*Journal of Pediatric Gastroenterology and Nutrition*
**49:**371–373 © 2009 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and
North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

# Letters to the Editor

## Commentary on ''Cytokeratin 18, a Marker of Cell Death, is Increased in Children With Suspected Nonalcoholic Fatty Liver Disease''

*To the Editor:* In a recent study published in the *Journal*, Vos et al (1) assessed the usefulness of plasma caspase–generated cytokeratin 18 (CK18) fragment (CK18-ASP396) as a novel biochemical marker for nonalcoholic fatty liver disease (NAFLD) in a pediatric cohort. Results showed that CK18-ASP396 levels were significantly higher in children with clinically suspected NAFLD compared with those of obese children without suspected NAFLD and healthy comparison lean children. The authors concluded that their results supported the potential utility of this biochemical test for the diagnosis of NAFLD in a pediatric setting.

We believe that this article raises a number of issues. The clinical definition of NAFLD used in the study by Vos et al (1) does not agree with other standard biopsy-proven definitions (2) and actually describes patients with chronic transaminases elevation of unknown origin. Although abdominal ultrasound is widely used for screening asymptomatic children with an incidental elevation of liver enzymes, ultrasound cannot establish reliably the diagnosis of nonalcoholic steatohepatitis or the degree of hepatic fat accumulation (3). The statistical analysis of the data demonstrating an association of CK18-ASP396 with NAFLD is likely to be underpowered and prone to type I error because of the arbitrary nature of clinical classification of patients. We therefore wonder whether the authors' conclusions would be the same if children with clinically suspected NAFLD who did not undergo biopsy ($n = 14$) would be analyzed separately from those with biopsy-proven nonalcoholic steatohepatitis (''group IIIa'' in the study of Vos et al, $n = 6$). Accordingly, it is possible that elevated levels of CK18-ASP396 in the entire group of 20 patients classified as having NAFLD would be chiefly driven by the values observed in the 6 children with definite nonalcoholic steatohepatitis. Another issue concerns the classification of obese children without transaminases elevation as comparison subjects without suspected NAFLD. In this regard, it should be noted that most pediatric NAFLD has been reported in children with elevated body mass index (4), and the absence of transaminases elevation does not exclude per se the presence of NAFLD (5).

For a better understanding of the pathophysiological link between CK18-ASP396 and NAFLD in pediatric patients, we should be diligent in using definitions that are consistent with the guidelines. We believe that the authors' findings are biased by several methodological issues that cast a shadow on the impact of their conclusions. However, we also believe that further exploration of CK18-ASP396 in pediatric NAFLD is of extreme importance to lessen the number of biopsies performed and to provide a reliable noninvasive method to diagnose NAFLD in children.

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### REFERENCES

1. Vos MB, Barve S, Joshi-Barve S, et al. Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2008; 47:481–5.
2. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–21.
3. Mishra P, Younossi ZM. Abdominal ultrasound for diagnosis of nonalcoholic fatty liver disease (NAFLD). *Am J Gastroenterol* 2007;102:2716–7.
4. Valva P, De Matteo E, Galoppo M, et al. Apoptosis markers in liver biopsy of nonalcoholic steatohepatitis in pediatric patients. *Hum Pathol* 2008;39:1816–22.
5. Yilmaz Y, Ulukaya E, Dolar E. Serum M30 levels: A potential biomarker of severe liver disease in nonalcoholic fatty liver disease and normal aminotransferase levels. *Hepatology* 2009;49:697.

## Author's Response

*To the Editors:* We thank Dr Yilmaz and colleagues for their comments on our manuscript. We appreciate their

support of our recommendation that further study of cytokeratin 18 (CK18) and CK18-ASP396 fragments is warranted in pediatric nonalcoholic fatty liver disease (NAFLD). Most of the available information in this area to date has been in adult studies (1,2). We undertook our analysis to provide pilot data to justify a biopsy-based study of CK18 in children. The limitations of our cohort and methods were discussed in the original article (in brief: lack of liver biopsies in most subjects and small sample size). We chose to use a widely accepted definition (3) for ''suspected'' or ''clinical NAFLD'' in our study because of the small number of biopsied patients. Given this, we carefully limited our conclusion to the determination that further research is warranted on both CK18 and CK18-ASP396 fragments in pediatric NAFLD. One of our original concerns had been that because CK18 is found in cells of epithelial origin, it could be elevated due to other diseases in children (eg, in respiratory illness and tumors). CK18 has been studied in pediatric respiratory syncytial virus (4) as well as tumors of several kinds (5,6). In fact, in our small cohort of 28 normal children we found 1 ''healthy'' child with a substantial elevation of CK18. This will need to be studied further in larger studies because it could limit the use of CK18 and/or CK18-ASP396 fragments in populations not already known to have NAFLD. Another interesting finding in our article is that CK18-ASP396 fragments did not seem to be as predictive of our groups as CK18. This is not the case in the previous adult studies (1,2). Especially when one considers the major differences that have been identified in pediatric NAFLD pathology compared with adult NAFLD pathology (7), it will be interesting to study both CK18 and CK18-ASP396 fragments in well-controlled pediatric studies with larger cohorts of biopsied subjects.

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## REFERENCES

1. Wieckowska A, Zein NN, Yerian LM, et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006;44:27–33.
2. Diab DL, Yerian L, Schauer P, et al. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. *Clin Gastroenterol Hepatol* 2008;6: 1249–54.
3. Schwimmer JA, McGreal N, Deutch R, et al. Influence of gender, race and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005;115:e561–5.
4. Bem RA, Bos AP, Bots M, et al. Activation of the granzyme pathway in children with severe respiratory syncytial virus infection. *Pediatr Res* 2008;63:650–5.
5. Greystoke A, Cummings J, Ward T, et al. Optimisation of circulating biomarkers of cell death for routine clinical use. *Ann Oncol* 2008;19:990–5.
6. de Haas EC, di Pietro A, Simpson KL, et al. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. *Neoplasia* 2008;10:1041–8.
7. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005;42: 641–9.

# Nutrient Composition vs Food Ingredients in the Treatment of Hospitalized and Severely Malnourished Children

*To the Editor:* In the March 2008 issue of *JPGN*, Bernal et al presented their experience in implementing the World Health Organization (WHO) guidelines for the treatment of severe malnutrition (1). They present the composition of the formulas that they used. We would like to share with readers the results of a recent trial comparing 2 F100s that differ only by the ingredient composition: The commercially available F100 (Nutriset, France) made with skim milk powder, vegetable fat, whey powder, maltodextrin, sugar, and a mineral and vitamin complex, and an F100 made of whole goat's milk (2), date concentrated juice, cassava starch, colza oil, and a mineral and vitamin complex (INRA, France).

In Madagascar, 33% of children suffer from weight insufficiency and 11% from severe malnutrition (3). In our hospital, severely undernourished children are treated according to the WHO guidelines, using the commercially available F100 (4); however, mortality remains high: $>10\%$.

In a randomized clinical trial we included 61 severely malnourished children without serious additional pathology. They received either F100 Nutriset (group A, $n = 33$) or F100 INRA (group B, $n = 28$) at the rate of $100\,mL \cdot kg^{-1} \cdot day^{-1}$ during the period of rehabilitation and then $200\,mL \cdot kg^{-1} \cdot day^{-1}$ until weight-for-height was $>80\%$.

No statistical difference was found in inclusion parameters (weight-for-height, $74\% \pm 3.6\%$ National Center for Health Statistics) and mid-upper arm circumference ($113 \pm 14$ mm). The results did not show any significant difference between the 2 groups on the following outcome variables: duration of hospitalisation in days ($12.6 \pm 1.5$ in group A vs $11.1 \pm 1.0$ in group B, $P = 0.43$), duration in days to obtain a weight-for-height greater than 80% ($8.2 \pm 0.96$ vs $8.5 \pm 0.91$; $P = 0.73$), and weight gain in grams per kilogram of body weight and per day ($9.2 \pm 1.9$ vs $8.6 \pm 1.9$; $P = 0.95$). Thus, within Madagascar conditions, the availability and cost of food ingredients, as well as technological constraints are probably more important than the type of food used to prepare F100. As recently presented in *JPGN* by Ferguson et al (5), optimal

combinations of local foods are unlikely to achieve the nutrient density of F100. Within such constraints, the cost of F100 is dependent on the world food market. Alternatively, F100 composition as recommended by WHO may not be optimal for use in all regions of the world. Clinical studies with locally available food may ultimately improve the knowledge and treatment of children suffering from severe malnutrition.

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## REFERENCES

1. Bernal C, Velãsquez C, Alcazar G, et al. Treatment of severe malnutrion in children: experience in implementing the World Health Organization guidelines in Turbo, Columbia. *J Pediatr Gastroenterol Nutr* 2008;46:322–8.
2. Razafindrakoto O, Ravelomanana N, Rasolofo A, et al. Goat's milk as a substitute for cow's milk in undernourished children: a randomized double-blind clinical trial. *Pediatrics* 1994;94 (1):65–9.
3. UNICEF. *http://www.unicef.org/infobycountry/madagascar_statistics.html*. 2007. Accessed September 2, 2008.
4. World Health Organization. *Management of Severe Malnutrition: A Manual for Physicians and Other Senior Health Workers*. Geneva: WHO; 1999.
5. Ferguson E, Briend A, Darmon N. Can optimal combinations of local foods achieve the nutrient density of the F100 catch-up diet for severe malnutrition? *J Pediatr Gastroenterol Nutr* 2008;46:447–52.