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Comments on the letters by Bruce Quinn and Douglas Miller:

Michael D. Norenberg

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Synaptophysin Staining for Ganglioglioma

Patel et al (1) recently evaluated neuroradiologic features in a subset of their series of 78 spinal cord gangliogliomas, a series that triples the published cases extant only a few years ago (2). As their key premise, the authors claim they now view certain patterns of synaptophysin immunostaining as unique for diagnosis of gangliogliomas. They state that this synatophysin staining is always invoked when an astrocytoma vs. ganglioglioma diagnosis is disputed after thorough review of all available hematoxylin-eosin stains. They emphasize that in a 1993 study, ''21 [gangliogliomas] originally diagnosed as astrocytomas at our institution were only recognized as gangliogliomas by using immunohistochemical analysis, and 23 of 25 gangliogliomas originally examined at outside institutions were called astrocytomas until we performed immunohistochemical studies.'' Biopsies that are determined to be nondiagnostic for ganglioglioma vs. entrapped neurons, even in the eyes of experienced neuropathologists, are exactly the cases in which any interpretation contributed by positive neuronal synaptophysin staining is most likely to be deceptive (3, 4).

The authors fail to cite a detailed four-page study published 2 years ago that challenged the concept that any pattern of synaptophysin immunostaining was unique to spinal cord gangliogliomas (3). Instead, the authors refer to an incorrect edition of a general pathology textbook (their reference 51). They provide the reader with other potential cause to be skeptical of their synaptophysin criteria; an article that does not use synaptophysin staining is cited (their reference 53). To prove their contention that synaptophysin-positive neurons never occur in spinal cord white matter, and must always appear in a background of fine neuropil, the authors cite an abstract (5). The citations in this part of the paper are incorrect or irrelevant. Readers interested in the validity of synaptophysin staining for ganglioglioma diagnosis should refer directly to Zhang and Rosenblum; a more recent article of mine extends this study by analyzing synaptophysin immunostaining in the diencephalon and brain stem (4).

Patel et al argue that synaptophysin-positive neurons are unique to gangliogliomas. This is simply not the case. Synaptophysin-positive neurons are widely distributed in the normal spinal cord (3), and clusters of synaptophysin-positive neurons can be found literally embedded in white matter in the normal medulla and cervicomedullary region (4). Figure 1A–B shows single and clustered synaptophysin-positive neurons in the white matter of normal cervical spinal cord. Such neurons were easily found in adult cord obtained 6 hours postmortem; tissues were fixed for 4–6 hours to simulate fixation conditions likely to be encountered in the type of neurosurgical biopsy tissues immunostained by Patel et al. There is no question that native neurons, once entrapped in tumors, can be intensely synaptophysin-positive. Figure 1C–D illustrates the dorsal medulla of an adult woman with malignant lymphoma. This case shows large, synaptophysin-positive native neurons becoming entrapped as tumor infiltrates and effaces the medial nucleus ambiguous. Again, there is no synaptophysin-positive neuropil background as Patel et al claim must exist when native neurons are entrapped in infiltrating tumor (Fig. 1D). Thus, synaptophysin-positive neurons may be found in spinal cord gray matter (3), in white matter, and in unpredictably synaptophysin-negative or synaptophysin-positive background as various zones of spinal cord are infiltrated or effaced by gliomas.

The authors find that spinal cord gangliogliomas are the second-most common intramedullary spinal cord tumor. The authors propose that most spinal cord gangliogliomas show only limited neuronal foci and have previously been misdiagnosed as astrocytomas because of undersampling. The basic proposition seems unlikely, but other authors have found that only 15% of gangliogliomas have purely astrocytic zones (6). Large autopsy series of brain and spinal cord tumors have never reported higher rates of gangliogliomas (7). Some of the neurora-

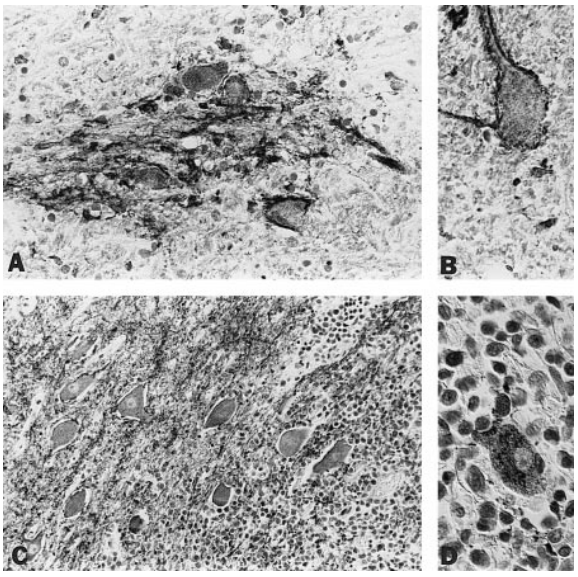

Fig 1. Immunohistochemical study† of a normal cervical spinal cord of a 75-year-old man (*A* and *B*), and of a dorsal medulla of a 79-year-old woman with malignant lymphoma (*C* and *D*).

A, A small cluster of spinal cord neurons, with cellular and superficial synaptophysin reactivity, is embedded in white matter. (original magn. ×330)

B, A cervical spinal cord neuron, synaptophysin-positive, is embedded in white matter. (original magn. ×500)

C, Native neurons of dorsal medulla, synaptophysin-positive, span the borderzone with lymphoma. (original magn. ×330)

D, Neurons are slightly deeper in the tumor. (original magn. ×500)

† For staining methods, see reference 4.

diologic findings of Patel et al can be explained. The most biologically infiltrative astrocytomas, studied in increasingly large resections, are more likely to engulf native neurons focally, a circumstance that is congruent with the authors' findings of only focal neoplastic neurons in their numerous gangliogliomas. Tumors less likely to show edema on neuroimaging would possibly be more likely to maintain surviving, entrapped, synaptophysin-positive native neurons at least focally within a large resection volume. Thus, a ganglioglioma diagnosis could select for the larger and more freely infiltrative tumors that might share characteristic neuroimaging and high-recurrence patterns. Patel et al mention that, in their series of 174 spinal cord tumors, not one pilocytic astrocytoma was recognized. Nonetheless, the most circumscribed, least infiltrative astrocytomas (perhaps those entities interpreted by other groups as common spinal cord pilocytic astrocytomas) would be less likely to show entrapped neurons, and would have different imaging and recurrence characteristics.

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Reply:

Quinn, in his comments on the pathologic diagnostic procedures described in our paper, distorts our position. He ignores part of our record of published criteria and methods, and criticizes our discussion of the extant literature in regard to synaptophysin immunostains of normal and abnormal neuronal cells in tumors of the spinal cord. An unfortunate set of typographic errors in our citations, missed in editing the proofs, add to this problem, although the text should have made our intentions clear. We appreciate the opportunity to correct these citation errors and clarify the record.

Quinn states that we ''fail to appropriately cite'' the Zhang and Rosenblum study of synaptophysin staining in normal human spinal cord (1). In fact, in the Discussion, we clearly indicate on page 884, the second column, ''Recently, the diagnostic utility of this pattern [of synaptophysin immunostaining] has been questioned by Rosenblum and others.'' Our references 51 and 53, however, have unfortunate errors. We apologize for these, and regret any confusion they may have created. Reference 51 is to a chapter by Rosenblum in a major surgical pathology text. In the reference list, the citation is correct except that the year is given as ''1966'' when it should be ''1996.'' Reference 53 should have referred to the Zhang and Rosenblum article cited by Quinn (1). That this was our intention was clearly understood by Dr. Norenberg in his accompanying commentary (2) in which he also discusses this controversy, and notes, ''while Patel et al reasonably address these concerns, appropriate caution is still necessary.''

Otherwise, we stand by our diagnoses. As noted, the tumors diagnosed come from a large series of pediatric intramedullary spinal cord tumors. The data from our histologic analyses have been presented at a meeting (3), but are currently still under review, and thus remain unpublished. Two neuropathologists (Douglas C. Miller, Lucy B. Rorke) evaluated all of the cases, and agreed on the diagnoses. Most of the diagnoses of ganglioglioma were made prior to examination of synaptophysin stains, and these stains were adjunctive or confirmatory in this majority of the cases we report. We found, as stated in our paper, that the radical resections of these tumors by our senior neurosurgical author (Fred Epstein) provided us with much more tissue from spinal cord tumors than is typically made available for pathologic analysis at other centers. This was of great importance in recognizing gangliogliomas. Quinn expresses doubt that neoplastic neuronal cell clustering in gangliogliomas occurs sufficiently often to obscure the diagnosis in smaller samples and cites one study (4) to affirm his position. As cited in our paper, most published series of gangliogliomas point to the tendency for the neuronal cells to cluster within entire zones lacking significant neuronal elements. Our references include the relatively large sets published by Diepholder and Isimbaldi (5, 6), and the more recent ones by Hirose and Büttner (7, 8). The Wolf article Quinn cites (4) in fact states that in 21 (33%) of 64 tumors the neurons were sparse, recognizing neuronal cells by special staining aided the diagnosis of ganglioglioma. These included immunostains for synaptophysin.

We emphasized the importance of radical resection specimens to the diagnosis of gangliogliomas in our original series (9); 20 biopsies were performed outside of our center. Biopsies showed only pure gliomas, and the identification of the tumors as gangliogliomas followed examination of radical resection specimens obtained at New York University Medical Center. In our discussion we explicitly stated that 71% of radically resected gangliogliomas in our series were identified by he-

matoxylin-eosin staining alone, whereas even with our own pathologic review, only 9% of biopsies ultimately shown to come from gangliogliomas were correctly identified from the biopsy itself. We concluded that, ''the increased sensitivity and accuracy of histopathologic diagnosis provided by the synatophysin immunostaining pattern, and extensive sampling were both important in diagnosing gangliogliomas.''

Furthermore, we carefully stated that the perikaryal surface immunoreactivity for synaptophysin we used to diagnose gangliogliomas was ''virtually unique'' for gangliogliomas, and we noted the limited exceptions in the spinal cord by citing Zhang and Rosenblum (1). We noted identical results that have been reported by others (5, 6, 10), and can now add a study by Wolf et al (4) and two newer studies, by Hirose et al and Büttner et al (7, 8). Quinn misconstrues the term ''virtually unique'' for ''literally unique'' in his current letter.

Quinn's own studies, as reflected in the illustrations accompanying his letter, do not distinguish cytoplasmic immunoreactivity from this perikaryal surface pattern (see his Fig. 1A, C, and D). We have never used cytoplasmic staining for the diagnosis of ganglioglioma because it has long been clear that any interruption in axonal transport in native neurons results in perikaryal and axonal cytoplasmic immunopositivity for synaptophysin. For Quinn to use these ''positive'' neurons as part of his argument is inappropriate. Our observations on a much larger number of specimens do not match Quinn's findings of neuropil positivity around ''normal'' neuronal nuclear clusters in the cord or stem. He has furnished only high-power photomicrographs; there is no way to verify his observations from his figures, except to note that Figure 1A has an immunopositive neuropil.

One of the main points we have consistently made about synaptophysin immunostaining for the diagnosis of ganglioglioma is that, in some cases, the large cells in the tumor samples are originally thought to be large tumor astrocytes, and it is only with the immunostains that they are recognized as neurons. The issue of trapped normal neurons in a pure glioma vs. neoplastic neurons of a ganglioglioma can indeed be difficult, but in our experience, was rarely a problem. For Quinn to express such doubt about our entire set of diagnoses based on an argument that synaptophysin-positive neurons might be normal trapped elements is a gross misperception of the nature of the pathologic materials on which our diagnoses are based.

Quinn expresses surprise at the number of gangliogliomas we reported, which ''triple the published cases extant only a few years ago.'' As Quinn well knows, the senior neurosurgical author (Fred Epstein) has a unique practice with an international referral base for which he performs a large number of spinal cord intramedullary tumor resections each year. Indeed, from 1978 through 1994 there were 226 such operations on 174 children alone, with a substantial additional number not yet quantified from our records in adults. We have viewed our opportunity to review these pathologic specimens as a unique opportunity, as this must be the largest such collection of surgically resected intramedullary cord tumors anywhere.

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Comments on the letters by Bruce Quinn and Douglas Miller:

Patel et al (1) described an unusually large number of spinal cord gangliogliomas. This finding prompted the letter by Dr. Quinn who challenged the accuracy of diagnosis because it appeared to him that these were largely based on the pattern of synaptophysin immunochemistry. Although Miller, as well as other pathologists, found the latter pattern of immunohistochemistry useful, Zhang and Rosenblum (2) and Quinn (3) have challenged the

accuracy of synaptophysin staining for the diagnosis of ganglioglioma. Miller responds that synaptophysin immunohistochemistry was only used adjunctively or for confirmation. It is fair to conclude that reliance on synaptophysin immunohistochemistry alone for the diagnosis of ganglioglioma is not justified at this time. This issue is clearly controversial that we hope future studies will clarify. I, however, see little reason to doubt the diagnoses that have been rendered by two senior, highly experienced neuropathologists with extensive experience in neurooncology (Drs. Miller and Rorke). Miller and Rorke based their diagnoses largely on more traditional histologic evaluation, and immunochemical study for glial fibrillary acidic protein to distinguish ganglion cells from abnormal appearing astrocytes.

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