Efficacy of a Single Oral Dose of 200,000 IU of Oil-soluble Vitamin A in Measles-associated Morbidity

Francisco J. Rosales, 1 Chris Kjolhede, 1 and Steven Goodman 2 3

The authors assessed the efficacy of the World Health Organization (WHO) recommendation of 200,000 IU of vitamin A in oil to treat acute non-xerophthalmic measles patients. Acute measles patients who did not require hospitalization were enrolled in a randomized, double-masked, clinical trial of vitamin A (n = 90) versus placebo (n = 110) carried out in Ndola, Zambia, in 1991. Measles-associated morbidity was defined by the presence of signs and symptoms of acute respiratory infection. Daily evaluations for the first 3 days were followed by weekly visits for a month at urban health centers. Baseline demographic, clinical, and biochemical characteristics were similar in both groups. Cross-sectional analysis of morbidity status, by group, at each weekly evaluation showed no significant differences until week 4, when more placebo-treated patients had cough or pneumonia (p = 0.005). However, longitudinal analysis, which looked at changes among individuals and controlled for initial health status, showed more equivocal results. The odds ratio for the development of pneumonia in patients with measles cough in vitamin A-treated subjects was 0.73 (95% confidence interval [CI] 0.30–1.80). The odds ratio for the development of measles-associated cough or pneumonia in asymptomatic measles patients was 0.52 (95% CI 0.24–1.13), in favor of vitamin A, but the odds ratio for failing to improve from pneumonia in vitamin A-treated subjects was 1.23 (95% CI 0.68–2.3), a result in favor of placebo. These results suggest that the evidence for the efficacy of one dose of vitamin A in oil to prevent measles complications is not as strong as that previously shown for two 200,000 IU doses of water-miscible vitamin A, and that the WHO recommendation may need to be reexamined. Am J Epidemiol 1996;143:413–22.

Supplementation with two oral doses of 200,000 IU of water-miscible vitamin A during measles infection significantly reduces measles morbidity and mortality (1). However, the World Health Organization (WHO) and United Nations International Children’s Emergency Fund (UNICEF) have jointly recommended administration of a single oral dose of 200,000 IU (or 100,000 IU in infants) of vitamin A, at the time of initial measles diagnosis, to non-xerophthalmic children who live in areas where measles case-fatality rates are greater than 1 percent (2). Recently, the American Academy of Pediatrics has recommended that US physicians follow the WHO guidelines in the treatment of children with measles (3). Unfortunately, the efficacy of a single oral dose (200,000 IU) of vitamin A in oil has never been determined.
of this lack of supporting evidence, some researchers have questioned the efficacy of the WHO/UNICEF recommendation (4), and others have suggested that it may be used in treating acute measles patients who do not require hospitalization (5).

This study was conducted to assess whether or not a single oral dose of vitamin A in oil would reduce measles-associated morbidity in measles patients who do not require hospitalization.

MATERIALS AND METHODS

Study design

The study was a randomized, doubled-masked, placebo controlled clinical trial conducted at urban health centers in Ndola, Zambia. A 1:1 randomization scheme was used to allocate vitamin A or placebo treatment. Four multidose dispenser bottles that each dispensed on average 1.06 ml were used to deliver study treatments. Two of the bottles contained vitamin A in oil, 190,000 IU per ml, and vitamin E (40 mg/ml) (Hoffmann La-Roche, Basel, Switzerland). The other two bottles had a local vegetable oil with <0.2 IU per ml of vitamin A. The vitamin A content in the bottles was determined independently by Lancaster Laboratories Inc., in Lancaster, Pennsylvania. Allocation of bottle codes for each series of 100 subject identification numbers was accomplished using a table of random numbers. The physician would supply the bottle code for each consecutive identification number, and a nurse would administer the assigned treatment to the child under the supervision of a study team member.

All procedures were approved by the Ethical Committee of the Tropical Diseases Research Centre in Zambia, and by the Committee on Human Volunteers, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.

Subject eligibility and standard treatment

Children with recent onset of measles (i.e., children with clinical signs and symptoms which indicated prodromal or effervescent measles) were eligible to participate in this study once their parents provided signed informed consents. Children diagnosed with measles by clinical officers were evaluated by a study physician to confirm measles diagnosis based on the Centers for Disease Control recommended clinical definitions for suspected, probable, and confirmed cases (6). Two weeks after baseline evaluation, these clinical definitions were confirmed by a fourfold increase in measles antibody titer among surviving patients. Measles cases were evaluated by a physician, including an eye examination. Subsequently, those children who required hospitalization based on WHO recommendations for acute respiratory infection (i.e., children with difficult breathing or impaired feeding) (7), or for diarrhea (i.e., children with severe dehydration) (8) were treated with vitamin A (200,000 IU in oil) and referred to the local hospital. In addition, measles cases with xerophthalmia, severe undernutrition defined by mid-upper-arm-circumference (MUAC) <12.5 cm, or those whose parents refused to participate in the study were immediately treated with vitamin A (200,000 IU in oil), and referred to the hospital as indicated. These patients were excluded from the study.

At baseline, subjects were provided standard treatment as recommended by the Essential Drug Programme Manual for Health Workers in Zambia (9). Subjects received aspirin or paracetamol, tetracycline eye ointment, intramuscular procaine penicillin injections for 5 days, oral rehydration salts if necessary, gentian violet (topical treatment for mouth ulcers), and a children’s cough mixture.

Morbidity definitions

Diarrhea was defined as >3 liquid or loose stools/day (8). Croup-like syndrome included the presence of stridor or a hacking/barking cough (7). Clinical status for acute respiratory infection (ARI) was categorized as “asymptomatic,” “cough alone,” or “pneumonia.” Pneumonia was defined with the use of diagnostic algorithms that included cough and a respiratory rate above an age-specific threshold. For children 6–60 months of age, respiratory thresholds recommended by WHO were used (7). For age groups 60–120 months, the threshold respiratory rate was >30/minute, and for age groups older than 120 months it was >20/minute. These were determined by obtaining the observed median respiratory rate value for each age group at baseline, and comparing them with reported respiratory rates in children older than 60 months with asthma or cystic fibrosis before and after standard treatment (10, 11). The clinical significance of these thresholds is that they are highly correlated with airway obstruction, hyperinflation, and poor oxygenation (10, 11). The advantage of using these clinical definitions is that they can be used without sophisticated training and equipment, and employed by physicians and non-physicians without losing reliability (12).

Measles morbidity and measles-associated morbidity were defined based on their time of occurrence from enrollment (acute measles). That is, any respiratory infection, diarrheal episode, or croup-like syndrome that occurred within one week of enrollment was considered “measles morbidity” or caused by measles virus, and thereafter as “measles-associated
morbidity,” or due to secondary infections (13). This classification controlled for possible etiologic differences in morbidity between the first week after rash onset and subsequent weeks during convalescence. “Measles mortality” was defined as any death that was not related to cholera or human immunodeficiency virus (HIV) infection that occurred during the month-long observation period.

Procedures and measurements

Morbidity evaluation. Enrolled subjects were examined daily for 3 days during the first week, and then weekly for four additional weeks. These evaluations included a week interim history for cough, diarrheal stool counts, and treatment compliance (e.g., feeding practices and eye ointment applications). In addition, a physical examination was conducted to assess respiratory function, hydration, and nutritional status. These examinations were performed by study personnel. Any time that enrolled patients developed signs or symptoms of severe disease (7, 8) they were given vitamin A (200,000 IU in oil) and referred to the local hospital. They were included in the analysis until hospital referral. At the end of one-month follow-up, each child received a large single dose of vitamin A (200,000 IU) and an eye examination which included a conjunctival impression cytology sample.

Blood samples and serum retinol. At baseline and 2 weeks later, patients had 5 ml of blood drawn for determinations of retinol and measles antibody titer. This sample was protected from light and placed inside a cold-box at 8°C and allowed to coagulate. Within hours, serum was separated at the Tropical Diseases Research Center, and stored at -20°C. Serum retinol levels were determined by high pressure liquid chromatography using the methodology recommended by Bieri et al. (14).

Antibody titer. Measles hemagglutination inhibition test (HAI) was used to determine measles antibody titer as previously reported (15). A fourfold increase in titer, from baseline to week 2, was used to further confirm measles diagnosis.

Anthropometric measurements

Patients were weighed at enrollment and at each weekly visit with a spring dial CMS scale (Weighing Equipment Limited, London, England) to the nearest 0.1 kg, and a Seca floor scale (Seca Corporation, Columbia, Maryland) to nearest 0.5 kg for children heavier than 25 kg (16). The rate of weight change for the 4-week follow-up was determined by the slope of the regression line of weekly body weight on time.

Statistical analyses

The t-test statistic was used to assess the difference between independent means. Non-parametric and Fisher’s exact tests were used when necessary. For categorical variables, the difference in proportions was assessed by chi-square statistics (17).

Measles morbidity and measles-associated morbidity were assessed based on ARI clinical status. This was done because the majority of patients with diarrhea also had ARI at baseline, and, subsequently, their number was markedly reduced compared with patients who suffered from cough or pneumonia alone; however, they remained similarly distributed between the treatment groups (see table 2). Thus, the effect of a single dose of vitamin A was assessed by comparing weekly distributions of subjects with ARI by treatment groups using chi-square statistics.

A Markov chain analysis of disease progression was used to examine the probabilities of clinical improvement or worsening within each week (18). For each visit, ARI status for each subject was categorized as “asymptomatic,” “cough only,” or “pneumonia.” From each of these states, a subject could have a transition—a change in health status. If the initial status was “asymptomatic,” a transition (to cough or pneumonia) always represented worsening. If the initial state was “pneumonia,” a transition (to cough or asymptomatic status) always indicated improvement. If the initial state was “cough,” a transition could go in either direction, either worsening to “pneumonia” or improving to “asymptomatic.”

These groupings allowed each weekly change to be represented as three 2 × 2 tables, with the rows representing the two treatment groups, and the columns, the nature of the health transition. One table was created for each of the three baseline states. For asymptomatic patients, an odds ratio was calculated from a 2 × 2 table that contrasted the probability of worsening versus staying asymptomatic. For pneumonia patients, the odds ratio represented the odds of remaining with pneumonia divided by the odds of improving. For patients with cough, the analysis was carried out in two ways. The primary analysis was done only among those subjects whose health status changed (i.e., excluding those who remained with a cough), so the odds ratio represented the contrast between the odds of worsening and the odds of improving. A second analysis grouped together those who remained with a cough with those who improved to an asymptomatic state, so the odds ratio represented the odds of worsening versus not worsening. There were not enough data in this study to implement a second order Markov model, which would have included both the initial state and the preceding one.
Finally, logistic regression models (19) were used to combine these odds ratios across weeks, and to adjust for confounders. The confounders adjusted for included age, rate of weight change (i.e., the slope of the regression line of weekly body weight on time), or a more restrictive measles definition (i.e., patients with a fourfold increment in antibody titer). This produced three summary odds ratios, one for each health state. An odds ratio of 0.8 for an initial state of "asymptomatic" would mean that patients on vitamin A had, across all weeks, an average odds of worsening that was 80 percent of the odds of worsening for those on placebo.

Only those variables that showed a substantive change in the estimate of the effect of vitamin A or a significant change in the likelihood ratio of the logistic regression model were included in the multivariate model. One model assumption was that the relative odds of response was independent of which week was examined. This assumption was evaluated by inspection of the weekly odds ratios, and by including an interaction term for treatment and week in the model. Another assumption is that, after adjustment for the initial health state, transitions of the same individual at different times were independent.

Data were analyzed using SPSSPC+/4.0 software (SPSS Inc., Chicago, Illinois), and SAS 6.0 (SAS Institute Inc., Cary, North Carolina).

RESULTS
Enrollment and baseline characteristics

The study lasted for 7 months, during which time 260 clinical measles cases were identified by clinical personnel. However, 59 were not enrolled for various reasons, including 13 with a desquamating measles rash (i.e., non-acute measles cases) and 15 with severe undernutrition (MUAC <12.5 cm). Another three cases had a recent history of vitamin A intake, six lived outside Ndola city, and six were severely ill children. Sixteen cases were excluded because parents refused to participate in the study or they had incomplete baseline information, but none of the 59 had clinical vitamin A deficiency (i.e., xerophthalmia). Of 201 measles cases whose baseline blood was collected, five were positive for HIV by both enzyme-linked immunosorbent assay (ELISA) and Western blot test (three in the vitamin A group and two in the placebo group). Four of the HIV-positive measles cases were infants younger than age 9 months and were included (one died during the study), and the other HIV-positive case was a young man who was excluded from the study, leaving two HIV-positive cases in the vitamin A group.

Thus, 200 children with acute measles were enrolled in this study. Of the 200 children, six children missed more than half their weekly visits, giving a 3 percent dropout rate. Another 10 children missed one or two visits; these were included in the analysis. The characteristics of the 200 enrolled patients are displayed in table 1. Demographic risk factors for severe measles, such as age at infection in infants, undernutrition, and measles secondary cases were balanced between the treatment groups at the time of enrollment. Clinical characteristics and immunologic status related to measles infection were also approximately equal in the treatment groups, and they showed that these children were acute measles patients. The geometric mean titer was 1:15 and 1:14 in the placebo and vitamin A groups, respectively, at baseline. Two weeks later, the geometric means were 1:73 and 1:62 in placebo and vitamin A groups, respectively. The change in antibody titer, from enrollment to week 2, increased significantly (paired t-test, p < 0.01) within each group, but the titer at week 2 was not significantly different between the groups (15).

### TABLE 1. Distribution of baseline characteristics, by treatment group, in a randomized, double-masked, clinical trial of vitamin A versus placebo, urban health centers, Ndola, Zambia, 1991*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 110)</th>
<th>Vitamin A (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>44.5</td>
<td>44.4</td>
</tr>
<tr>
<td>Age in months (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>30.0</td>
<td>35.2</td>
</tr>
<tr>
<td>12 and &lt;60</td>
<td>42.7</td>
<td>38.5</td>
</tr>
<tr>
<td>&gt;60</td>
<td>27.3</td>
<td>26.4</td>
</tr>
<tr>
<td><strong>Undernutrition (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-age &lt;−2 Z-score†</td>
<td>35.5</td>
<td>35.6</td>
</tr>
<tr>
<td>Measles cases (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>15.0</td>
<td>17.0</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal temperature °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.8 ± 0.2†</td>
<td>38.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Rash (%)</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>Mean day of rash onset</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Koplik spots (%)</td>
<td>30.9</td>
<td>35.6</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol*§ (µg/dl)</td>
<td>11.90 ± 1.05</td>
<td>12.75 ± 1.06</td>
</tr>
<tr>
<td>HAI II (ln of 1/titer)</td>
<td>2.75 ± 0.09</td>
<td>2.62 ± 0.08</td>
</tr>
</tbody>
</table>

* No contrasts were statistically significant. n, number of patients in treatment group.
† Z-score distribution was calculated using Centers for Disease Control and Prevention Anthropometric Software (CDC, Atlanta, Georgia). A cut-off value of <−2 Z-score was considered undernutrition.
‡ Mean ± standard error.
§ Retinol, normal value >20 µg/dl.
II HAI, hemagglutination inhibition test for antibody titer to measles hemagglutinin protein.

The vitamin A status of all the children enrolled in this study showed that these patients were suffering from measles-induced hyporetinemia (i.e., serum retinol <20 μg/dl) (table 1). The mean serum retinol at baseline, however, was not significantly different between the groups. Two weeks later, the serum retinol levels increased significantly in both groups. In the placebo group (n = 77), serum retinol (μg/dl) was 18.96 + 1.07 (mean ± standard error (SE)) versus 21.63 + 1.07 in the vitamin A group (n = 78). The increment was higher among vitamin A-treated patients at week 2 than in placebo-treated children, but it did not reach statistical significance (unpaired t-test, p = 0.18). The increment of serum retinol concentration among placebo-treated patients during convalescence was analogous to that observed by others in placebo-treated measles patients (20).

**Morbidity**

The temporal distribution of morbidities by treatment group is shown in table 2. At baseline, the majority of children with acute measles suffered from more than one morbidity, e.g., diarrhea with cough or pneumonia. The rest had either cough or pneumonia alone, and very few had diarrhea (1–3 percent) or croup alone. During the following weeks, the percentage of patients who suffered from diarrhea associated with ARI was minimal compared with those who suffered from cough or pneumonia alone. Therefore, the assessment of vitamin A supplementation on measles-associated morbidity was solely based on ARI clinical status.

Table 3 shows the cross-sectional analysis of ARI clinical status by week and treatment group. At baseline, the distribution of clinical status was similar between the vitamin A and placebo groups, and showed that most of the patients had either cough or pneumonia. The same was observed during subsequent 2 weeks. By week 3, most patients were asymptomatic, 81 percent in the vitamin A group versus 74 percent in the placebo group; however, this difference did not reach statistical significance. By week 4, the major differences between the treatment groups were among asymptomatic patients and those with pneumonia (p = 0.005) in favor of vitamin A supplementation.

However, this cross-sectional analysis did not provide information on whether the significant difference at week 4 was due to a reduced number of patients with pneumonia, or to an increased number of asymptomatic patients at the end of the study, and did not take into account the previous status of patients that reached these states. Thus, to examine the effect of treatment on the movement of individual patients between health states, and to assess whether or not there was a trend throughout the study, a longitudinal analysis that incorporated the transition probabilities between ARI clinical statuses was used.

By time period, the numbers of pairs of observation in vitamin A- and placebo-treated patients, respectively, were as follows: from enrollment to the week 1 visit, 79 and 90 pairs; from week 1 to week 2, 78 and 87 pairs; from week 2 to week 3, 75 and 82 pairs, and from week 3 to week 4, 73 and 82 pairs.

The chance of not improving from measles-associated pneumonia is illustrated in figure 1. Each bar represents the probability, if one had pneumonia at the start of an interval, of ending the interval with pneumonia. For example, of the 62 children with pneumonia at baseline in the placebo group, 38.7 percent still had pneumonia at week 1, in contrast to 47.9 percent of the 48 with pneumonia at baseline in the vitamin A group. This corresponds to an odds ratio of about 1.4 (95 percent confidence interval (CI) 0.7–3.1) in favor of placebo for the period of the first week for patients with pneumonia at enrollment. The aver-

---

**TABLE 2.** Percentage of patients with single or multiple morbidities from enrollment to week 4, by treatment group, in a randomized, double-masked trial of vitamin A versus placebo, urban health centers, Ndola, Zambia, 1991

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Baseline (n = 90)</th>
<th>Week 1 (n = 90)</th>
<th>Week 2 (n = 78)</th>
<th>Week 3 (n = 75)</th>
<th>Week 4 (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diarrhea</strong></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>Cough</strong></td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td><strong>Pneumonia</strong></td>
<td>27</td>
<td>38</td>
<td>39</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td><strong>Croup</strong></td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Diarrhea + ARI</strong></td>
<td>42</td>
<td>38</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><strong>Fever, coryza, and conjunctivitis</strong></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Asymptomatic</strong></td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>22</td>
<td>48</td>
</tr>
</tbody>
</table>

* n, number of patients per treatment group.
† Only one morbidity.
‡ ARI, acute respiratory infection clinical status (cough or pneumonia).
TABLE 3. Cross-sectional distribution (in percent) of acute respiratory infection (ARI) status, by treatment group, in a randomized, double-masked trial of vitamin A versus placebo, urban health centers, Ndola, Zambia, 1991

<table>
<thead>
<tr>
<th>ARI status</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin A</td>
<td>Placebo</td>
<td>Vitamin A</td>
<td>Placebo</td>
<td>Vitamin A</td>
</tr>
<tr>
<td></td>
<td>(n = 90)*</td>
<td>(n = 110)</td>
<td>(n = 75)</td>
<td>(n = 90)</td>
<td>(n = 75)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>3</td>
<td>1</td>
<td>22</td>
<td>23</td>
<td>49</td>
</tr>
<tr>
<td>Cough</td>
<td>33</td>
<td>31</td>
<td>42</td>
<td>41</td>
<td>19</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>63</td>
<td>68</td>
<td>37</td>
<td>36</td>
<td>32</td>
</tr>
</tbody>
</table>

*p, number of patients per treatment group.  
† p value indicates the significance of the difference between vitamin A-treated and placebo measles patients, a chi-square statistic with 2 degrees of freedom.

FIGURE 1. The chance of not improving from measles-associated pneumonia during each weekly interval, by treatment group: urban health centers, Ndola, Zambia, 1991. A change from pneumonia to cough or asymptomatic state represented improvement. Higher bars represent unfavorable outcomes. Numbers in parentheses indicate numbers of subjects at risk.

age odds ratio for remaining with pneumonia over the three intervals was 1.2 (95 percent CI 0.68–2.3), after adjusting for age and the rate of change in body weight, an effect in the direction of placebo advantage.

Figure 2 shows the risk of developing measles-associated pneumonia among patients with cough who changed health status. From baseline to week 1, the majority of patients who changed their status developed pneumonia, eight of 13 patients in the placebo group versus five of 11 patients in the vitamin A group, while in the last intervals, most patients who changed became asymptomatic (from week 2 to week 3, six of seven subjects in the placebo group, 13 of 13 in the vitamin A group). Vitamin A-treated patients appeared to have a lower risk of changing to pneumonia than placebo patients. The odds ratio based on the estimate from the logistic regression comparing the three intervals, adjusted for age and change in body weight, was 0.73 (95 percent CI 0.30–1.80). The analysis performed to compare those who worsened to pneumonia with those who remained with cough or who improved showed almost the identical result.

Figure 3 illustrates the risk of developing any measles-associated morbidity among those who were asymptomatic at the beginning of a week. In comparison with table 3, which shows that the percentage of asymptomatic children increased steadily with each interval, figure 3 provides a more comprehensive picture of the probability of worsening. It indicates that among the children who became asymptomatic by week 1, about 40 percent relapsed by week 2; however, after week 2, most patients (around 90 percent) tended to remain asymptomatic. Vitamin A-treated children had a lower risk of relapsing than placebo children. The odds ratio for developing ARI morbidity was 0.52 (95 percent CI 0.24–1.13, p = 0.09) after
adj)usting for age and the rate of change in body weight.

Because the cross-sectional analysis showed a large shift between weeks 3 and 4, we examined in detail what occurred in that interval. Among the eight children with pneumonia in the placebo group at week 3, two improved, but six still had pneumonia at week 4. In addition, of the 60 patients who were asymptomatic at week 3, four developed pneumonia by week 4, for a total of 10 pneumonia cases in the placebo group at the end of the 4 weeks (table 3). In contrast, in the vitamin A group, all three with pneumonia at week 3
became asymptomatic by week 4, and none of the 60 asymptomatic children developed pneumonia. The Fisher’s exact test, for the improvement from pneumonia from week 3 to week 4 was marginally significant at \( p = 0.06 \) in favor of vitamin A, although this pattern was not seen in the first 2 weeks (figure 1). The \( p \) value for worsening from an asymptomatic status from week 3 to week 4 was \( p = 0.21 \), in favor of vitamin A (figure 3). Thus, the week 4 results derived from a few pneumonia patients in the placebo group that did not get better, and a few asymptomatic patients in that group who developed pneumonia. Because these patterns involved small numbers of subjects, and were not entirely consistent with the patterns seen in earlier weeks, the longitudinal analysis suggests that the evidence for vitamin A benefit is weaker than the cross-sectional analysis indicates.

Severe morbidity and mortality

A total of 14 subjects were referred to the local hospital after enrollment because of severe measles. These subjects were divided equally between the two groups. There were 21 deaths; seven deaths were related to cholera, one to HIV infection, and the rest were measles-associated. Among the latter, seven occurred in the placebo group and six in the vitamin A group, for a case fatality rate of 6.5 percent.

DISCUSSION

To our knowledge, this is the largest study reported so far among non-hospitalized patients on the effect of vitamin A treatment of measles infection. The clinical outcomes were rigorously defined and measured. However, the results are far more equivocal than those seen in several other, smaller studies. While a cross-sectional analysis seemed to indicate a significant vitamin A benefit, none of the three longitudinal analyses showed a statistically significant degree of benefit, and one of the three even had a point estimate in the direction of vitamin A harm, unlikely though that is. This type of analysis is very important, because it enables us to obtain a more in-depth look at the course of disease and possible therapeutic benefit, and to link the results with prior knowledge about pathogenesis. It has been suggested that the efficacy of vitamin A depends on the relative severity of disease (21), and this analysis allowed us to examine separately the effect of vitamin A on the evolution of illness from any one of three starting states. It also allowed a separate look at the effects in acute and convalescent measles. Our discussion will focus on the reasons why such results may have occurred in this study, and on their implications.

Vitamin A treatment during measles may be beneficial either by reducing the effects of measles infection (therapeutic effect), or by preventing the subsequent development of secondary infections (protective effect), or both. Morbidity during or immediately after rash onset is presumably related to measles virus, while morbidity that occurs 1–2 weeks after rash onset may be caused by secondary bacterial infections (13).

Previous studies generally have found a greater therapeutic effect than we observed. Barclay et al. (22) studied and followed 180 acute measles cases during their hospitalization, including some less than 9 months and others older than 5 years. Eighty-eight of the enrolled patients in the study by Barclay et al. received 200,000 IU of vitamin A in oil per day for 2 days. These authors found a 50 percent reduction of mortality in vitamin A-treated subjects. Hussey and Klein (1) studied 189 children aged less than 13 years with acute measles, who had illness severe enough to require hospitalization. Ninety-two received 200,000 IU of water-miscible vitamin A on each of 2 days, and were followed for their in-hospital stay. Hussey and Klein (1) observed a 60 percent reduction of measles-associated morbidity (i.e., pneumonia or diarrhea) after 10 days posttreatment with vitamin A. Coutsoudis et al. (23) enrolled 60 acute measles cases between 4 and 24 months of age who had illness severe enough to warrant hospitalization. Twenty-nine received 200,000 IU (infants, 100,000 IU) of water-miscible vitamin A on days 1, 2, and 8 of their hospitalization, and another dose at day 42 post-discharge. The researchers evaluated these children during their in-hospital stay and at 6 weeks and 6 months post-discharge. They found a 90 percent reduction of measles morbidity (i.e., pneumonia and diarrhea combined) after 7 days posttreatment.

Previous studies differed from the present study in setting, patient follow-up, disease severity, patient age, vitamin A preparation, and analytic approach. In the earlier studies, the patients studied were in-hospital under controlled conditions, and their follow-up was relatively brief (1, 22, 23). Coutsoudis et al. (23) followed subjects for 6 months, but with only two visits during this period, and they based their inferences on morbidity status from reported histories of illness. Our study looked at ambulatory patients followed closely for one month with daily and weekly visits to urban health centers. Thus, our study reflected patient-care conditions under which the majority of measles cases are diagnosed and treated in less developed countries. From a public health perspective, the assessment of vitamin A therapy under these conditions is of great interest.
Other studies treated more severe patients (e.g., requiring hospitalization) than those seen in this study. There is the possibility that more severe clinical cases are more likely to benefit from vitamin A treatment. However, if the favorable effect of vitamin A during measles is mediated by replenishing the measles-induced hyporetinemia (i.e., plasma retinol <20 μg/dl) (1, 23), the patients in our study should have benefited from receiving vitamin A. Eighty percent of patients had serum retinol levels less than 20 μg/dl, and, among them, half had levels below 10 μg/dl. Thus, an explanation for the modest effect of vitamin A observed in this study is not that our cases were less hyporetinemic than in other studies. In any case, the evidence that the effect of vitamin A is mediated by hyporetinemia is far from clear either in this study or in other studies. The beneficial effect of vitamin A treatment observed in other studies has not been associated with undernutrition, sex, socioeconomic status, type, or etiology of morbidity, nor with vitamin A status before infection (1, 20, 22, 23).

Our study also differs from previous clinical trials in the age of enrolled patients. Most other studies have primarily studied children under 5 years of age (putatively, vitamin A is more effective in younger children) (1, 22, 23). The only study of which we are aware that could address this question demonstrated a significant reduction of measles morbidity and mortality in patients ranging from infants to 13-year-old children, but it did not find an association between vitamin A efficacy and younger age at infection, although younger children (<24 months old) were at higher risk of morbidity (1). In our study, measles patients of all ages attending an urban health center were enrolled, and there was no interaction of treatment effect with age, although the power to detect such an interaction was not high.

All other studies used a cross-sectional analysis to assess their outcomes. In this study, a cross-sectional analysis suggested a vitamin A effect by the end of the trial (p = 0.005), albeit not before then. However, when these data were examined using transition analysis, which allowed tracking of individual patients and controlling for their baseline clinical status, the pattern of recovery and illness appeared to be more complex. It showed that the risk of measles-associated morbidity, and specifically measles-associated pneumonia, was highest before week 2, whereas the chance of recovering from measles-associated pneumonia was higher after week 2. This natural progression of measles disease suggested that for vitamin A supplementation to reduce measles morbidity, it needed to exert an effect before week 2 because afterwards measles severity declined. We found no pattern of measles morbidity reduction with vitamin A supplementation in that early period. This suggests that the positive effect observed at the end of the study, in the cross-sectional analysis, could be artifactually strong.

The final possible explanation for these results is that the effect of vitamin A may depend on the dosage or preparation. In our study, measles patients received a single dose of 200,000 IU vitamin A in oil, as recommended by WHO for non-xerophthalmic measles patients (2). Vitamin A preparations in oil and in water are pharmacokinetically different. The data from studies by Srikantia and Reddy (24), Sivakumar and Reddy (25) and Reddy et al. (26) indicate that about 50 percent of a water-miscible vitamin A product is available in serum for peripheral tissues immediately after dosing, whereas from an oil-soluble preparation, only 33 percent is available. Serum retinol levels in humans are higher at 4 and 24 hours post-dosing with water-miscible vitamin A than with an oil-soluble preparation (24). Barclay et al. (22) found a 50 percent reduction in measles mortality (p = 0.13), whereas Hussey and Klein (1) showed a 80 percent reduction in measles mortality (p < 0.05). These studies used different vitamin A preparations.

Reversal of hyporetinemia during measles disease by multiple large doses of water-miscible vitamin A presumably replenishes depleted peripheral tissues, and reduces morbidity and mortality (1, 20). In our study, 200,000 IU of vitamin A in oil may not have been sufficient to raise their serum retinol, or the preparation we used (vitamin A in oil) may have been mostly stored in the liver, as indicated by Srikantia and Reddy (24). A decreased bioavailability of vitamin A in the present study is suggested by a reduced increment in serum retinol after vitamin A treatment. Serum retinol concentration increased approximately by 70 percent after vitamin A treatment; however, Coutsoudis et al. (20) observed a 215 percent increment of serum retinol concentration post-vitamin A treatment.

It is not possible to say definitely from these data whether the failure to attain statistical significance in the transition analysis was due to too few events (because this was a healthier population than previously studied), an absence of a vitamin A effect in this setting, or a vitamin A effect smaller than that found in other studies. These findings must be interpreted within the context of related trials of vitamin A during measles (1, 22, 23) and studies of the pharmacokinetics of different preparations of vitamin A (24–26). In this context, we believe they indicate that multiple doses of water-miscible vitamin A may have a greater effect than a single dose of 200,000 IU in oil, although that comparison needs to be studied directly. We
therefore feel that the current WHO recommendation may need to be reexamined. The recommendation best supported by the current evidence is that children with measles, including ambulatory cases, should receive two daily doses of 200,000 IU of water-miscible vitamin A.

ACKNOWLEDGMENTS

This research project was funded through Cooperative Agreement No. DAN-5116-A-00-8051-00 between Johns Hopkins School of Hygiene and Public Health, Division of Human Nutrition and the US Agency for International Development, Office of Nutrition. Additional support was received from the Task Force for Sight & Life, Hoffman-La Roche, Basel, Switzerland.

The authors thank Dr. Alvaro Muñoz for his advice and supervision on the use of transition analysis and Dr. George Graham for his helpful comments on the manuscript.

REFERENCES