001). TB patients have considerable weight loss and vitamin and mineral deficiencies at the time of diagnosis (Kennedy et al. 1996; Karyadi et al. 2000; Mugusi et al. 2003). Despite appropriate TB treatment, it is unlikely that a patient will be able to regain normal body weight or achieve nutritional status equivalent to a healthy person, even after successful completion of treatment. For HIV-positive patients, supplementation with antioxidants, vitamins, and minerals may help to fill the immunological and nutritional gaps that are created by the concomitant diseases.

### Table 5. Baseline characteristics of the 422 patients followed up for survival status at 8 months compared to 77 not followed up†

<table>
<thead>
<tr>
<th>Survival status</th>
<th>Followed up (n 422)</th>
<th>Not followed up (n 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td>n</td>
</tr>
<tr>
<td>Female sex</td>
<td>40·8</td>
<td>172</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35·6</td>
<td>34·5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18·2</td>
<td>18·0</td>
</tr>
<tr>
<td>HIV (g/l)</td>
<td>104·7</td>
<td>102·4</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>33·2</td>
<td>28·7</td>
</tr>
<tr>
<td>Smoking</td>
<td>10·9</td>
<td>8·1</td>
</tr>
<tr>
<td>Infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture intensity‡</td>
<td>42·9</td>
<td>181</td>
</tr>
<tr>
<td>Viral load</td>
<td>3·95</td>
<td>3·79</td>
</tr>
<tr>
<td>CD4 count (cells/μl)§</td>
<td>406</td>
<td>356</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>34·7</td>
<td>146</td>
</tr>
<tr>
<td>Hookworm</td>
<td>19·5</td>
<td>15·8</td>
</tr>
<tr>
<td>Malaria parasitaemia</td>
<td>4·5</td>
<td>2·7</td>
</tr>
<tr>
<td>Study intervention*</td>
<td>25·4</td>
<td>107</td>
</tr>
<tr>
<td>Placebo/placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo/MVM</td>
<td>23·7</td>
<td>100</td>
</tr>
<tr>
<td>Zn/MVM</td>
<td>24·4</td>
<td>103</td>
</tr>
<tr>
<td>Placebo/MVM</td>
<td>23·7</td>
<td>100</td>
</tr>
<tr>
<td>Zn/MVM</td>
<td>24·4</td>
<td>103</td>
</tr>
</tbody>
</table>

MVM, multi-vitamin/mineral.
*Distribution between intervention groups was significantly different between those followed up and those not followed up.
† For details of procedures, see pp. 763–764.
‡ Twenty-two were only microscopy-positive: seven were culture-negative, and culture data were missing for fifteen.
§ Among HIV-infected only.
★ Test for interactions: MVM £ Zn,
★ Test for interactions: MVM £ Zn £ HIV,
P = 0·03; MVM £ Zn £ HIV £ P = 0·45.
†† Test for interactions: MVM x Zn, P = 0·66.
‡‡ Test for interactions: MVM x Zn, P = 0·30.

### Discussion

The deteriorating TB treatment outcome in the wake of the HIV pandemic is a major concern (UNAIDS, 2004; World Health Organization, 2004), and new adjunctive interventions are needed (Harries et al. 2001). TB patients have considerable weight loss and vitamin and mineral deficiencies at the time of diagnosis (Kennedy et al. 1996; Karyadi et al. 2000; Mugusi et al. 2003). Despite appropriate TB treatment, it is unlikely that a patient will be able to regain normal body weight or achieve nutritional status equivalent to a healthy person, even after successful completion of treatment. For HIV-positive patients, supplementation with antioxidants, vitamins, and minerals may help to fill the immunological and nutritional gaps that are created by the concomitant diseases.

### Table 6. The effects of different combinations of zinc and multi-vitamin/mineral (MVM) supplementation on cumulative mortality by 8 months among 422 pulmonary tuberculosis patients†

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>Placebo + placebo</th>
<th>Placebo + Zn</th>
<th>MVM + placebo</th>
<th>MVM + Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n 422)</td>
<td>n 107</td>
<td>n 112</td>
<td>n 100</td>
<td>n 103</td>
</tr>
<tr>
<td>No. of deaths (n 52)</td>
<td>16</td>
<td>15</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>15·0</td>
<td>13·4</td>
<td>13·0</td>
<td>7·8</td>
</tr>
<tr>
<td>RR (95 % CI)‡</td>
<td>0·90 (0·47, 1·72)</td>
<td>0·67 (0·44, 1·71)</td>
<td>0·52 (0·23, 1·16)</td>
<td></td>
</tr>
<tr>
<td>HIV-negative (n 241)</td>
<td>n 65</td>
<td>n 60</td>
<td>n 55</td>
<td>n 61</td>
</tr>
<tr>
<td>No. of deaths (n 10)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>3·2</td>
<td>3·3</td>
<td>3·6</td>
<td>6·6</td>
</tr>
<tr>
<td>RR (95 % CI)‡</td>
<td>1·08 (0·16, 7·45)</td>
<td>1·18 (0·17, 8·12)</td>
<td>2·13 (0·40, 11·21)</td>
<td></td>
</tr>
<tr>
<td>HIV-positive (n 181)</td>
<td>n 42</td>
<td>n 52</td>
<td>n 45</td>
<td>n 42</td>
</tr>
<tr>
<td>No. of deaths (n 42)</td>
<td>14</td>
<td>13</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>33·3</td>
<td>25·0</td>
<td>24·4</td>
<td>9·5</td>
</tr>
<tr>
<td>RR (95 % CI)‡</td>
<td>0·75 (0·40, 1·42)</td>
<td>0·73 (0·38, 1·43)</td>
<td>0·29* (0·10, 0·80)</td>
<td></td>
</tr>
</tbody>
</table>

RR, relative risk.

Significantly different from the placebo + placebo group: *P = 0·016.
† For details of procedures, see pp. 763–764.
‡ Test for interactions: MVM x Zn, P = 0·75; MVM x HIV, P = 0·02; Zn x HIV, P = 0·03; MVM x Zn x HIV, P = 0·45.
§ Test for interactions: MVM x Zn, P = 0·66.
‖ Test for interactions: MVM x Zn, P = 0·30.
weight and vitamin and mineral status on a typical diet based on cereals, tubers or legumes, which has low content and bioavailability of vitamins and minerals (Ramakrishnan & Huffman, 2001). The vitamin and mineral deficiencies may impair full restoration of lean body mass and immune functions, and survival.

We therefore assessed the effect of adjunctive Zn and MVM supplementation given throughout the 8 months of TB treatment. As previously reported, we found no beneficial effect on the primary outcome, sputum conversion which was assessed over the 2-month intensive phase (Range et al. 2005). However, effects on weight gain up to 7-month follow-up, and all-cause mortality during the full 8 months of treatment and supplementation were also assessed.

There were no overall reductions in mortality with Zn and MVM supplementation. With only ten deaths, the effect of the study interventions could not be assessed among TB patients not infected with HIV. However, among HIV co-infected patients, both Zn and MVM supplementation were associated with roughly a halving of the risk of death, although not statistically significant, but those receiving the combined supplement had a significant 70% reduction in risk of death. Due to the deviating interaction ratios it may be considered justified to report the effect of the combined interventions despite the lack of statistical interaction between interventions, since power to detect interactions are often lacking in factorial trials (McAlister et al. 2003).

Survival status could only be ascertained for 85% of the study participants for the full 8-month treatment period. Nevertheless, the median observation time for those lost to follow-up was above 6 months, and they were similar to those followed up with respect to age, sex, body composition, and the proportion with heavy culture intensity and HIV co-infection, although the mean HIV viral load among infected participants was lower. Among those lost to follow-up, however, a greater proportion was allocated to MVM and a lower proportion to Zn. If the mortality rate among those lost to follow-up was the same irrespective of treatment allocation, then the effect of MVM would be overestimated and the effect of Zn supplementation underestimated. The proportion receiving both Zn and MVM was not different among those lost to follow-up compared to those followed up, so the risk of selection bias seems to be small, and not able to explain the findings. Both patient and investigator were blinded as to the treatment allocation, and data analysis was done prior to breaking the code, based on intention-to-treat. Compliance was not systematically assessed, but the supplement was given together with the TB drugs, i.e. under direct observation during the first 2 months and by self-administration during the last 6 months.

This is the first reported randomised trial on the effects of MVM supplementation on mortality among TB patients. However, the effects seen among HIV-infected individuals are in accordance with recent data from randomised trials among HIV-infected individuals in Thailand and Tanzania. The trial from Thailand showed that a daily MVM supplement may reduce mortality among HIV-infected individuals with low CD4 cell counts. The supplement containing twenty-one vitamins and minerals in multiples of RDA was given daily to 481 HIV-infected individuals with CD4 counts between 50 and 550 (10\(^9\) cells/l; Jiamton et al. 2003). After 48 weeks, seventy-nine (16%) were lost to follow-up and twenty-three (5%) had died. The death rate was lower in those allocated to MVM (mortality hazard ratio 0.53; 95% CI 0.22, 1.25), but only significantly so among those with CD4 counts below 100 (0.26; 95% CI 0.07, 0.97). There was no effect of the intervention on viral load. Similarly, a recent randomised, two-by-two factorial trial among 1078 pregnant HIV-infected women from Dar es Salaam, Tanzania, assessed the effect of a daily vitamin A and multivitamin supplement given throughout pregnancy and for several years after. While vitamin A supplementation increased mother-to-child HIV transmission, multivitamin supplementation increased maternal CD4 cell counts (Fawzi et al. 1999), reduced mother-to-child HIV transmission in subgroups (Fawzi et al. 2002), and also reduced viral load and risk of AIDS and AIDS-related deaths among the mothers themselves (Fawzi et al. 2004).

The supplement we used was similar to the supplement given to pregnant HIV-infected women in Dar es Salaam (Fawzi et al. 1999), but with more vitamin E and less vitamin C, but with addition of vitamin D and preformed vitamin A, as well as selenium and copper. We were particularly interested in the mineral Zn, since it is known to be essential to the immune system (Shankar & Prasad, 1998) and host defence to respiratory tract and other infectious diseases (Bhutta et al. 1999, 2000). Therefore, and due to the concern that Zn may increase progression of HIV in US patients (Tang et al. 1993, 1996), we assessed the effect of Zn separately, using a two-by-two factorial design. The dose of Zn (45 mg) was considered appropriate, given the high anti-nutrient content of a typical diet in developing countries, and the high requirements during synthesis of lean body mass.

As in the trial in Bangkok, but in contrast to that in Dar es Salaam, the effect of our intervention was apparently not mediated by reduced viral replication, although viral load was only measured over the first 2 months. In contrast, the effect on survival was accompanied by considerable effects on weight gain. Interestingly, neither Zn nor MVM alone had any effects on weight gain, whereas the combined intervention increased weight gain by almost 2.5 kg. This is plausible, since several nutrients are required for the synthesis of new tissues (Golden, 1992).
Supplementation and tuberculosis mortality 769

Vitamin and mineral supplementation may considerably increase survival during treatment of sputum-positive TB patients co-infected with HIV. Nevertheless, the finding should be confirmed, because the effect is only seen on secondary outcomes in a subgroup of patients, and because compliance data are not available. Also, the effects may differ between different settings, due to interactions with other nutrients and co-infections. The effect should also be assessed among sputum-positive TB patients without HIV co-infection, and among sputum-negative pulmonary TB patients.

The cost is around US$8 for the full supply of supplements, which can easily be delivered to the patients together with TB drugs. If proven beneficial, then MVM supplementation could be a feasible and cost-effective adjunctive intervention during TB treatment.

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References


