The Time Delay Between *In Vivo* Imaging and *Postmortem* Data Poses a Caveat on "No Link" Findings

I read with interest the article by Honkanen and colleagues[1] on the question of correlation between striatal dopaminergic innervation and dopamine transporter (DAT) imaging as assessed by putamen tyrosine hydroxylase–positive axon counts and DAT single-photon emission computed tomography (SPECT). In their 14 patients with neuropathologically confirmed Parkinson's disease or atypical parkinsonism, specific binding ratios (SBRs) from SPECT did not correlate with the total putamen tyrosine hydroxylase-positive fiber counts. This is very surprising given that DATs are densely present on dopaminergic axons with varicosities,[2,3] and nearly all striatal

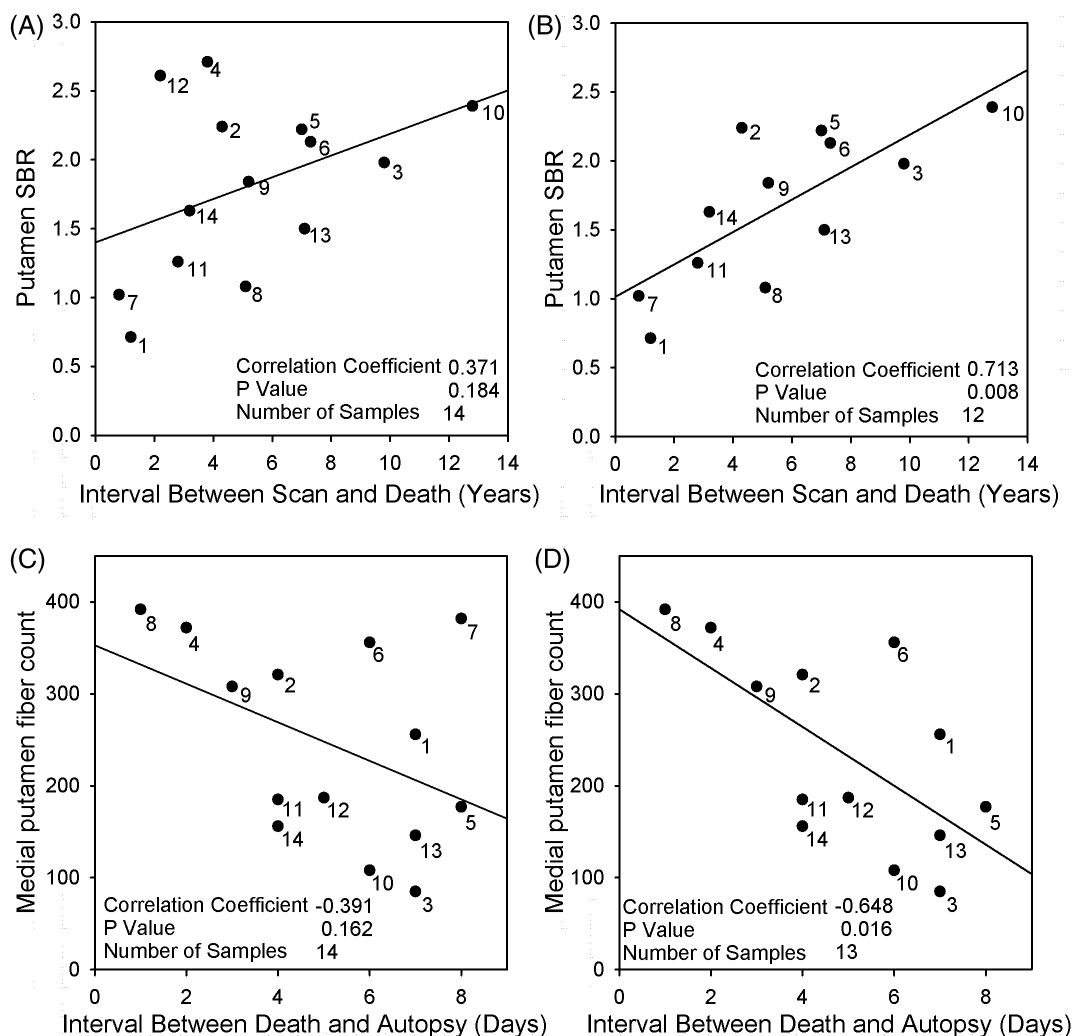

FIG. 1. (A,B) Scatter plot presenting a positive correlation between the putamen SBR calculated on DAT SPECT data and the interval between the time of the respective scan and death for all 14 cases (A) and for 12 of these cases (B). (C,D) Scatter plot presenting a negative correlation between the medial putamen fiber counts and the interval between death and autopsy for all 14 cases (C) and for 13 of these cases (D).

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tyrosine hydroxylase is contained in axons of the dopaminergic mesostriatal pathways.[4]

The key to the discrepancy between lack of correlation in Honkanen and colleagues and the well-established cellular coexpression of DAT and tyrosine hydroxylase in the striatum may lay in the fact that a correlation was investigated between an *in vivo* (SBR) and a *postmortem* parameter (tyrosine hydroxylase–positive axon counts) with a considerable time interval in between. Obviously, a long interval between scan and death means an imaging analysis earlier in the disease process with still high SBRs for DAT and therefore lets one expect higher SBRs. Furthermore, a long interval between death and autopsy affects the tyrosine hydroxylase–positive axon counts: Impairment in immunohistochemical stains can be caused by postmortem delay attributed to autolysis and enzymatic activation.[5] The individualization of the data, together with the individual intervals between scan and death and between death and autopsy in Honkanen and colleagues, enables the reader to make a correlation between the *in vivo* and *postmortem* findings and the two time intervals. And, in fact, it turns out that the data of SBRs and intervals between scan and death reveal a positive correlation coefficient of 0.371 with a P value of 0.184 (Fig. 1A), and omission of two cases results in a highly significant correlation of 0.713 with a P value of 0.008 (Fig. 1B; Spearman rank-order correlation). Furthermore, as predicted, there is a negative correlation coefficient of –0.391 between tyrosine hydroxylase–positive fiber counts and interval between death and autopsy (varying between 1 and 8 days!) with a P value of 0.162 calculated for the 14 patients (Fig. 1C), and omission of just one case results in a significant correlation of –0.648 with a P value of 0.016 (Fig. 1D).

If the two parameters, SBR and fiber counts, are biased to such a high extent by time variables, it is not surprising that no correlation was detected between them, and, for such a low number of cases, this problem cannot be resolved by calculations using death to autopsy as covariates. Therefore, the conclusion that striatal DAT imaging may not reflect striatal dopaminergic projections axons cannot be drawn from this study.

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References

1. Honkanen EA, Saari L, Orte K, Gardberg M, Noponen T, Joutsa J, Kaasinen V. No link between striatal dopaminergic axons and dopamine transporter imaging in Parkinson's disease. Mov Disord 2019 Jun 24. https://doi.org/10.1002/mds.27777. [Epub ahead of print].
2. Ciliax BJ, Heilman C, Demchyshyn LL, et al. The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci 1995;15:1714–1723.
3. Freed C, Revay R, Vaughan RA, et al. Dopamine transporter immunoreactivity in rat brain. J Comp Neurol 1995;359:340–349.
4. Graybiel AM, Hirsch EC, Agid YA. Differences in tyrosine hydroxylase-like immunoreactivity characterize the mesostriatal innervation of striosomes and extrastriosomal matrix at maturity. Proc Natl Acad Sci U S A 1987;84:303–307.
5. Lundström Y, Lundström P, Popova SN, Lindblom RPF, Alafuzoff I. Detection of changes in immunohistochemical stains caused by postmortem delay and fixation time. Appl Immunohistochem Mol Morphol 2019;27:238–245.

Reply to: The Time Delay Between *In Vivo* Imaging and *Postmortem* Data Poses a Caveat on "No Link" Findings

We thank Dr. Christian Pifl for his interest in our article[1] and for his comments.[2] We agree that the inevitable delays in clinicopathological studies are an important factor to consider when evaluating the results of correlations with clinical parameters. This is the reason why we chose to present raw individual values and delays for the reader to evaluate. However, we do not agree with the interpretation that the conclusions are not valid because of delays and explain our arguments below.

In our primary analyses, there were no correlations between putamen-specific binding ratios and total putamen fiber counts in all patients (r = 0.00, P = 1.0, n = 14), in PD patients (r = 0.07, P = 0.86, n = 10) or in patients with shorter intervals (mean, 3.2 years) between single-photon emission computed tomography and death (r = 0.21, P = 0.62, n = 9). Although there were no significant correlations, we controlled for possible confounding effects of scan-to-death, death-to-autopsy and death-to-neuropathology intervals by including them as covariates in the analyses. It is important to point out that the letter[2] showed scatterplots in just one selected medial putaminal region (numerical values included as additional data for the reviewers of the article, available on request), whereas our main result in the article is the TH+ axon count in the whole putamen (although fibers were calculated in 9 subregions). Nevertheless, the correlations were clearly nonsignificant (panels A and C), and similar correlations can be detected by chance in 16%–18% of tests, even when not considering a multiple-comparison problem. The correlations became significant only after selectively removing data points based on their outcome variable values (in panels B and D).

It is crucial to note that our results do not imply that there are no dopamine transporter (DAT) in striatal dopaminergic axons. Rather, they point out that when a tropane-derivate in vivo DAT tracer, such as FP-CIT or β-CIT, is used in patients with fully established motor-PD, the tracer binding may not reflect the number of dopaminergic neuron fibers. However, we acknowledge the limitations of our study (see the discussion

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