

## Some Brief Remarks on Statistical Significance and “Trends” in Gerontological Research

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**Abstract**—Drawing on his editorial experience at relevant scientific journals and his experience in teaching the basics of scientific article formatting/design at Lomonosov Moscow State University, the author analyzes the current state of statistical analysis of results of the gerontological research aimed at understanding the mechanisms of aging and search for drugs that slow down this process and suppress the development of age-related diseases (geroprotectors, anti-aging drugs). It is emphasized that in many cases, such analysis is either not performed at all or is performed incorrectly. Specifically, authors periodically analyze data that does not follow a normal distribution using a parametric Student’s *t*-test. Moreover, many researchers, having failed to obtain reliable results ( $p > 0.05$ ), increasingly replace the term “significance” in their articles with the term “trend,” which seems completely unacceptable. Unfortunately, this problem affects not only gerontology but all biomedical research. It is suggested that greater attention should be paid to this issue by both reviewers of relevant articles and editors of the journals in which they are published. In addition, the importance of teaching the basics of statistical processing of research results to students and PhD students of biomedical institutes and relevant university schools is emphasized.

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In a recent article [1], I outlined my perspective on the problem of the “correct” definition of aging in the current situation with gerontological research, the success of which we constantly await, but never see [2]. However, of course, this is not the only problem that, in my opinion, is hindering the progress of our “hopeless struggle with senility,” as my recently deceased friend and colleague, Professor V.N. Anisimov, put it [3]. Of no small importance is the correct selection of control objects for experimental gerontological studies [4, 5], who must be healthy and free of genetic abnormalities, as well as exist in normal living conditions. The approaches to statistical data processing used by authors in such studies are also very important. And I would like to focus on the latter issue in this short paper.

Circumstances have developed in such a way that I am heavily involved with two biomedical journals, *Advances in Gerontology* and *Moscow University Biological Sciences Bulletin*. Both journals receive a large number of articles from specialists from a wide range of countries. Unfortunately, in most of these papers, we encounter serious problems related to the statistical analysis of the obtained data. Some of these problems were listed in one of our recent publications [6], initiated by the lecture course “Basics of scientometrics

and the preparation of scientific publications” for PhD students in the School of Biology at Lomonosov Moscow State University (last year, this course became university-wide).

In these lectures, we’ve devoted considerable attention to the need for accurate statistical processing of experimental data published in scientific articles. In fact, in biomedical experiments—for example, on rats—one animal is always heavier than another, even if we are using a completely homogeneous group of individuals. However, these weight differences should be random, and the distribution of animals by this parameter should be normal, i.e., obey the Gaussian distribution. And if we compare control and experimental groups (for example, studying the effect of a potential geroprotector) using the parametric Student’s *t*-test, this approach assumes a normal distribution of animals by the parameter under study in both groups. In high-quality programs designed for statistical data analysis—for example, SigmaPlot (Grafiti LLC, US)—when attempting to run a Student’s *t*-test, the computer first analyzes the data for normality and then evaluates the significance of differences between the experimental and control groups only if the distribution of the physiological parameters under study does not significantly differ from normality. However, it appears that

most researchers limit themselves to the Student's *t*-test built into MS Excel, which doesn't include any normality check. And they are often simply unaware of the existence of nonparametric tests of statistical analysis.

I once asked one of my wonderful teachers from the School of Biology at Lomonosov Moscow State University, the world-renowned biochemist Professor A.D. Vinogradov, why he had stopped publishing in a fairly popular, highly ranked scientific journal. In response, he showed me several illustrations from articles published in that journal, featuring histograms with bars of varying heights lacking the error bars that indicate dispersion in the data. As Vinogradov said, “If there are no error bars, then there are no correct results.” But for some reason, readers of such articles don't seem to mind. Moreover, as the author of one microbiology paper submitted to one of our journals told me, “In microbiology, statistical analysis of the obtained data is not customary.” And this despite the fact that the paper presented the results of a comparison of the effects of various culture media on bacterial growth! How one could draw any conclusions from such a paper without using statistical methods is beyond me. Moreover, in response to our comments, the author sent us several of his previously published articles in quite respectable journals, where statistical processing of the results was indeed absent!

It is well known that to obtain reliable/significant results, the number of animals used in gerontological studies must be sufficiently large. When constructing survival curves, at least 50 animals must be used in each group. Otherwise, the “tails” of such curves will be unworkable (there will be too few animals for analysis). When testing geroprotectors using biological age indicators, the number of objects in both the control and experimental groups might be smaller, but still sufficient to draw accurate conclusions about the effect of the drug under study on aging and lifespan. The same applies to the number of cells, culture flasks, and hemocytometers (Goryaev's chambers) in cyto-gerontological experiments [7, 8]. I don't believe the explanations of some colleagues who tell me things like, “These animals are very expensive and rare, so we had no more than 2–3 individuals in each group” are justified. However, they, nevertheless, easily publish their results in high-ranked journals, especially if these journals receive large APCs from the authors and, sadly, are interested in turning a blind eye to problems with statistical processing of the results [9, 10]. However, even if the animals are not “very expensive,” the authors may not mention at all their number in the experiments, but still have no problem publishing in one of the most prestigious scientific journals [9].

And the most important thing is what the title of this article states. The sheer number of scientific articles whose authors, having failed to obtain significant results ( $p > 0.05$ ), calmly note the “obvious trend” they have discovered regarding the effect of the drug

being studied on certain living organisms or cultured cells is simply appalling. I especially like the statement: “Yes, we have  $p > 0.05$ , but just a little bit greater, so we think we have definitely detected an effect.” I have always believed that the absence of significant differences between the experimental and control groups simply indicates that such differences simply don't exist. If the authors truly have reason to believe that differences should exist, then they need to significantly increase the sizes of the control and experimental groups and rerun the experiments. Moreover, as noted above, colleagues' reactions to such comments are surprising: “But we've already published similar results in very reputable journals, and they were all accepted without issue.” We checked—they really did publish them, and in very reputable journals from Q1. It is unclear where the reviewers and editors were looking in such cases.

As is well known, very expensive and lengthy longitudinal studies can yield far more interesting and important for gerontology results than cross-sectional studies. This is how correlations are identified between various physiological indicators or people's habits and their morbidity, mortality, and lifespan (longevity). However, the factor analysis and statistical analysis of the results obtained in such studies must be performed by highly qualified specialists in the field of mathematical processing of biomedical data. I certainly do not consider myself one of them, so I still do not fully understand why gerontologists consider the relatively small correlation coefficients obtained in such studies worthy of attention. For example, I find it strange that, according to data from “The Duke First Longitudinal Study,” work satisfaction is a strong predictor of longevity for men ( $r = 0.28$ , which, in my opinion, is too low) [11]. However, I also don't quite understand the approaches that lead many gerontologists to consider minimally significant differences in the survival curves of control and experimental groups of animals in experimental gerontological studies to be of interest: “Yes, both average and maximum lifespan increased by only 0.8%, but this increase, according to statistical analysis, is significant and indicates the effectiveness of the geroprotector we studied.” I don't rule out the possibility that I simply lack the qualifications to understand such results.

If we try typing “Statistical analysis in gerontology” into Google Scholar, we get a very large number of links to various scientific papers. However, in the vast majority of cases, the words “Statistical analysis” will lead us only to the “Materials and Methods” sections, which simply describe the procedures for processing the relevant data, as well as the statistical programs used for such processing. Finding serious articles on the Internet devoted to the methodological issues of using statistical analysis specifically in gerontological research is not easy, although it is still possible—even in the form of specialized monographs [12, 13].

Unfortunately, in them I was still unable to find all the answers to the questions posed in this article.

However, entering the words “Statistics in biomedical research” into the Google Scholar search bar already yields several million links to articles and books [14–18] that could well be used by gerontologists. For example, the book *Nursing Research and Statistics* [18]. However, the vast majority of authors at the journals I work with apparently have little interest in such sources of information.

### CONCLUSIONS

The situation with statistical data analysis in experimental gerontological studies, unfortunately, seems unsatisfactory to me. In a large number of studies, the results obtained are either not subjected to statistical analysis at all or are analyzed incorrectly, and in many cases, the authors easily replace the concept “significance” with the concept “trend.” And this is happening in studies devoted to the search for drugs against aging and age-related diseases! Erroneous conclusions in such studies can cost us dearly. And countless examples of this can be easily found online. Just think of the endless missteps in the search for drugs against Alzheimer’s disease [19]!

I believe this issue needs to be given greater attention by both reviewers of relevant articles and editors of the journals in which they are published. Furthermore, I would like to emphasize the need to teach the basics of statistical analysis of research results to students and PhD students at biomedical institutes and relevant university schools, including those who intend to pursue experimental gerontology in the future.

However, I could very well be wrong in my understanding of the issue discussed in this article. I would very much like to hear the opinions of “real” experts in this field. We would be happy to publish their perspective on this issue in the journal *Advances in Gerontology*.

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### CONFLICT OF INTEREST

The author of this work declares that he has no conflicts of interest.

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