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37-Kilodalton/83-Kilodalton RNase L Isoform Ratio in Peripheral Blood Mononuclear Cells: Analytical Performance and Relevance for Chronic Fatigue Syndrome

A French group has reported results (5) supporting the use of the RNase L 37-kDa/83-kDa ratio (37/83 R) in peripheral blood mononuclear cells (PBMC) as a diagnostic test for chronic fatigue syndrome (CFS). More recently (6), the same group cautioned about the diagnostic value of the 37/83 R, based on a small patient follow-up study which was likely to indicate analytical variability among duplicate assays, lack of reproducibility over time, and a weak correlation with the multidimensional fatigue inventory (MFI) score. Because of our long-term experience with this assay, we would like to offer some comments.

First, we tested the analytical performance of the 37/83 R assay according to CLSI (formerly NCCLS) procedure EP5-A (4), with control samples at three different levels made of extracts of the monocytic U937 cell line spiked with various concentrations of recombinant RNase L. The guideline protocol involves assaying the samples in duplicate twice daily over a total period of 20 days. The results summarized in Table 1 indicate that both within- and between-run variation does not exceed 13%. In another series of experiments, we assayed eight patient samples in duplicate (average 37/83 R ranging from 0.5 to 245). Although in accordance with the NCCLS protocol results, the variation did not exceed 12% for samples with 37/83 R levels up to 20, and it rose significantly to 30% and more for samples with 37/83 R levels above 20. This should be expected, because beyond this level, more than 70% of the 83-kDa isoform is cleaved, and consequently, the faint 83-kDa band is difficult to scan with accuracy. Thus, in our opinion, the lower level of correlation between the duplicate assay results observed with the CFS group versus those with the controls (6) reflects the prevalence of high 37/83 R levels in the CFS group rather than a low test reproducibility as claimed by these authors. This is further supported by the good correlation found for the control group ($r = 0.95$). During validation, the lowest detectable ratio measured with a sample containing the 83-kDa isoform only was estimated (3 independent experiments with 26 replicates each) to be 0.13 ± 0.06 (average $\pm$ three standard deviations). Thus, the clinical cutoff ratio of 0.4 found by the authors (5, 6) to best discriminate CFS patients from controls falls within the measurable range.

Second, although long-term unexplained fatigue is a hallmark symptom, CFS is a complex clinical condition, and other important symptoms reflect an exacerbated inflammatory response. Because the inflammatory protease elastase has been shown to be responsible for the cleavage of 83-kDa RNase L into the 37-kDa isoform (2), one would expect the 37/83 R assay to reflect inflammatory activity in the immune system. In a recent study, we compared the 37/83 R with human leukocyte elastase activity (Molecular Probes kit) with 52 CFS PBMC samples. The correlation was highly significant ($r^2 = 0.76$; $P < 0.001$), supporting this proposal. In our opinion, both fatigue and RNase L cleavage result from similar dysfunctions in the interferon-activated pathways that occur upstream of RNase L activation and exert their effects on different targets (3). Consequently, we do not feel particularly concerned by the weak correlations observed between the MFI and 37/83 R (6). The poor reproducibility of the test results over time (6) might reflect changes in the immune inflammatory status rather than in fatigue. In particular, the authors do not provide any information concerning the drugs CFS patient might have been using during the longitudinal study, which is critical as some drugs may act on the inflammatory response.

Finally, by combining the results of both studies by this group (5, 6) with ours (1), which included 57 CFS patients and 28 healthy controls, we note that both diagnostic sensitivity (76%) and specificity (65%) remain attractive for such a poorly defined pathological condition.

TABLE 1. Precision profile of the 37/83 R assay determined according to the NCCLS EP5-A protocol (3)

Category for precision estimate (relative SD)	% Variation for avg 37/83 R of:		
	0.40	2.17	3.95
Within-run	12.5	10.1	10.9
Between-run	10.0	12.9	6.6
Between-day	57.5	25.3	18.2
Total	57.5	29.9	22.3

REFERENCES

1. **De Meirleir, K., C. Bisbal, I. Campine, P. De Becker, T. Salehzada, E. Demettre, and B. Lebleu.** 2000. A 37-kDa 2-5A binding protein as a potential biochemical marker for chronic fatigue syndrome. Am. J. Med. **108:**99–105.
2. **Demettre, E., L. Bastide, A. D'Haese, K. De Smet, K. De Meirleir, K. P. Tiev, P. Englebienne, and B. Lebleu.** 2002. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. J. Biol. Chem. **277:**35746–35751.
3. **Englebienne, P., C. V. Herst, M. Frémont, T. Verbinnen, M. Verhas, and K. De Meirleir.** 2002. The 2-5A pathway and signal transduction: a possible link to immune dysregulation and fatigue, p. 99–130. *In* P. Englebienne and K. De Meirleir (ed.), Chronic fatigue syndrome, a biological approach. CRC Press, Boca Raton, Fla.
4. **National Committee for Clinical Laboratory Standards.** 1999. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS document EP5-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
5. **Tiev, K. P., E. Demettre, P. Ercolano, L. Bastide, B. Lebleu, and J. Cabane.** 2003. RNase L levels in peripheral blood mononuclear cells: 37-kilodalton/83-kilodalton isoform ratio is a potential test for chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. **10:**315–316.
6. **Tiev, K. P., M. Birant, M. Ziani, J. Cabane, E. Demettre, and B. Lebleu.** 2005. Variability of the RNase L isoform ratio (37 kilodaltons/83 kilodaltons) in diagnosis of chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. **12:**366.

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Authors' Reply

Our communication (3) did not intend to be polemic but factual in pointing to the variability over time of the 37/83 R assay in a longitudinal study. We agree, however, that only a small cohort of CFS patients and matched controls has been studied, and extended further such studies would be welcome.

The assay in itself is not questioned and is well controlled in our hands. It was indeed originally set up in the laboratory of B. Lebleu before being transferred to RED Laboratories (1).

Moreover, any large 37/83 ratios were not found in our experiments (1, 3, 4) and should not be a problem as suggested by Englebienne et al.

The analytical performance of the 37/83 assay has been tested according to the CLSI (formerly NCCLS) procedure on a laboratory cell line supplemented with recombinant RNase L. This is very far from a clinical situation in which endogenous RNase L is assayed in patients' PBMC. It is worth pointing out here that variability was much higher for samples collected from CFS patients than for those from healthy controls. Moreover our patients did not take any immunosuppressive or corticosteroid therapy.

We entirely agree with Englebienne et al. when they propose that RNase L truncation probably reflects increased (or activated) proteolysis, which itself probably reflects an inflammatory condition. This was indeed reported in a collaborative study between our group and the Belgian group (2). Future studies should indeed aim at tracking the cause of increased proteolysis in CFS patients and might hopefully lead to a better diagnostic test.

REFERENCES

1. **De Meirleir, K., C. Bisbal, I. Campine, P. De Becker, T. Salehzada, E. Demettre, and B. Lebleu.** 2000. A 37 kDa 2-5A binding protein as a potential biochemical marker for chronic fatigue syndrome. Am. J. Med. **108:**99–105.
2. **Demettre, E., L. Bastide, A. D'Haese, K. De Smet, K. De Meirleir, K. P. Tiev, P. Englebienne, and B. Lebleu.** 2002. Ribonuclease L proteolysis in peripheral blood mononuclear cells of chronic fatigue syndrome patients. J. Biol. Chem. **277:**35746–35751.
3. **Tiev, K. P., M. Briant, M. Ziani, J. Cabane, E. Demettre, and B. Lebleu.** 2005. Variability of the RNase L isoform ratio (37 kilodaltons/83 kilodaltons) in diagnosis of chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. **12:**366.
4. **Tiev, K. P., E. Demettre, P. Ercolano, L. Bastide, B. Lebleu, and J. Cabane.** 2003. RNase L levels in peripheral blood mononuclear cells: 37-kilodalton/83-kilodalton isoform ratio is a potential test for chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. **10:**315–316.

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