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 expected within-patient variation in these analytes 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 analyte 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 biologic autocorrelation were modeled using the Exponential (Auto)correlation 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 SD_{within} denotes the overall within-subject standard deviation, then $SD_{within}\times\sqrt{(nugget)}$ can be interpreted as the analytical standard deviation, while

  $$SD_{change}(d) = SD_{within}\times\sqrt{(2 - 2\times correlation(d))}$$

  can be used to determine whether or not a change over d 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 examining the relationship between (normalized) residuals and fitted values, and by applying the Shapiro-Wilk test to the (normalized) residuals.
- Data collected, in Sydney, Australia, as part of study to develop a method for detecting illicit use of ESA by athletes, was used to compare the responses of Hb and %retics to ESA. We were unable to locate comparable data on dialysis patients starting ESA therapy.

RESULTS

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

Transformation	Test for constant variance		Test for normality
	correlation (r)	p-value	p-value
%retics	0.215	4.2 10^{-5}	2.2 10^{-5}
sqrt(%retics)	0.018	0.739	0.002
ln(%retics)	-0.198	0.0002	2.0 10^{-5}

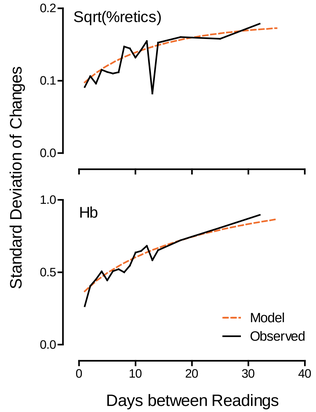

Figure 2: Plot of the standard deviations of within-patient changes in sqrt(%retics) and untransformed Hb values against days between observations. Model estimates closely fit observed values.

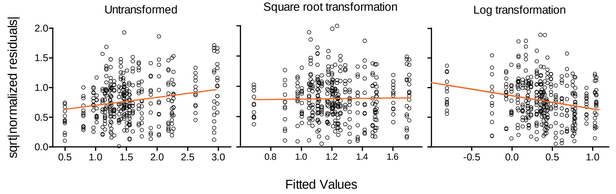

Figure 1: Sqrt|normalized residuals| plotted against fitted values using raw and transformed %retics data. Square-root transformation yielded the most constant variance.

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

Parameter				Test for Autocorrelation	Analytical SD
	SD_{within}	Range	Nugget	p-value	= $SD_{within}\times\sqrt{nugget}$
Sqrt (%retics)	0.128	16.42	0.248	< 0.001	0.064
Hb	0.740	33.22	0.098	< 0.001	0.232

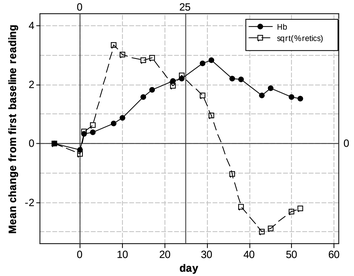

Figure 3: ESA induced changes in Hb & sqrt(%retics) from the Sydney EPO administration trial Standardized, ESA-induced changes from baseline in means of Hb and sqrt(%retics); first and last ESA injections at days 0 and 25, respectively.

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.

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