Within-Patient Variation of Hemoglobin and Reticulocytes: Implications for Evaluating ESA Responsiveness in Dialysis Patients

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INTRODUCTION

Statistical techniques that assess reticulocytes (retics) or hemoglobin (HB) to detect illicit ESA use in athletes (Sharpe et al. Hematologica 2006) may be applicable in evaluating responsiveness to ESA therapy in dialysis patients. However, little quantitative information is available to guide the assessment of within-patient variation in these analyses in dialysis patients.

OBJECTIVE: To determine, for retics and HB, the appropriate transformation, if any, needed to stabilize variance and render the distribution closer to normal; and, to evaluate the relationship between the length of the interval between blood draws and the magnitude of analyze variation.

METHODOLOGY

- We conducted a prospective, single-arm clinical trial.
- 30 in-center HD patients receiving stable ESA doses underwent HB (g/dL) and retics (%) determinations on 12 consecutive dialysis days, and within-patient results evaluated for variance and distribution with and without transformation.
- In addition to including terms for between and (overall) within-patient variation, the potentially separate effects of analytical variation, and within-patient biological autocorrelation were modelled using the Exponential/Autocorrelation Structure (correlation(d) = (1-nugget)×e-d/range, where d is the time in days between readings, nugget reflects analytical variation, and range reflects the rate at which within-patient autocorrelation approaches zero.
- If SDchange(d) denotes the overall within-subject standard deviation, then SDwithin=√(SDchange(d)) can be interpreted as the analytical standard deviation, while SDwithin×√nugget can be used to determine whether or not a change over days is unusually large.
- The suitability of the assumed autocorrelation structure was assessed by comparing model results to those obtained by calculating the SD of changes directly from the data.
- The effect of data transformation on the homoscedasticity and normality of within-patient variation was assessed by comparing model results to those obtained by calculating the SD of changes directly from the data.
- Our work is based. We thank DaVita Clinical Research (DCR), and specifically acknowledge Karen Spach, PhD of DCR for her editorial contribution, in preparing this poster. DCR is committed to advancing the knowledge and practice of kidney care.

RESULTS

Table 1. Tests comparing effect of no transformation, square root transformation, or natural log transformation on within-patient variation.

<table>
<thead>
<tr>
<th>Transformation</th>
<th>Test for constant variance</th>
<th>Test for normality</th>
</tr>
</thead>
<tbody>
<tr>
<td>% retics</td>
<td>0.023 × 10^-4</td>
<td>2.0 × 10^-4</td>
</tr>
<tr>
<td>sqrt(% retics)</td>
<td>0.038 0.739 0.002</td>
<td></td>
</tr>
<tr>
<td>ln(% retics)</td>
<td>-0.198 0.0002 2.0 10^-4</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: sqrt(normalized residuals) plotted against fitted values using raw and transformed %retics data. Square-root transformation yielded the most constant variance.

Table 2. Parameter estimates for sqrt(%retics) and HB models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test for Autocorrelation</th>
<th>Analytical SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>sqrt(% retics)</td>
<td>-0.198 0.0002 2.0 10^-4</td>
<td></td>
</tr>
<tr>
<td>HB</td>
<td>0.128 0.248 &lt; 0.001 0.232</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Plot of the standard deviations of within patient changes in sqrt(%retics) and untransformed HB values against days between observations. Model estimates closely fit observed values.

Figure 3: ESA induced changes in HB & sqrt(%retics) from the Sydney EPO administration trial (standardised, ESA induced changes from baseline in means of HB and sqrt(%retics); first and last ESA injections at days 0 and 25, respectively.

SUMMARY of RESULTS

- Square-root transformation (sqrt) of %retics produced the most constant variance (correlation between variability and mean closest to zero) and showed the least departure from normality (highest p-value) compared with log transformation or no transformation. Table 1, Figure 1.
- HB results did not improve with transformation (data not shown).
- The standard deviation of within-patient changes in both retics and HB increased with length of the interval between lab draws (highly significant autocorrelation, p<0.001; Table 2, Figure 2).
- The increase in %retics is considerably greater than in HB during days 8-17 after the first injection (Figure 3).

KEY LEARNINGS

- Quantitative assessment of ESA responsiveness in dialysis patients will require square root transformation of %retics and adjustment for length of time between lab draws for both %retics and HB.
- These are the first steps needed to evaluate use of statistical anti-doping tools in athletes for diagnostic testing in ESRD patients.

Our sincere appreciation to the teammates in our nearly 1600 clinics who work every day not only to take care of patients but also to ensure the extensive data collection on which our work is based. We thank DaVita Clinical Research (DCR), and specifically acknowledge Karen Spach, PhD of DCR for her editorial contribution, in preparing this poster. DCR is committed to advancing the knowledge and practice of kidney care.

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