

Remodeling Oversuppression and the Handling of Missing and Single labels

David W. Dempster

Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York and
Department of Pathology, College of Physicians and Surgeon of Columbia
University, New York, NY, USA

The clinical significance of long-term therapeutic inhibition of bone turnover is unknown. Given the impracticality and ethical problems associated with long-term fracture trials we will be constrained to rely more and more heavily on surrogate markers of bone strength and quality. The iliac crest biopsy will continue to play an important role in this endeavor. The use of tetracycline labels and the measurement of the dynamic parameters of bone formation that they afford allows direct and quantitative assessment of bone turnover rate in the bone biopsy. The theory and practice of administering and measuring tetracycline labels were developed 50 years ago by Frost and others. Usually, a double label is given during the weeks immediately preceding the biopsy with a typical sequence being 3 days ON, 10-12 days OFF and 3 days ON. The biopsy is taken 5 days after the last labeling day. When unstained sections are viewed under polarized light, the labels that were incorporated at sites of new bone formation can be visualized and quantified.

There are three categories of labels depending on the prevailing rate of new bone formation: double, single or absent. When bone formation rate is normal or elevated and there is no inhibition of mineralization, both double and single labels are usually present and the quantification of dynamic parameters is straightforward. The extent of double and single labels can be measured to obtain the mineralized perimeter (Md.Pm) and the average distance between the double labels can be measured and divided by the inter-label interval to obtain the mineral apposition rate (MAR). The bone formation rate (BFR) can then be calculated as the product of Md.Pm and MAR.

Excluding conditions with mineralization defects, when the bone turnover rate is low it is not uncommon to find only single labels and perhaps no labels in the biopsy. In this situation the quantification of dynamic parameters becomes more complicated and there is no general consensus on how it should be done. Some authors (1,2) suggest that if only single labels are present in a biopsy, MAR should be assigned a value that approximates the lowest measurable value in the population under study. This allows BFR to be calculated and datasets do not become imbalanced by the exclusion of samples with only single labels from group means. Sometimes there are no double labels present in the cancellous envelope but there are some in the intracortical or endocortical envelopes. In that case, the measured value for MAR in one of these envelopes could be used to calculate BFR in cancellous bone (1). Other authors elect to exclude subjects with only single labels from the calculation of BFR. This has been the case in several studies involving potent antiresorptive agents.

Our group recently compared the two most common methods (i.e., imputation for MAR or exclusion) for dealing with the single label phenomenon in alendronate-treated subjects (3). We compared the values obtained using both methods in 46 subjects who were either treated with alendronate (ALN) or were treatment-naïve (Rx-naïve). Following tetracycline labeling biopsies

were obtained from 46 postmenopausal women, aged 66.1 ± 1.3 years, with low bone mass and/or fractures who had been treated with ALN ($n=24$) for 6.6 ± 0.6 years or from 22 treatment-naïve subjects ($n=22$). MAR, Md.Pm and BFR were quantified using 2 methods: by imputing a value of 0.3 microns/day for subjects with only single labels and a value of 0 for subjects with no labels or excluding these subjects from the calculation of group mean values. MAR and BFR were significantly lower with the imputation method in the ALN group, whereas there were no differences between the results obtained by the 2 methods in the Rx-naïve group. Furthermore, MAR was significantly lower in ALN-treated versus Rx-naïve by the imputation method. We concluded from this study that the method used to evaluate MAR has a profound effect on the results for MAR and BFR and can lead to a different conclusion regarding the effects of ALN on MAR. We recommended that the method used be clearly stated in all publications and that, preferably, results obtained by both methods be presented.

The intent at the 2010 Sun Valley meeting is to discuss this issue with the panel and attendees in the hope of reaching a consensus recommendation on how to deal with the issue of single and missing labels should be handled in future clinical studies. This recommendation will have important implications for the interpretation of future studies of potent antiresorptive agents and evaluating the clinical significance of long-term therapeutic inhibition of bone turnover.

References

1. Foldes J, Shih MS, Parfitt AM. Frequency distributions of tetracycline-based measurements: implications for the interpretation of bone formation indices in the absence of double-labeled surfaces. *J Bone Miner Res.* 1990;5:1063-7.
2. Hauge E, Mosekilde L, Melsen F. Missing observations in bone histomorphometry on osteoporosis: implications and suggestions for an approach. *Bone.* 1999;25:389-95.
3. Dempster D, Zhou H, Bostrom M, Nieves J, Cosman F, Lindsay R. To impute or not to impute: That is the question. *J Bone Miner Res* 2009;24, Suppl. 1:S (abstract)