Cancellous Bone Volume Is an Indicator for Trabecular Bone Connectivity in Dialysis Patients

Junichiro James Kazama,* Ryo Koda,* Suguru Yamamoto,* Ichiei Narita,* Fumitake Gejyo,* and Akihide Tokumoto†

*Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; and †Kamifukubara Medical Clinic, Yonago, Japan

Background and objectives: A new assessment system for bone histology, termed the turnover-mineralization-volume system, is advocated for patients with chronic kidney disease-related mineral and bone disorder. The system measures cancellous bone volume (BV/TV) as a third major evaluation axis; however, the physiologic significance of BV/TV remains unclear.

Design, setting, participants, & measurements: Conventional bone histomorphometry was performed in 75 iliac bone samples obtained from dialysis patients. In 47 of the 75 samples, the remaining samples were subjected to direct microfocus x-ray computed tomographic observation. Quantitative morphologic examinations, including micro-bone mineral densitometry, and marrow space star volume, Euler number, and node-strut analyses, were performed in the virtual three-dimensional space reconstructed from the microfocus x-ray computed tomographic images.

Results: The levels of BV/TV were comparable in each of the conventional bone histomorphometric criteria. No significant correlations were found between BV/TV and other parameters. Two- and three-dimensional BV/TVs were significantly correlated with cancellous bone mass but not with cortical bone thickness or cortical bone mass. Two- and three-dimensional BV/TVs were significantly correlated with trabecular bone connectivity as determined by marrow space star volume, Euler number, and node-strut analyses.

Conclusions: In dialysis patients, BV/TV is not dependent on bone turnover or bone mineralization. BV/TV is unlikely to indicate the balance between bone formation and bone resorption. Instead, it reflects trabecular bone connectivity, and improved trabecular bone connectivity is physiologically beneficial in terms of bone quality. The turnover-mineralization-volume system offers an advantage over the conventional system for the assessment of bone quality.


Chronic kidney disease (CKD) is associated with various forms of metabolic bone disorders (1), which are accompanied by mineral and parathyroid metabolic abnormalities. These abnormalities are considered to be symptoms of a systemic syndrome called CKD-related mineral and bone disorder (CKD-MBD) (2).

The current gold standard for assessing bone status in CKD-MBD is bone histology (3). Bone histology is conventionally classified into five categories on the basis of tetracycline labeling-dependent bone histomorphometric findings. In this conventional classification, the histologic findings are classified according to two major assessment criteria: bone turnover and bone mineralization (4). However, the Kidney Disease: Improving Global Outcomes recently advocated the addition of cancellous bone volume (BV/TV) as a third major evaluation criterion for bone histology in CKD-MBD patients. The concept of this system, called turnover-mineralization-volume (TMV) classification (2,5), has become widely recognized.

The Kidney Disease: Improving Global Outcomes considers BV/TV to be the result of a balance between bone formation and bone resorption. However, the target for bone histomorphometric analysis is currently confined to cancellous bone area in most cases. Therefore, BV/TV actually indicates the balance between cancellous bone formation and cancellous bone resorption, which may not be identical to the balance between bone formation and bone resorption. The significance of knowing the balance between cancellous bone formation and cancellous bone resorption remains unknown.

Thus, the justification of the use of BV/TV in the TMV system has not yet been demonstrated. First, it has not been confirmed whether BV/TV is independent of bone turnover and bone mineralization. If significant associations were found between these factors, the significance of BV/TV as an evaluation criterion would be limited.

Moreover, the reason why BV/TV should be included in this classification has not been fully explained. It must be clarified whether BV/TV can theoretically enhance the balance between bone formation and bone resorption. It is unknown whether BV/TV reflects cortical bone mass, which is the major determin-
nant of bone strength. Other factors that affect bone strength are referred to as bone quality (6), which includes the chemical and structural properties. In terms of chemical properties, bone turnover and bone mineralization can be assessed by conventional bone histomorphometry. However, it is unknown whether BV/TV is associated with the structural properties of bone quality. The validation of the TMV system in terms of these aspects has not yet been performed.

On the basis of this background, we performed three-dimensional quantitative morphologic analyses of biopsied iliac bone samples obtained from CKD stage 5D (CKD5D) patients. We performed microfocus computed tomography (MCT) and novel three-dimensional image analysis (7–9). The aims of this study were (1) to confirm whether BV/TV, a new assessment criterion applied to the TMV system, is independent of bone turnover and bone mineralization, and (2) to determine whether and/or how BV/TV could be used to predict bone strength.

Materials and Methods
This study was approved by the ethics committee of Niigata University (no. 455).

Patients
Chronic hemodialysis patients who had undergone iliac bone biopsy at either Niigata University Hospital or Sanin Rosai Hospital for clinical reasons were enrolled in the study. Subjects provided written consent that biopsied bone samples would also be used in this study after being assessed for routine clinical purposes.

Bone Biopsy
Twenty days and 10 days before the bone biopsy, the patients were orally administered with 1000 mg of tetracycline for 2 days. A cylindrical piece of bone tissue was removed from the left iliac bone by trephine (8 mm in diameter) under local anesthesia. The biopsy specimens were fixed in 80% ethanol for 3 days at room temperature, immersed in Villanueva bone staining solution (Wako Pure Chemical Industrial, Ltd., Osaka, Japan) for 4 days, and then embedded in methyl methacrylate resin (MMA). Sections (10 μm thick) were processed from the block with a microtome, as described previously (10). The remaining tissues blocks were subjected to direct MCT observation (7).

Conventional Tetracycline-Dependent Bone Histomorphometry
The sections were observed at ×128 magnification with fluorescence microscopy (Optiphot; Nikon, Tokyo, Japan), and morphometry was performed using a semiautomatic image analyzer with Bone Morphometric Software version 3.0 (System Supply Co., Nagano, Japan) (10). Each morphometric parameter was defined, measured, and calculated according to the criteria of the American Society for Bone and Mineral Research Histomorphometry Nomenclature Committee (11). However, to avoid confusion with parameters derived from three-dimensional bone histomorphometry, the parameters measured by conventional two-dimensional bone histomorphometry in the sliced samples are expressed as sParameter in this manuscript (for example, BV/TV measured by the conventional two-dimensional bone histomorphometry is expressed as sBV/TV).

According to the histomorphometric data, each case was classified into one of five groups, as described below:

- Mild change type (MC): 10% < sOV/BV; and sFb.V/TV = 0; and osteoid volume (sOV/BV) < 10%;
- Osteitis fibrosa type (OF): 50% per year < sBFR/BV; 0.5% < sFb.V/TV; and sOV/BV < 10%;
- Osteomalacia type (OM): 10% < sOV/BV; and mineral apposition rate (sMAR) = 0 μm/day.
- Mixed change type (MX): 0.5% < sFb.V/TV; and 10% < sOV/BV.
- Adynamic bone type (AB): sOV/BV < 5%; and sBFR/BV = 0% per year.

Cases that could not be classified into any of these five groups were excluded from the analysis.

MCT
MCT and image analyses were performed in the remaining MMA tissue blocks after processing the tissue sections for conventional bone histomorphometry. The methods are described in detail elsewhere (7). In brief, MMA blocks were placed on the stage of the MCT (ScanXmate-A080S; Comscantecno, Yokohama, Japan) (8), and tomographic images were generated under the following settings: energy, 80 kV; current, 250 μA. Serial x-ray tomographic images were generated every 35.255 μm.

Reconstruction of Virtual Three-Dimensional Image
Virtual three-dimensional images were reconstructed from the serial tomographic data with an image analyzer (Ratoc System Engineering Co. Ltd., Tokyo, Japan) (12). The three-dimensional BV/TV, namely, the ratio of space occupied by the mineralized bone in the inner space 500 μm distant from the cortical bone, was defined as vBV/TV.

Cancellous Bone/Cortical Bone Mineral Density
Bone mineral density was calculated for every 35.255 × 35.255 × 35.255 μm pixel in the virtual three-dimensional image using an image analyzer (TRI/3D-BON-BMD; Ratoc System Engineering Co. Ltd.). Cancellous bone mineral density (CaBMD) and cortical bone mineral density (CoBMD) were defined as the mean pixel bone mineral density for the cancellous bone area and the cortical bone area, respectively. Cancellous bone mineral content (CaBMC) was the mineral content in the bone marrow area, and it was defined as CaBMD × vBV/TV. Because the vBV/TV was nearly 100% for almost the entire area of cortical bone, we defined cortical bone mineral content (CoBMC) as CoBMD × relative cortical bone thickness. Cortical bone thickness was measured as the diameter of bulbs that internally attached to both cortical bone surfaces. The relative cortical bone thickness was defined as the ratio of cortical bone thickness to the maximum cortical bone thickness in the 47 samples.

Marrow Space Star Volume Analysis
Marrow space star volume analysis (V*m) was defined as the mean volume of a specific region of the bone marrow space that could be seen, unobscured, in all possible directions from a random point (13,14). Lower V*m values indicate better trabecular bone connectivity.

V*m was calculated as follows: V*m = π/3 × mean (L³), where L is the distance from a random point to the point intercepted by the trabecular bones.

Euler Characteristic
The Euler number was defined as the difference between the number of particles present in the spaces occupied by trabecular bones and the number of bone marrow spaces surrounded by the trabecular bones.
When a trabecular bone is regarded as a topological manifold (T), its Euler characteristic can be obtained by the Euler-Poincare formula, \( EN(T) = b_0(T) - b_1(T) + b_2(T) \), where

\[ EN(T) = \text{The Euler number of T}, \]
\[ b_0(T) = \text{The zero-dimensional Betti number of T}, \]
\[ b_1(T) = \text{The one-dimensional Betti number of T}, \]
\[ b_2(T) = \text{The two-dimensional Betti number of T}. \]

Lower EN/TV values indicate better trabecular bone connectivity.

Node-Strut Analysis

Node-strut analysis (NSA) defines the following parameters: a node (Nd) is a point at which three or more trabecular bones join; a terminus (Tm) is a free ending of trabecular bone; and a trabecular strut is a winding line that connects any combination of Nd, Tm, cortical bones, or boundaries of the region of interest. These parameters are expressed as NdNd and NdTm, for example (17,18). Larger values of the following parameters indicate better trabecular bone connectivity; the number of nodes per tissue volume (NdNd/TV; per cubic millimeter), the node-to-node strut length per tissue volume (NdNd/TSL; millimeters per cubic millimeter), and the node-to-node strut length per total strut length (NdNd/TVL; percent).

NSA was originally a two-dimensional morphometric method. To apply NSA to three-dimensional samples, a new image analysis technique was developed. In brief, the reconstructed three-dimensional cancellous bone structure was converted into a winding ribbon-like structure consisting of the centers of the bulbs that internally attach to the bone surface, and thereafter conventional NSA was performed on the ribbon-like structure (7).

Data and Statistical Analyses

Data are expressed as means ± SD, and a \( P \) value less than 0.05 was considered to be statistically significant. Differences between groups were calculated using the Mann-Whitney test. For simple correlation studies, the Pearson correlation coefficients were calculated.

**Table 1.** Clinical characteristics of 75 patients who contributed biopsied iliac bone samples that were assessed for conventional tetracycline labeling-dependent bone histomorphometry

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>OF</th>
<th>OM</th>
<th>MX</th>
<th>ABD</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n ) (men:women)</td>
<td>14 (8:6)</td>
<td>18 (8:10)</td>
<td>7 (2:5)</td>
<td>14 (7:7)</td>
<td>22 (13:9)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>47.7 ± 12.7</td>
<td>46.7 ± 12.6</td>
<td>51.3 ± 14.3</td>
<td>49.4 ± 14.7</td>
<td>52.6 ± 13.5</td>
</tr>
<tr>
<td>Dialysis vintage, M</td>
<td>27.4 ± 45.2</td>
<td>60.2 ± 92.8</td>
<td>8.9 ± 14.8</td>
<td>14.0 ± 36.9</td>
<td>37.5 ± 49.8</td>
</tr>
<tr>
<td>Primary disease</td>
<td>CGN 12</td>
<td>CGN 14</td>
<td>CGN 3</td>
<td>CGN 13</td>
<td>CGN 16</td>
</tr>
<tr>
<td></td>
<td>DMGS 1</td>
<td>DMGS 3</td>
<td>DMGS 3</td>
<td>DMGS 0</td>
<td>DMGS 6</td>
</tr>
<tr>
<td></td>
<td>Others 1</td>
<td>Others 3</td>
<td>Others 0</td>
<td>Others 0</td>
<td>Others 0</td>
</tr>
<tr>
<td></td>
<td>UK 0</td>
<td>UK 1</td>
<td>UK 1</td>
<td>UK 1</td>
<td>UK 0</td>
</tr>
<tr>
<td>Intact parathyroid hormone, pg/ml</td>
<td>220.4 ± 108.9</td>
<td>778.1 ± 429.7</td>
<td>253.7 ± 136.6</td>
<td>849.7 ± 577.6</td>
<td>88.9 ± 59.6</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>9.2 ± 1.3</td>
<td>9.4 ± 1.2</td>
<td>7.8 ± 1.2</td>
<td>8.8 ± 1.1</td>
<td>9.4 ± 1.2</td>
</tr>
<tr>
<td>P, mg/dl</td>
<td>4.9 ± 1.5</td>
<td>6.8 ± 1.2</td>
<td>5.4 ± 1.3</td>
<td>5.7 ± 1.7</td>
<td>5.5 ± 2.0</td>
</tr>
<tr>
<td>sBV/TV, %</td>
<td>17.7 ± 3.3</td>
<td>18.3 ± 5.7</td>
<td>18.7 ± 6.6</td>
<td>19.9 ± 6.6</td>
<td>19.2 ± 4.1</td>
</tr>
<tr>
<td>sOvB, V/TV, %</td>
<td>5.1 ± 3.0</td>
<td>8.9 ± 2.4</td>
<td>21.2 ± 5.4</td>
<td>20.3 ± 4.6</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>sFbV, V/TV, %</td>
<td>0 ± 0.17</td>
<td>6.0 ± 7.4</td>
<td>0 ± 8.8</td>
<td>8.8 ± 12.6</td>
<td>0</td>
</tr>
<tr>
<td>sMAR, μm/d</td>
<td>0.62 ± 0.17</td>
<td>0.85 ± 0.40</td>
<td>0 ± 0.59</td>
<td>0 ± 0.25</td>
<td>0</td>
</tr>
<tr>
<td>sBFr/BV, %/yr</td>
<td>28.2 ± 11.3</td>
<td>111.8 ± 92.4</td>
<td>0 ± 68.1</td>
<td>61.8 ± 51.8</td>
<td>0</td>
</tr>
</tbody>
</table>

CGN, chronic glomerulonephritis; DMGS, diabetic glomerulosclerosis; UK, unknown.
sumably because of the significant correlation between sBV/TV and vBV/TV. However, sBV/TV and vBV/TV were not significantly correlated with cortical bone thickness, CoBMD, or CoBMC.

The Three-Dimensional Quantitative Analyses of Trabecular Bone Microstructure

Both V*m and EN/TV showed significant negative correlations with sBV/TV and vBV/TV (Figures 4 and 5). Finally, by NSA, N.Nd/TV, NdNd/TV, and NdNd/TSL were significantly and positively correlated with sBV/TV and vBV/TV (Figure 6).

Discussion

The TMV classification proposes the bone morphometric parameter BV/TV (sBV/TV in this manuscript) as its third assessment criterion. In this study, we compared sBV/TV with sFb.V/TV, sOV/BV, and sBFR/BV, which have been used to assess bone turnover and bone mineralization in conventional two-dimensional bone histomorphometry. However, we found no correlations between these parameters. Moreover, the sBV/TV values were comparable among the five conventional histomorphometric groups that are principally classified by bone turnover and bone mineralization, even though we excluded borderline cases by using strict criteria for bone histomorphometric classification to emphasize the characteristics of each histomorphometric group (Table 1). Thus, sBV/TV is independent of bone turnover or bone mineralization; therefore, it is theoretically acceptable to use sBV/TV as the third assessment criterion in the TMV system.

Generally, the main determinant of bone strength is considered to be bone mass (19,20). Strictly speaking, BV/TV is not identical to cancellous bone mass. The cancellous bone mass should be calculated as the product of cancellous bone volume and cancellous bone mineral density. Therefore, in theory, BV/TV and cancellous bone mass may not necessarily show a significant correlation (21,22) because the distribution of bone mineral is not homogeneous (Figure 2). However, we found that BV/TV and CaBMC show a strong correlation (Figure 3), indicating that sBV/TV reflects the cancellous bone mass in CKD patients.

Nevertheless, we must also consider that it is the cortical bone mass rather than cancellous bone mass that critically affects bone strength. In fact, excluding information obtained from the cancellous bone area improved the accuracy of bone mineral densitometry results in estimating the risk of bone fracture (23,24). Even if BV/TV reflects cancellous bone mass,

Table 2. Clinical characteristics of 47 patients who contributed biopsied iliac bone samples that were subjected to MCT observation and image analyses

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>OF</th>
<th>OM</th>
<th>MX</th>
<th>ABD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men:women)</td>
<td>11 (5:6)</td>
<td>11 (5:6)</td>
<td>5 (2:3)</td>
<td>7 (4:3)</td>
<td>13 (6:7)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>44.1 ± 13.1</td>
<td>49.6 ± 15.3</td>
<td>55.2 ± 12.8</td>
<td>53.6 ± 18.8</td>
<td>54.4 ± 14.0</td>
</tr>
<tr>
<td>Dialysis vintage, M</td>
<td>CGN 9</td>
<td>CGN 8</td>
<td>CGN 2</td>
<td>CGN 6</td>
<td>CGN 9</td>
</tr>
<tr>
<td>Primary disease</td>
<td>DMGS 1</td>
<td>DMGS 2</td>
<td>DMGS 2</td>
<td>DMGS 0</td>
<td>DMGS 4</td>
</tr>
<tr>
<td></td>
<td>Others 0</td>
<td>Others 0</td>
<td>Others 0</td>
<td>Others 0</td>
<td>Others 0</td>
</tr>
<tr>
<td>Intact parathyroid hormone, pg/ml</td>
<td>213.5 ± 93.0</td>
<td>722.4 ± 382.3</td>
<td>191.6 ± 105.3</td>
<td>914.9 ± 539.7</td>
<td>104.0 ± 68.2</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>9.2 ± 1.4</td>
<td>8.9 ± 1.1</td>
<td>7.9 ± 1.5</td>
<td>8.5 ± 1.1</td>
<td>9.5 ± 1.3</td>
</tr>
<tr>
<td>P, mg/dl</td>
<td>5.0 ± 1.5</td>
<td>7.0 ± 1.2</td>
<td>5.5 ± 1.6</td>
<td>6.6 ± 1.5</td>
<td>5.7 ± 2.4</td>
</tr>
<tr>
<td>sBV/TV, %</td>
<td>17.4 ± 3.6</td>
<td>18.3 ± 7.5</td>
<td>18.6 ± 8.1</td>
<td>19.8 ± 6.6</td>
<td>19.2 ± 4.1</td>
</tr>
<tr>
<td>sOV/BV, %</td>
<td>5.1 ± 3.6</td>
<td>8.1 ± 2.1</td>
<td>21.2 ± 5.4</td>
<td>20.3 ± 4.6</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td>sFb.V/TV, %</td>
<td>0</td>
<td>4.5 ± 8.1</td>
<td>0</td>
<td>6.5 ± 6.7</td>
<td>0</td>
</tr>
<tr>
<td>sMAR, μm/d</td>
<td>0.62 ± 0.17</td>
<td>0.77 ± 0.46</td>
<td>0</td>
<td>0.67 ± 0.09</td>
<td>0</td>
</tr>
<tr>
<td>sBFR/BV, %/yr</td>
<td>29.1 ± 10.0</td>
<td>131.2 ± 116.5</td>
<td>0</td>
<td>79.0 ± 39.7</td>
<td>0</td>
</tr>
</tbody>
</table>
we cannot conclude that increased BV/TV, namely, the positive balance between cancellous bone formation and cancellous bone resorption, has a benefit in the patient, because the role of cancellous bone mass is yet unknown.

On the other hand, sBV/TV and vBV/TV were not associated with cortical bone thickness, which is the major determinant of cortical bone volume. Therefore, BV/TV does not indicate the balance between cortical bone formation and cortical bone resorption. Thus, it is unlikely that BV/TV is a proper indicator of the balance between bone formation and bone resorption. Moreover, because sBV/TV or vBV/TV did not indicate CoBMC, estimation of bone mass is also unlikely to offer a reasonable explanation for using BV/TV in the TMV system.

Trabecular bone connectivity is a structural property of cancellous bone that affects cortical bone strength (25). Generally, bone with a well-connected trabecular bone network protects cortical bone by preventing the retention and/or accumulation of local damage by diffusing it immediately through the trabecular bone network. In fact, studies have revealed that bones

Figure 2. Distribution of bone mineral density in the iliac bone block obtained from a CKD5 patient (65-year-old man; osteitis fibrosa type). (A) Tomographic image. (B) Reconstructed virtual three-dimensional image. The color of each pixel represents the bone mineral density in the $35.255 \times 35.255 \times 35.255 \, \mu m$ cubic volume. The bone mineral density was heterogeneously distributed in the calcified tissue.

Figure 3. BV/TV and cancellous bone mass. sBV/TV and vBV/TV were significantly correlated with CaBMC but not with CaBMD.

Figure 4. Three-dimensional analysis of the V*m. sBV/TV and vBV/TV were significantly and negatively correlated with V*m.

Figure 5. Three-dimensional analysis of EN/TV. sBV/TV and vBV/TV were significantly and negatively correlated with EN/TV.
with a well-developed trabecular bone network have increased bone strength (26–29). Trabecular bone connectivity is considered to be an important component of bone quality (30), and the need for its assessment in CKD patients has recently become noticed (24). Thus, the ability to obtain information about trabecular bone connectivity from bone biopsy samples is of considerable value (31,32).

However, programs that assess trabecular bone connectivity are not readily available for many institutions. Moreover, because trabecular bone connectivity is actually a three-dimensional property, there are methodological limitations in assessing trabecular bone connectivity from sliced bone samples (7,33). Thus, trabecular bone connectivity is not currently routinely determined as part of the bone histomorphometric examinations performed on CKD-MBD patients.

Previous reports suggested that BV/TV might enhance trabecular bone connectivity (34,35). Therefore, we examined the ability of sBV/TV, a common trabecular bone microstructural parameter that is routinely calculated in the bone morphometric study for CKD-MBD patients, to estimate trabecular bone connectivity. As a result, sBV/TV was significantly correlated with the results of three representative quantitative trabecular bone connectivity analyses obtained from virtual three-dimensional samples (Figures 4 to 6). Thus, sBV/TV represents the ratio of space occupied by trabecular bone in the region of interest. Increases in the volume of trabecular bone may increase the possibility of contact with nearby trabecular bone, which results in the development of trabecular bone connectivity. However, additional morphometric examinations are required to confirm the mechanism involved in the significant correlation between BV/TV and trabecular bone connectivity in these dialysis patients.

In conclusion, BV/TV is a cancellous bone microstructural parameter that is not dependent on bone turnover or bone mineralization. Therefore, it is appropriate for use the parameter as a third assessment criterion in the TMV system. Because BV/TV did not indicate cortical bone volume, it seems quite unlikely that this parameter represents the balance between bone formation and bone resorption. On the other hand, BV/TV is a practical indicator of cancellous bone connectivity, which is one of the components of bone quality. By using BV/TV as the third evaluation criterion, the TMV classification offers an advantage because it can assess bone quality from a more general aspect than is possible with the conventional system. Further clinical studies are needed to confirm whether this novel bone histology evaluation system will be more useful than the conventional system.

Acknowledgments

The authors are grateful to Ms. Akemi Ito for her excellent technical assistance in conventional bone histomorphometry. This study was partially supported by research grants from the Kidney Foundation Japan and ROD21 research foundation (to J. J. Kazama).

Disclosures

None.

References


Figure 6. Three-dimensional NSA. sBV/TV and vBV/TV were significantly correlated with N.Nd/TV, NdNd/TV, and NdNd/TSL.