Correspondence

To the editor:

Units of analysis in accelerated telomere shortening in glycosylphosphatidylinositol (GPI)–negative compared with GPI-positive granulocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH) detected by proaerolysin flow-FISH

The article by Beier et al1 makes an important contribution to the understanding of paroxysmal nocturnal hemoglobinuria (PNH) bone marrow failure syndrome. We are concerned about application of the statistical technique, which was potentially a consequence of a misconception about the study design. We argue that the unit of analysis should have been cell populations rather than patients. In the “Introduction,” the authors state their hypothesis that the glycosylphosphatidylinositol (GPI)–negative hematopoietic stem cells have growth advantage over the GPI-positive ones. Furthermore, the authors clearly state that the objective of the study was to selectively analyze telomere length in GPI-positive and GPI-negative cells from patients diagnosed with the syndrome. The study was executed by comparing telomere lengths from 3 cell populations, GPI-negative and GPI-positive hematopoietic stem cells, and cells from the healthy donors. Finally, in the “Results and discussion,” the authors state that their data support the hypothesis of the growth advantage of the GPI-negative hematopoietic stem cells over GPI-positive ones.

The above argument suggests that the hypothesis-driven unit of analysis should have been the cell populations rather than the patients. Therefore, the appropriate statistical application would have been the single-factor analysis of variance with a post hoc test of significance, such as Tukey HSD (honestly significant difference).2 This statistical approach would have tested for differences among all possible combinations of the GPI-negative/GPI-positive cells from patients with the syndrome and cells from the healthy donors while guarding against a type I error. If the study hypothesis had focused on the differences between patients and the healthy individuals, then the unit of statistical analysis should have been the 2 groups and the application of the Student t test of significance would have been correct. Because of the long-standing relationship between statistics and medicine and because of the inherent variability of biologic data, appropriate experimental designs and statistical techniques are required to draw unbiased conclusions.

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The authors declare no competing financial interests.

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Response:

Telomere shortening in patients with paroxysmal nocturnal hemoglobinuria (PNH) detected by proaerolysin flow-FISH

As pointed out in the correspondence by Stark and Schultz, the aim of our study was the analysis of telomere length in glycosylphosphatidylinositol (GPI)–negative and GPI-positive cells from patients with paroxysmal nocturnal hemoglobinuria (PNH). In order to understand the study design (together with the statistical analysis of the data), 2 important aspects need to be emphasized. First, telomere length represents a highly age-dependent parameter that, in addition, is characterized by substantial, mostly genetic interindividual variability.1,2 Secondly, clone size (ie, the proportion of GPI-negative cells in patients with PNH) is highly variable.3(Tab1) Based on these considerations, we believe that the statistics used in our study are correct and we see no reason to adopt a statistical approach that would ignore the random factor “patient” in a one-way analysis of variance. For the comparison of telomere lengths between GPI-positive and GPI-negative cells in the PNH patients, we applied a paired t test because, as expected based on the strong genetic components of variability in telomere length among individuals, there was a highly significant correlation of telomere lengths between GPI-positive and GPI-negative cells ($r = 0.94, P < .001; n = 14)$.

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References


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