Potential magnitude of the misclassification of a population's trace element status due to infection: example from a survey of young Peruvian children[1,2]

Kenneth H Brown, Claudio F Lanata, Michelle L Yuen, Janet M Peerson, Betzabe Butron, and Bo Lönnerdal

ABSTRACT To examine the effects of concurrent infection on population-based assessment of trace element status, we collected data on clinical signs and laboratory indicators of infection when obtaining blood for serum zinc, copper, and ferritin analyses in 153 Peruvian children aged 11–19 mo. Fifty-two (34.7%) of the children had some reported sign of infection and 43 (28.3%) had elevated C-reactive protein concentrations or leukocytosis. Children with any evidence of infection had marginally lower mean (±SD) serum zinc concentrations (7.0 ± 2.3 vs 7.5 ± 2.0 μmol/L, $P = 0.16$) and significantly greater serum copper (24.7 ± 4.7 vs 22.7 ± 4.2 μmol/L, $P = 0.006$) and serum ferritin concentrations (10.0 ± 12.9 vs 3.9 ± 4.4 μg/L, $P < 0.001$) than did those without infections. Infection caused an underestimation in the rate of low copper status by 1 percentage point and low iron status by 12 percentage points. Thus, the effect of concurrent infections is of variable magnitude and may differ by nutrient, nutritional status of the population, and prevalence and severity of infections. *Am J Clin Nutr* 1993;58:549–54.

KEY WORDS Nutritional assessment, iron deficiency, zinc deficiency, copper deficiency, nutrition and infection, trace elements

Introduction

Clinical studies of infected patients and research in both humans and laboratory animals subjected to either experimental infections or to other inflammatory stimuli have identified many metabolic responses that affect circulating concentrations of specific acute-phase proteins (1, 2). Several of these acute-phase proteins bind trace elements, and serum concentrations of zinc, copper, and iron are modified by the presence of a systemic inflammatory response (1). Despite this knowledge there is little information regarding the implications of these metabolic phenomena for the assessment of the micronutrient status of different populations. If, for example, a sizable proportion of a particular population is infected at the time of assessment of their nutritional status, the rates of apparent low or high status may be confounded by the presence of infection, potentially leading to errors in the estimated prevalence of trace element deficiencies.

These issues are especially important in developing countries, where the prevalence of infection is usually considerably higher than in more affluent ones. Moreover, suboptimal nutritional status occurs more commonly in poor communities. Thus, any misclassification bias introduced by the presence of infection could be especially problematic for the assessment of nutritional status in these settings. To explore the potential magnitude of this source of misclassification bias, we conducted the present study of trace element status in a group of high-risk children who were already enrolled in frequent morbidity surveillance. We were therefore able to relate the presence of infections, with or without concurrent laboratory evidence of a systemic inflammatory response, to differences in the serum concentrations of selected trace elements, their carrier proteins, or both. The results of these analyses are the focus of this report.

Methods

Study site and subjects

The study was carried out in Canto Grande, a low-income periurban community on the eastern limits of Lima, Peru. The physical, socioeconomic, demographic, and health characteristics of different sectors of this community have been described previously (3–6).

The 153 children between 11 and 19 mo of age who were included in the present analyses were selected from a cohort of children already enrolled in a prospective field-based comparison of an experimental oral rotavirus vaccine or placebo, which had been provided at 2 mo of age. As part of the vaccine study, the children were included in active surveillance for diarrhea and other infections, and their venous blood was drawn at selected intervals for assessment of rotavirus antibody titers. One of the samples obtained at ~1–1.5 y of age was also used for the nutritional-status assessments described herein. The overall research protocol was approved by the institutional review boards of the

[1] From the Program in International Nutrition, Department of Nutrition, University of California at Davis, and the Instituto de Investigacion Nutricional, Lima, Peru.

[2] Reprints not available. Address correspondence to KH Brown, Department of Nutrition, University of California, Davis, CA 95616-8669.

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Instituto de Investigacion Nutricional in Lima, Peru, and of the Johns Hopkins University School of Hygiene and Public Health, Baltimore.

Morbidity surveillance and anthropometry

The children were visited in their homes twice weekly by a field worker who inquired systematically about the presence of specific symptoms and signs of illness during the period since the previous visit. The surveillance methods have been described previously in detail (4, 5, 7), as has the validity of reported fever (4) and of reported poor appetite (8). The surveillance data corresponding to the day of the blood drawing were used for the present analyses.

Diarrhea was defined as the excretion of three or more liquid stools in a 24-h period. Nondiarrheal febrile illnesses were categorized separately by using parental reports of fever. The presence of anorexia, defined as appetite reportedly decreased from normal, was also analyzed independently. Specific categories of respiratory illnesses were not considered separately, both because of the difficulty in distinguishing mild upper-respiratory symptoms from other nonrespiratory systemic diseases and because of the low prevalence of lower-respiratory illness that was observed.

At the time of each clinic visit the children were weighed unclothed by using a frequently standardized spring balance (Salter, West Midlands, UK) accurate to 0.1 kg. Recumbent length was measured to 0.1 cm by using a locally constructed wooden length board with fixed head plate and movable foot plate. Anthropometric data obtained within 20 d of the blood sampling were used for the present analyses and were compared with those of the North American reference population, expressed as Z scores (9).

Laboratory analyses

Blood was drawn from an antecubital vein by using appropriate precautions to prevent contamination with exogenous trace elements (10). The samples were obtained in the morning at the field clinic; the children were not necessarily in the fasting state. A portion of the blood sample was placed in a heparinized tube for measurement of hemoglobin, hematocrit, and total leukocyte count (WBC) by using standard methods. The remaining blood was centrifuged at $\approx 1000 \times g$ for 10 min at room temperature within 4 h and the serum was separated and stored at −70 °C before transport on dry ice and subsequent analysis of proteins and trace elements in Davis, CA.

Serum samples for zinc and copper were wet ashed with nitric acid for measurement of mineral content by flame atomic-absorption spectrophotometry (11). The cutoffs that were used to define adequate serum zinc and copper concentrations were 9.2 μmol/L (0.60 mg/L) and 15.7 μmol/L (1.00 mg/L), respectively. Regrettably, iron analyses could not be completed because of an initial failure in the method and insufficient remaining serum for repeated analyses. Serum α_2-macroglobulin, ceruloplasmin, and C-reactive protein (CRP) were measured by radial immunodiffusion (12) with commercially available kits (The Binding Site, Inc, San Diego); serum ferritin was quantified by using an enzyme-linked immunoassay (13) and commercially produced reagents (Medix, Foster City, CA). The normal range for α_2-macroglobulin was defined as 1500–4000 mg/L, for ceruloplasmin as 300–650 mg/L, for CRP as < 10 mg/L, and for serum ferritin as 7–140 μg/L. Anemia was defined as a hemoglobin concentration < 110 g/L.

Data analyses

Preliminary univariate descriptive statistics were completed for all variables. Bivariate analyses were subsequently conducted to explore relationships between *1*) fixed characteristics of the children (age, sex, and anthropometric status) and their illness and indicators of trace element status, *2*) reported clinical signs and laboratory indicators of infection, and *3*) all indicators of infection and indicators of trace element status. The bivariate analyses included correlation of continuous variables, contingency tables for categorical variables, and t tests for combinations. The overall relationships between the major indicators of trace element status (hemoglobin, serum zinc, copper, and ferritin) and the explanatory variables (age, sex, anthropometric status, socioeconomic status, presence of illness symptoms, and laboratory evidence of infection) were examined with stepwise-regression procedures. The children's socioeconomic status was classified according to two proxy variables indicating housing quality (materials of floor, walls, and roof) and number of possessions from a list of six specific items. All analyses were done with *PC-SAS* release 6.04 (14).

Results

A general description of the study subjects is presented in **Table 1.** Nineteen (16%) of the children were stunted (length-for-age ≤ 2 SD with respect to the international reference data) and one (0.8%) was wasted (weight-for-length ≤ 2 SD). Approximately 60% were anemic and ≈ 70% had low iron reserves, as indicated by serum ferritin concentrations < 7 μg/L. Whereas nearly 80% of the children had serum zinc concentrations < 9.2 μmol/L (0.60 mg/L), only two (1.3%) had serum copper concentrations < 15.7 μmol/L (1.00 mg/L).

TABLE 1
Characteristics of study subjects*

Characteristic	$\bar{x}$ ± SD	Minimum	Maximum
Age (mo)	14.7 ± 2.1	11.0	18.9
Weight (kg)	9.54 ± 1.13	7.00	12.85
Length (cm)†	74.8 ± 3.4	65.5	83.5
Length-for-age (Z score)†	−1.23 ± 0.89	−4.69	1.42
Weight-for-length (Z score)†	−0.07 ± 0.99	−2.70	2.94
Hemoglobin (g/L)	104.6 ± 12.3	76.0	135.0
Hematocrit (l)	0.338 ± 0.027	0.27	0.42
Serum ferritin (μg/L)	7.0 ± 10.2	0	59.9
Serum zinc (μmol/L)	7.3 ± 2.1	1.2	14.7
Serum α_2-macroglobulin (mg/L)	7582 ± 3180	2279	24009
Serum copper (μmol/L)	23.4 ± 4.6	4.1	37.1
Serum ceruloplasmin (mg/L)	10422 ± 5200	636	37128
Total leukocyte count (10^6/L)	10363 ± 3631	5100	33000
C-reactive protein (mg/L)	9.8 ± 16.8	0	148.3

* n = 153 except where otherwise indicated.
† n = 111.

TABLE 2
Prevalence of positive indicators of infection in the study population*

Indicator of infection†	Children	Total
	n	%
Reported signs of infection†		
Fever	13/149	8.7
Diarrhea	18/149	12.1
Anorexia	34/150	22.7
Any clinical indicator	52/150	34.7
Laboratory indicators		
Total leukocyte count >15000 × 10^6 cells/L	9/153	5.9
C-reactive protein >10 mg/L	37/153	24.2
Any laboratory indicator	43/153	28.1
Any clinical sign or laboratory indicator	69/147	46.9

* n = 147–153.
† *See* text for definitions of indicators.
‡ Information on signs of infection were not available from three children.

Fever was reported more commonly among children < 15 mo of age than among the older ones (13.8% vs 1.7%, $P = 0.012$), as was diarrhea (16.1% vs 5.1%, $P = 0.042$). The younger children also had significantly lower mean (±SD) serum zinc concentrations than the older ones (6.0 ± 2.0 vs 8.0 ± 2.1 μmol/L, $P = 0.007$), but there were no significant differences in any of the other indicators of trace element status by age group. Girls had higher hemoglobin and hematocrit values than boys (109 vs 101 g/L, $P < 0.001$ and 34.5% vs 33.2%, $P = 0.002$, respectively) and higher serum ferritin concentrations (9.2 ± 12.2 vs 5.1 ± 7.5 μg/L, $P = 0.002$). Diarrhea was more common among boys than among girls on the day of the blood drawing (17.3% vs 4.5%, $P = 0.015$). There were no other sex-specific differences in the indicators of trace element status or the illness rates.

As expected, highly significant correlations were observed between hemoglobin and hematocrit values ($R = 0.91$, $P < 0.001$). Of the 93 children classified as anemic, 71 (76%) had serum ferritin concentrations < 7 μg/L, compared with 61% of the nonanemic children ($P = 0.044$).

No significant relationship was found between serum zinc and α_2-macroglobulin concentrations ($R = 0.09$, $P = 0.27$), but the concentrations of copper and ceruloplasmin were significantly correlated ($R = 0.81$, $P < 0.001$). Serum zinc concentrations were significantly correlated with the Z score for length-for-age ($R = 0.25$, $P = 0.008$) and marginally with the Z score for weight-for-age ($R = 0.16$, $P = 0.059$), but not with the relative weight-for-length ($R = 0.05$, $P = 0.565$). Serum ferritin concentrations were negatively correlated with the weight-for-age Z score ($R = -0.19$, $P = 0.018$), but not with the other anthropometric indicators. Serum copper concentrations showed no significant correlations with anthropometric status.

The number and percentage of children with specific reported signs or laboratory evidence of infection are shown in **Table 2.** Approximately one-third of the subjects had some sign of infection, and about one-fourth had either leukocytosis (WBC > 15 000 × 10^6 cells/L) or elevated CRP concentrations, indicating the presence of a systemic acute-phase response. Nearly half of the children had either reported clinical or laboratory evidence of infection or both.

The relationships between the presence of clinical histories and laboratory signs of infection were explored by a series of independent two-way comparisons of each reported indicator vs the results of each of the laboratory tests. As shown in **Figure 1,** fever, anorexia, and any reported indicator of infection were each significantly associated with positive laboratory results (either elevated WBC or CRP). By contrast, diarrhea was not associated with elevated WBC or CRP concentrations. Notably, a considerable number of children without reported evidence of infection had positive laboratory results and vice versa. Thus, each set of indicators of infection was considered separately for possible relationships with apparent trace element status.

Selected indicators of trace element status and the mean concentrations of hemoglobin were compared for children with and without reported clinical signs of infection (**Table 3**). Children with reported anorexia had lower mean serum zinc concentrations ($P = 0.059$) and higher mean serum copper concentrations ($P = 0.005$) than did those without anorexia; no significant differences in the mean serum concentrations of these trace elements were present in relation to any of the other reported signs. The mean hemoglobin concentrations were slightly lower among children with fever ($P = 0.095$) or anorexia ($P = 0.042$) than in those who were free of these respective clinical signs, but there were no differences by presence of diarrhea. The mean serum ferritin concentrations were significantly greater among children with either fever ($P < 0.001$) or anorexia ($P = 0.003$); but, again, no significant differences were observed in relation to diarrhea. The mean serum concentrations of zinc, copper, and ferritin were also compared for children whose laboratory values were above or below the stated cutoffs. The results were generally similar when the six children with elevated leukocyte counts, but nonelevated CRP concentrations, were included in the group of children with elevated CRP concentrations; therefore, only the combined results are presented (Table 3). As was observed with the clinical histories, the mean serum zinc concentrations were significantly lower in those children with either leukocytosis or elevated CRP concentrations compared with those in whom these laboratory tests were negative ($P = 0.04$). The mean serum copper concentrations were greater in the ill subjects ($P = 0.004$). There were no significant differences in hemoglobin concentrations by illness status, but the mean serum ferritin concentrations were significantly greater in children with positive laboratory evidence of infection ($P < 0.001$). Interestingly, of the 22 anemic

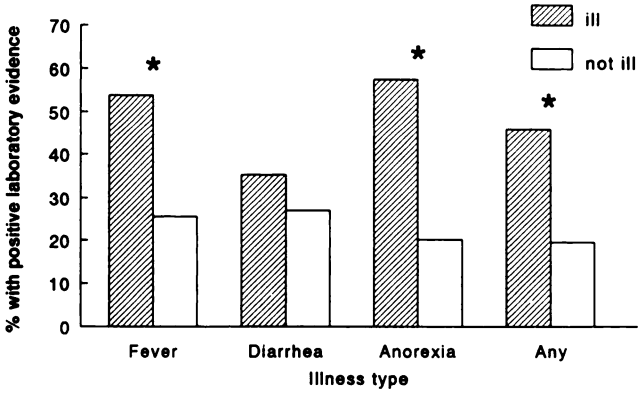

FIG 1. Relationship between presence of selected symptoms of illness and of positive laboratory indicators of infection.

TABLE 3
Indicators of trace element status by presence of illness, by different indicators of illness and illness status*

Indicator of trace element status	Reported signs		Laboratory tests		Reported signs or positive laboratory evidence of infection	
	Ill (n = 51)	Nonill (n = 97)	Ill (n = 43)	Nonill (n = 109)	Ill (n = 69)	Nonill (n = 78)
Serum zinc (μmol/L)	6.9 ± 2.1	7.5 ± 2.1	6.7 ± 2.4	7.5 ± 2.0†	7.0 ± 2.3	7.5 ± 2.0
Serum copper (μmol/L)	24.5 ± 4.9	23.1 ± 4.4†	25.2 ± 4.9	22.8 ± 4.2‡	24.7 ± 4.7	22.6 ± 4.2‡
Hemoglobin (g/L)	10.2 ± 1.2	10.6 ± 1.2†	10.4 ± 1.2	10.5 ± 1.2	10.5 ± 1.3	10.4 ± 1.2
Serum ferritin (μg/L)	9.8 ± 12.6	5.3 ± 7.3‡	12.7 ± 14.9	4.7 ± 6.3§	10.0 ± 12.9	3.9 ± 4.4§

* $\bar{x}$ ± SD. See text for definitions of illness categories. Serum ferritin concentrations compared as log transformed values.
†‡§ Significantly different from ill subjects (t test): †$P < 0.05$, ‡$P < 0.01$, §$P < 0.001$.

children with apparently adequate ferritin concentrations, 15 (68.2%) had clinical or laboratory evidence of infection. Only 23 (33.8%) of the 68 anemic children with expectedly low serum ferritin concentrations had laboratory evidence of infection (P = 0.005). Thus, it is possible that in at least some cases infections were responsible for the improbable observation that iron reserves were apparently normal in the anemic children.

To assess whether inherent characteristics of the children rather than the presence of infection could have explained the observed differences in their mean serum zinc, copper, and ferritin concentrations, we explored child age, sex, anthropometric status, and socioeconomic status as possible confounding variables. The only significant predictors of the children's serum zinc concentrations were their ages and length-for-age Z scores (**Table 4**). After these variables were controlled for the effect of infection was no longer statistically significant. Thus, the presence of infection did not in fact affect the interpretation of serum zinc concentrations in this population of young Peruvian children. By contrast, the presence of positive laboratory indicators of infection was the only factor significantly associated with the serum copper concentrations. Significant predictors of log serum ferritin concentrations were child sex, weight-for-length Z score, and the presence of fever, anorexia, or positive laboratory evidence of infection. Even after potential confounders were controlled for, the presence of infection influenced the population's apparent iron status.

To estimate the potential magnitude of the bias introduced by the presence of infection at the time of the assessment of the population's copper and iron status, we compared the cumulative distributions of serum copper and ferritin concentrations for children classified as either ill or nonill, as defined by the clinical histories, the laboratory values, or either of these two criteria. This enabled us to estimate the proportions of each group of children who had copper or iron-status indicators below the indicated cutoff values. An example of these analyses, with use of only the laboratory data to identify the presence of infection, is presented in **Figure 2.**

With the assumption that the children with no clinical or laboratory evidence of infection were representative of the entire universe of children in this community when free from infection, we then constructed theoretical frequency distributions to compare the rates of apparently depleted trace element status among either all of the children in the population without regard for their infection status or only among those who were free from infection, using each of the separate sets of infection indicators to identify children for exclusion (**Table 5**). The rates of depleted trace element status were estimated by using maximum likelihood techniques to define the expected distribution of serum values and the stated cutoffs for adequate status. We assumed that serum copper was normally distributed and serum ferritin was log normally distributed, as was suggested by the data. The effect of infection on the rate of apparently low zinc status was not explored because of the aforementioned results of the regression analyses.

TABLE 4
Results of regression analyses to predict serum zinc, copper, and ferritin concentrations

Dependent variable	Independent variables*	F	P
Serum zinc	Length-for-age Z score	7.58	0.007
	Child age	6.10	0.015
Serum copper	Laboratory evidence of infection	5.27	0.023
Serum ferritin (log)	Child sex	14.31	< 0.001
	Weight-for-length Z score	5.33	0.023
	Laboratory evidence of infection	12.52	< 0.001
	Presence of anorexia	4.66	0.033
	Presence of fever	4.39	0.038

* Independent variables explored in all models were child sex, age, length-for-age, and weight-for-length Z scores; two indicators of socioeconomic status; presence of diarrhea, fever, anorexia; and positive laboratory indicators of infection. Model R^2 values were 0.298 for log serum ferritin, 0.132 for serum zinc, and 0.079 for serum copper.

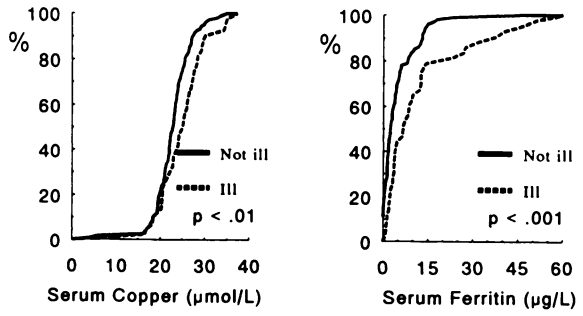

FIG 2. Cumulative distributions of serum copper and ferritin concentrations by illness status, defined by laboratory indicators of infection.

TABLE 5
Percentage of either all children or of noninfected children only with low copper and iron status, according to indicator of illness*

Trace element status	All children	Nonill children, by indicator used to exclude ill children		
		Clinical signs	Laboratory test†	Clinical signs or laboratory test†
Low copper status	5	6	5	6
Low iron status	70	76	80	82

* Based on theoretical distributions of serum copper and log serum ferritin concentrations.
† Positive laboratory evidence of infection.

The rate of low copper status was either zero or one percentage point less in noninfected children than in the whole population, depending on the exclusion criterion applied. Infection had only a minor impact on the assessment of the population's copper status because most of the children had serum copper concentrations well above the normal cutoff concentrations; the prevalence of low serum copper concentrations would have been only slightly underestimated if the presence of infection in the individuals examined had been ignored. The rate of apparently depleted iron reserves was 10 or 12 percentage points greater after the infected children were excluded from the analysis. If serum ferritin were to be used as the only indicator to assess the iron status of this population, the presence of infection should be taken into account to avoid a relatively sizable underestimate of the prevalence of low iron reserves.

Discussion

To estimate the potential magnitude of misclassification that may result from the failure to consider the effect of infection on the circulating concentrations of selected indicators of trace element status, we compared the serum zinc, copper, and ferritin concentrations of young Peruvian children with and without specific reported signs and laboratory evidence of infection. As expected, the mean serum concentrations of zinc were significantly lower and the mean concentrations of serum copper and serum ferritin were significantly greater among infected individuals, regardless of the type of indicator of infection that was used. However, the implications of these differences in mean serum concentrations of indicators of trace element status on the rates of children classified as depleted with regard to these nutrients was less dramatic. In particular, the presence of infection had no significant independent impact on the proportion of children with low zinc status and only minimal effect on the rate of low copper status. By contrast, the degree of underestimation of depleted iron reserves imposed by the presence of infection would be of moderate size.

One previous study of serum ferritin concentrations in pregnant and lactating Zairian women likewise found a positive relationship between mean serum ferritin concentrations and a laboratory indicator of an acute-phase response (15). However, no clinical indicators of infection were reported in that study, and no attempt was made to exclude other factors that might have confounded the possible relationship between infection and serum ferritin concentrations. Moreover, the significance of these findings with regard to assessment of the population's nutritional status was not discussed. Several other studies have examined the implications of infection for the interpretation of serum ferritin concentrations in clinical settings (16), but we are not aware of any population-based research on the relationship between concurrent infection and the assessment of zinc, copper, or iron status.

The magnitude of error in the classification of a population's trace element status that may result from the presence of concurrent infections depends on the prevalence of specific types and severity of infections, the particular nutrient being assessed, and the expected distribution of serum concentrations of the nutrients of interest among the noninfected individuals. Misclassification biases would tend to be greater in those populations or population subgroups having higher rates of more severe infections. In the current study nondiarrheal febrile illnesses and illnesses complicated by anorexia were both associated with positive laboratory evidence of infection and with changes in serum concentrations of indicators of trace element status, but diarrheal diseases were not. Thus, groups with higher rates of the former types of illness might be more subject to errors in the assessment of their trace element status.

In the present study children < 15 mo of age had higher rates of febrile illness than did the older ones. Thus, it is conceivable that populations of younger children may be more susceptible to assessment errors. On the other hand, recent analyses of data collected from another group of Peruvian children found that the incidence of infections complicated by anorexia increased with age, at least up to 12 mo, which was the oldest age group studied (8). Thus, the age-specific likelihood of under- or overestimation of the prevalence of trace element deficiencies is a complex issue that may vary according to the different disease patterns encountered in particular populations.

The results from this group of young Peruvian children indicate that the mean serum ferritin and serum copper concentrations were more affected by the presence of infection than was the mean serum zinc concentration. Despite the significant impact of infections on mean serum copper concentrations, illnesses did not produce important errors in the assessment of the population's apparent copper status because nearly all of the individuals had serum concentrations well above the critical cutoff value.

In this population we were able to estimate the approximate rate of over- or underestimates of the prevalence of trace element deficiencies that would occur if the children's infection status were ignored. When all children were considered, regardless of infection status, an estimated 70% had low serum ferritin concentrations and 5% had low serum copper concentrations, according to the theoretical frequency distributions that were described. When only the noninfected children are included in the analysis, these rates increase to 82% and 6%, respectively. Thus, the observed rates of infections (as diagnosed by either the clinical or laboratory indicators) resulted in an under-diagnosis of low iron status by 12 percentage points and of low copper status by only 1 percentage point. Similar estimates of misclassification of zinc status are inappropriate because the presence of infection was not independently associated with altered serum zinc concentrations in this population. The high observed prevalence of

low serum zinc concentrations probably indicates true low zinc status in these children (17).

These results have several practical implications for the design of field surveys to assess the trace element status of populations. First, to avoid completely the potentially confounding effect of concurrent infections, data on the presence of infection should be recorded whenever a population is examined. Unfortunately, neither reported signs of infection nor the selected laboratory tests alone appear to be capable of identifying all of the apparently ill children. The laboratory tests tended to be more strongly associated with the indicators of trace element status than with the clinical signs of infection, but the laboratory tests are also more expensive than the clinical histories. Thus, the choice of which method to use to evaluate the presence of infection may depend on the amount of resources available for the survey. It must also be recognized that the acute-phase response itself may be blunted in severely undernourished individuals (18), although there were very few severely malnourished children in the present study.

Second, because different subgroups of a population may be more or less likely to be infected, it would not be possible to exclude all infected individuals without risking the introduction of a selection bias into the sample. It is probably more appropriate to adjust the results statistically for the presence of infection once the survey is completed.

Finally, the results suggest that, with the exception of serum ferritin concentrations, the other indicators of trace element status that were examined in the present study are only minimally affected by infection. Thus, it may not be necessary to initiate special data-collection procedures for the diagnosis of infection to avoid biases in the classification of a population's zinc and copper status. Obviously, more information is needed from additional geographic settings and age and physiological-status subgroups and for other nutrients before definitive conclusions can be drawn.

References

1. Beisel WR. Magnitude of the host nutritional responses to infection. Am J Clin Nutr 1977;30:1236–47.
2. Kushner I. The acute phase response: an overview. Methods Enzymol 1988;163:373–83.
3. Lopez de Romaña G, Brown KH, Black RE. Health and growth of infants and young children in Huascar, Peru. Ecol Food Nutr 1987;19:213–29.
4. Lopez de Romaña G, Brown KH, Black RE, Kanashiro HC. Longitudinal studies of infectious diseases and physical growth of infants in Huascar, an underprivileged peri-urban community of Lima, Peru. Am J Epidemiol 1989;129:769–84.
5. Black RE, Lopez de Romaña G, Brown KH, Bravo N, Grados Bazalar O, Kanashiro HC. Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. Am J Epidemiol 1989;129:785–99.
6. Yeager BAC, Lanata CF, Lazo F, Verastegui H, Black RE. Transmission factors and socioeconomic status as determinants of diarrhoeal incidence in Lima, Peru. J Diarrhoeal Dis Res 1991;9:186–93.
7. Lanata CF, Black RE, Gilman RH, Lazo F, del Aguila R. Epidemiological, clinical, and laboratory characteristics of acute vs persistent diarrhea in periurban Lima, Peru. J Pediatr Gastroenterol Nutr 1991;12:82–8.
8. Brown K, Peerson J, Creed-Kanashiro H, Lopez de Romaña G, Black R. Validity and epidemiology of reported poor appetite in low-income, Peruvian infants. FASEB J 1992;6:A1649(abstr).
9. National Center for Health Statistics. Growth curves for children birth–18 years, United States. Vital Health Stat [11]. 1977;165.
10. Smith JC, Holbrook JT, Danford DE. Analysis and evaluation of zinc and copper in human plasma and serum. J Am Coll Nutr 1985;4:627–38.
11. Clegg MS, Keen CL, Lonnerdal B, Hurley LS. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet ashing. Biol Trace Elem Res 1981;3:107–15.
12. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235–54.
13. Uotila M, Ruoslahti E, Engvall E. Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J Immunol Methods 1981;42:11–5.
14. SAS Institute Inc. SAS/STAT user's guide. Release 6.03 edition. Cary, NC: SAS Institute Inc, 1988.
15. Kuvibidila S, Mbele V, Yu L, Warrier R, Lemonnier D. Usefulness of serum ferritin in evaluation of iron status in Zairen women of the reproductive age group. Am J Clin Nutr 1991;53:P22(abstr).
16. Witte DL. Can serum ferritin be effectively interpreted in the presence of the acute-phase response? Clin Chem 1991;37:484–5.
17. Udomkesmalee E, Dhanamitta S, Yhoung-Aree J, Rojroongwasinkul N, Smith JC Jr. Biochemical evidence suggestive of suboptimal zinc and vitamin A status in schoolchildren in Northeast Thailand. Am J Clin Nutr 1990;52:564–7.
18. Cruickshank AM, Hansell DT, Burns HJG, Shenkin A. Effect of nutritional status on acute phase protein response to elective surgery. Br J Surg 1989;76:165–8.