Cell Proliferation and Tumors of the Central Nervous System, Part II: Radiolabeling, Cytometric, and Immunohistochemical Techniques

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Abstract. A variety of techniques have been developed to evaluate cell proliferation. Many of these methods provide a more accurate means of assessing the true proliferation rate of a given neoplasm, as compared with the simple assessment of mitotic activity. Similar to the evaluation of mitotic activity, these methods are also subject to limitations associated with tumor heterogeneity and interobserver variability. This paper reviews a variety of methodologies including radiolabeling, flow cytometric, and immunohistochemical that have been used in recent years to evaluate cell proliferation in brain neoplasms. Factors that affect these methodologies and their practical application to routine practice of diagnostic neuropathology will be explored.

Key Words: Brain tumors; Bromodeoxyuridine; Cell proliferation; Ki-67; MIB-1; PCNA; Thymidine labeling.

INTRODUCTION

A variety of techniques have been employed in recent years to augment our ability to evaluate cell proliferation in central nervous system (CNS) neoplasms. Although generally more labor intensive and expensive than mitosis counting, these techniques often provide a more accurate reflection of the real cell proliferation of a given tumor and may provide additional useful information with regard to predicting tumor behavior. Methodologies employed have varied and have included a variety of radiolabeling techniques, flow cytometric analysis, and immunohistochemical stains. Most of these methodologies have been used at one time or another in the evaluation of CNS neoplasms in a research venue. The time required to perform some of these procedures and, in some instances, the cost, may limit their utility in routine practice and result in their obsolescence or continued relegation to the research arena. However, some of the immunohistochemical based markers, most notably Ki-67 or MIB-1, are relatively easy to perform, are fairly inexpensive, and can be performed in a reasonably short period of time. Consequently, these markers have proven useful in certain circumstances in the evaluation of CNS neoplasia.

Thymidine Labeling

Tritiated thymidine labeling (³H-TdR) is one of the earliest developed ancillary means of assessing cell proliferation. The technique has the advantage of serving as a direct morphologic assessment of cell proliferation. The technique is based on the cellular incorporation of a radioactively labeled DNA precursor and provides an accurate measurement of cells in the S-phase of the cell cycle, in contrast to the morphologic identification of mitotic figures of M-phase or the more broad cell cycle distribution of immunohistochemical markers such as Ki-67 and PCNA (Fig. 1a). The methodology also allows for the measurement of cell cycle duration (1). This can be accomplished by biopsying tissue at known intervals of time and assessing cell cycle segment durations from percent labeled mitosis curves generated from this data (double labeling technique using ¹⁴C-TdR) (2).

The earliest methods for assessing cell proliferation using this approach involved infusing patients, via a vascular route, with a radioactive reagent prior to surgery (3, 4). Tissue harvested at surgery is processed routinely with formalin-fixation and paraffin-embedding. In early work done by Hoshino, it was established that labeling indices of low-grade gliomas was generally lower as compared with glioblastoma multiforme (3, 4). More recently, an in vitro methodology involving tissue samples has been utilized (5, 6). Since the incorporation of radio-labeled tritiated thymidine requires actively proliferating cells, freshly excised tissue must be utilized. Tissue sections are incubated with the tritiated thymidine prior to routine fixation. Potentiation of tritiated thymidine uptake can be accomplished by incubation in a hyperbaric environment or by use of 5-fluorouridine or 2'-deoxy-5-fluorouridine. Microscopic slides are generated and developed by autoradiographic methods. A labeling index is determined by calculating the percentage of positive tumor cells. In an evaluation of 33 gliomas, including 12 glioblastoma multiforme, 7 anaplastic astrocytomas, and 14 low-grade astrocytomas, Broggi et al found a correlation between labeling indices and histologic grade (5).

Similar to other methodologies that will be discussed, issues of interobserver variation in terms of interpreting positivity, tumor heterogeneity, and tissue sampling are all important factors to be considered in the utilization of this methodology (7). A requirement for fresh tissue also requires forethought in employing this technique. Another serious drawback is requirement for use of a radioactive isotope, which has a long half-life of approximately...
Cell Cycle Distribution of Cell Proliferation Markers

(A) Tritiated thymidine/bromodeoxyuridine
(B) Histone mRNA in situ hybridization
(C) Flow cytometry
(D) DNA polymerase alpha
(E) Topoisomerase II-alpha
(F) p105
(G) PCNA
(H) Ki-67 / MIB-1

Shaded area corresponds to distribution of cell-cycle marker of cell proliferation.

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12 yr. The long incubation times required to develop the slides (often a week to 10 days) also precludes generation of information in a timely fashion that can be used in the clinical decision making process.

Bromodeoxyuridine

Bromodeoxyuridine (BrdU) is a thymidine analogue which, when initially developed, was technically similar to the tritiated thymidine procedure in that it used radioactive materials. More recently, however, monoclonal antibodies specific for 5-BrdU have been developed that do not require use of a radioisotope and autoradiography procedure (8). Similar to tritiated thymidine labeling, BrdU provides a measurement of the S-phase fraction of the cell cycle (Fig. 1a). Studies examining thymidine labeling in BrdU have noted similar results between the methodologies (9). Nonradioactive methodologies for evaluating BrdU include immunohistochemistry, immunofluorescence procedures, or flow cytometry. Meyer et al demonstrated that comparable results are attainable in CNS neoplasms between in vivo (radioactive) BrdU administration and the in vitro assay (10). Utilization of immunofluorescence or flow cytometric procedures obviously adds additional quality control variables to the procedure that need to be kept in mind. Again similar to thymidine labeling, the BrdU procedure requires fresh, viable tissue and some forethought in that regard. Certainly, the nonradioactive methodology is much quicker to perform and affords better definition of labeled nuclei, which may be sometimes obscured by silver grains when utilizing the radioactive technique. Although the results between in vitro and in vivo assays are generally comparable for tumors of the same grade, labeling indices using the in vivo method tend to be consistently higher (10). This can be due to a variety of factors, including limitation of tissue penetration. This is related to the time required for diffusion of the DNA precursor to reach the cells. Cells not at the periphery or surface of the slice may progress through DNA synthesis and can complete synthesis before the precursor can reach them. Delays in incubation may also result in a variability of labeling. Since only selective slices are labeled by the in vitro method, there clearly is a selection bias. This is further compounded by glioma heterogeneity; the selected tissue may not necessarily represent the most proliferative area of the tumor.

A number of studies have reported results with BrdU labeling in gliomas and have demonstrated a correlation between labeling indices and histologic tumor grade (11–14). In a series of 47 low-grade astrocytomas reported by Hoshino et al, parametric analysis showed that BrdU labeling indices of <1% were associated with shorter survival and that the labeling index was an important prognostic factor evaluating low-grade astrocytomas (15). Lamont et al, in an evaluation of 190 primary gliomas and 149 recurrent tumors, noted that the labeling index was predictive of patient outcome both initially and at the time of recurrence (14). Interestingly, in the highest-grade tumors (grade IV gliomas), the labeling index score was the only predictor for survival. Ritter et al evaluated a series of 55 primary and 49 recurrent glioblastomas from 98 patients and noted no association between labeling index and survival for either group of tumors (16). This seems to support the notion that there is a decreased association between BrdU labeling indices and patient survival in the high-grade tumors.

Nuclear Organizing Region Analysis

Nuclear organizing regions (NOR) are segments of DNA that code for ribosomal RNA (rRNA). NOR are situated on the short arms of chromosomes 13, 14, 15, 21, and 22. During interphase, the NOR forms 18S–28S loops of DNA that are associated with proteins, such as RNA polymerase I, and form rRNA. NOR comprise a portion of the nucleolus and are important in regulating protein synthesis, cell growth, and cell differentiation. Evaluation of NOR is not specifically a measurement of cell proliferation (cell cycle), but studies have demonstrated a correlation between NOR measurements and cell proliferation in tumors (17, 18).

One of the most common ways of visualizing NOR involves a silver staining methodology (AgNOR). The procedure is relatively easy to perform and uses formalin-fixed material. Drawbacks to the methodology include problems in interpreting overlapping AgNORs and issues related to thickness of tissue sections (thick sections may result in difficulty identifying AgNORs related to focusing; thin sections may result in lower counts). Similar to other counting methodologies, selection bias regarding sections that are chosen for evaluation, tumor heterogeneity, selection of cells to count, and interobserver variability can all affect observed results. Utility of the method may lie more in its ability to discriminate between nonneoplastic conditions (gliosis) and tumor (glioma). Several studies have suggested a difference in the amount and quality of AgNOR between normal and reactive CNS...
cells versus tumor cells (19–21). Korkolopoulou noted a significant correlation between PCNA labeling indices and AgNOR numbers with respect to histologic grade in the 82 CNS tumors they evaluated with both techniques (22). In general, the direct correlation between AgNOR and cell proliferation is variable and not as strong as the correlation between AgNOR and tumor grade (22–26). The correlation with grade (and to some degree cell differentiation) may be reflective of increased protein production in higher grade neoplasms and the increased likelihood of aberrant nucleoli related to genetic abnormalities that are often more prevalent in higher grade tumors. The correlation of AgNOR with cell proliferation may be related indirectly to higher rates of protein production associated with a rapidly proliferating tumor (which is more likely higher grade). Nicoll and Candy showed no correlation between AgNORs and postoperative survival in a series of glioblastoma multiforme (27).

**Histone In Situ Hybridization**

Similar to tritiated thymidine and bromodeoxyuridine, histone H3 and/or H4 mRNA in-situ hybridization can be used to specifically evaluate proliferating cells confined to the S-phase of the cell cycle (Fig. 1b) (28–30). Histones (H2A, H2B, H3, H4) are a group of nuclear proteins whose synthesis is restricted to the S-phase of the cell cycle. Histone mRNA is very rapidly degraded, with a half-life of only 10 min (31); thus, visualization of histone mRNA is quite specific for the S-phase. The technique has the advantage of not requiring radioactive material and it can be easily performed on formalin-fixed material. Disadvantages of the technique may be related to loss of mRNA or failure of the probe to reach target mRNA when archival material is used, resulting in undercounting.

Stenger et al showed a correlation of histone H3 gene expression with mitotic index in a series of pediatric brain tumors (32). Rautiainen and colleagues demonstrated a strong correlation between histone (H2B, H3, H4 cocktail) mRNA-labeling indices and mitotic index in a series of 71 astrocytomas, suggesting that most astrocytoma cells which enter the S-phase ultimately undergo mitosis as well (33). The same study also showed a correlation with PCNA and MIB-1 immunostaining and in multivariate survival analysis, the histone mRNA labeling index was an independent prognostic marker.

**Flow Cytometry/Image Cytometric Analysis**

Flow cytometric and image cytometric analysis of cell proliferation is predicated on an assessment of DNA content of cells (34). Staining of DNA is accomplished using a Feulgen reaction or a fluorescent marker such as propidium iodide. The stains bind stoichiometrically to DNA; the intensity of staining being directly related to the amount of DNA in the cell being evaluated. Therefore, these techniques can be used to identify cells with increased DNA content (S-phase and G2/M-phases) but cannot distinguish cells in the G1-phase of the cycle from G0 (Fig. 1c). The results are plotted out in histogram form, reflecting DNA content and the number of cells evaluated.

Either fresh or formalin-fixed archival materials may be evaluated (35). Use of fresh tissue generally results in better resolution and decreased cellular debris. Cellular debris or cell fragmentation may fall in the S-phase area. The amount of tissue required is more substantial than usual image cytometric or immunohistochemical analysis, which generally require only a section (slide) of tumor. Therefore, small tissue cores, such as might be generated via a stereotactic biopsy procedure, do not typically provide enough cells for proper analysis. In larger specimens, flow cytometric analysis has the advantage of providing an evaluation of large numbers of cells (thousands) in a relatively short period of time, avoiding selection biases and providing a truer measurement of cell proliferation. Thresholds for positivity can be set, thereby eliminating problems of observer assessment of individual results (e.g. whether immunostaining of a particular cell is sufficient to interpret as positive). The procedure may not distinguish between cell types, neoplastic versus nonneoplastic; particularly with “blindly” triaged fresh tissue, one makes the assumption that the specimen being evaluated is representative of the lesion. Another significant drawback is the cost of the equipment required to perform such analyses. Although flow cytometry machinery is generally accessible at most academic institutions and large group practices, it is not immediately available in smaller practice situations. Another problem is that the presence of aneuploid peaks, which may result in overlap between various peaks, makes accurate determination of the S-phase difficult if not impossible.

Correlation between flow cytometric results and immunohistochemical results, using markers such as Ki-67/MIB-1 or PCNA, have been generally good. Immunohistochemical markers have the advantage of allowing for morphologic assessment of what one is evaluating and, theoretically, the ability to allow one to distinguish neoplastic from nonneoplastic cells. However, this may not always be as easy to do as one would think, as evidenced by well known differential diagnostic conundrums such as gliosis versus glioma, microgial cells versus tumor cells, and resident oligodendroglial cells versus nonneoplastic oligodendrogial cells. Artefactual underestimation of S-phase due to contamination by nonneoplastic cells is particularly problematic in diploid tumors because of the overlapping peaks of tumor cells and nonneoplastic cells.

Differences between studies may result due to various specimen preparation methods and differences in analysis.
programs used. Thus, the establishment of precise “cutoff points” for purposes of predicting tumor grade or behavior should be done with caution and are probably significant only for the institution that generated the data. A number of studies have evaluated brain tumors via flow cytometry (36–43). Sallinen et al noted that flow cytometric S-phase proliferative fraction was associated with histologic grade and patient survival in an analysis of 50 astrocytomas (36). Studies by Coons et al have likewise demonstrated correlations between S-phase proliferative fractions and survival in astrocytomas and oligodendrogliomas (37, 38).

Immunohistochemical Markers

A variety of immunohistochemical markers have been developed to evaluate cell proliferation. Some, such as PCNA and Ki-67/MIB-1, are widely used; others, including DNA polymerase alpha, p105, and topoisomerase II-alpha, are less commonly employed.

DNA Polymerase Alpha: DNA polymerase alpha is a cell cycle-related enzyme that is expressed in cells during the G1-, S-, G2- and M-phases (Fig. 1d) (44, 45). Its utility is somewhat limited by the fact it requires fresh-frozen tissue because the epitope does not survive formalin-fixation and routine histologic processing. There is limited data available regarding this antibody and brain tumors. Shibuya et al demonstrated a correlation between DNA polymerase alpha staining and histologic grade in a series of 67 gliomas (astrocytomas and oligodendrogliomas) (46). Correlation with other markers of cell proliferation (Ki-67 and BrdU) were evaluated and the best correlation was with Ki-67 labeling indices (46).

DNA Topoisomerase II-Alpha: There is some indication that DNA topoisomerase II-alpha may also serve as a cell proliferation marker. The topoisomerase II-alpha is required by the cell to untangle DNA strands at the end of DNA synthesis prior to the cell entering mitosis (47). Topoisomerase is synthesized in the late G1- and S-phases of the cell cycle and is present throughout the G2- and M-phases of the cycle (Fig. 1e); the topoisomerase is degraded as the cell enters the G2-phase of the cycle (48). Antibodies specific for topoisomerase II-alpha that work with paraffin-embedded tissue sections are available and there is indication that immunoreactivity may correlate with cell proliferation, as evidenced by correlation with MIB-1 immunoreactivity (49–51). In 1999, Holden and Townsend examined topoisomerase II-alpha expression in 26 patients with astrocytoma (51). They observed low levels of immunostaining in low-grade tumors versus higher-grade lesions. They also noted a correlation between topoisomerase II-alpha immunostaining and MIB-1 labeling indices and strong correlation between immunostaining and patient outcome: an average topoisomerase II-alpha labeling index of 8.8 in patients alive 2 yr after diagnosis versus average labeling index of 30.5 in patients dead from disease after 2 yr (51).

p105: Another antibody, which has not been extensively studied in CNS neoplasms, is p105. p105 is a nuclear-associated protein localized to interchromatin granules in a region within the nuclear matrix associated with RNA synthesis. p105 may play a role in the production of mature RNA transcripts necessary for cell cycle progression (52, 53). Antibody to p105 appears to be expressed in cells that are present in the G1-, S-, G2- and M-phases of the cell cycle but not in the G0-phase (Fig. 1f). The antigen appears to survive routine histologic processing. Interpretation of staining results may be somewhat problematic. Staining of all tumor cell nuclei have been reported in some series, suggesting that expression by immunohistochemistry may be detectable in all phase of the cell cycle with variable intensity of expression (54). Therefore, the intensity of staining may be more important than whether staining is absent or present. This results in considerable subjectivity and limits the potential utility of this antibody for routine evaluation of tumors. Again, only limited data is available on p105 antibody and CNS neoplasia. Appley et al examined a variety of CNS neoplasms including gliomas, meningiomas, and pituitary tumors, and quantitated p105 by flow cytometric analysis (55). High-grade tumors appeared to have increased p105, higher proliferative indices, and poor clinical outcome. It appears that the utility of p105 may not be very great in evaluating immunostained tissue sections and it may be better analyzed in a flow cytometric framework.

Proliferating Cell Nuclear Antigen (PCNA): PCNA and Ki-67/MIB-1 antibodies have proved in recent years to be the most widely utilized and useful immunohistochemical markers of cell proliferation. PCNA is a non-histone nuclear protein that functions as a factor for DNA polymerase delta. More than 10 different monoclonal antibodies to PCNA, recognizing genetically distinct forms of the protein and localized to different areas within the cell nucleus, have been isolated and characterized (56). PCNA levels increase in the mid-G1-phase of the cell cycle and remain elevated throughout the S-phase and begin to decrease in the G2- and M-phases (Fig. 1g). Early PCNA antibodies required fresh-frozen or alcohol-fixed tissues; more recently, commercially available antibodies that work with formalin-fixed materials are widely available. Like some of the other previously discussed markers, PCNA may be found in low levels in noncycling G0 cells, and decreased expression may be observed in noncycling cells during periods of DNA repair or in cells that are under the influence of growth factors. In a series of brain tumors examined by both PCNA and Ki-67 immunohistochemistry, Louis et al suggested that PCNA was a more specific S-phase marker.
but an overall less sensitive cell proliferation marker versus Ki-67, i.e. Ki-67 labeling indices were a better assessment of the tumor growth fraction (57). Caution must be exercised when comparing different PCNA antibodies generated to different clones, in that they may define different epitopes and therefore have slightly different cell cycle distributions. Different epitopes may also be variably labile and subject to differences in fixation methods.

The relatively long half-life of PCNA (approximately 20 h) may be responsible for an over-estimation of proliferation rates in some instances, since cells that have recently left the cell cycle could still have residual non-degraded PCNA protein present. A number of studies have demonstrated correlation between PCNA labeling indices and histologic grade, other cell proliferation markers, and patient survival/prognosis (58–64).

**Ki-67 and MIB-1:** In 1983, Gerdes et al developed the Ki-67 antibody by immunizing mice with nuclei of Hodgkin’s lymphoma cell line L428 (65). The specific epitope associated with the antibody is not known. The antigen associated with the antibody is encoded for on a gene situated on chromosome 10. The antigen is present in the nuclei of cells in the G1-, S-, G2-, and M-phases of the cell cycle (Fig. 1h). Resting cells in the G0-phase do not express the Ki-67 antigen (66). The primary limitation of the Ki-67 antibody is that it requires fresh-frozen tissue; cytologic preparations for evaluation cannot be used with formalin-fixed, paraffin-embedded material.

Morphologic studies have suggested that the antigen is a component of the nuclear matrix of the cell (67). In 1992, Cattoretti et al reported a new monoclonal antibody (MIB-1) to the Ki-67 antigen that was able to work with formalin-fixed material (68). MIB-1 has been shown to benefit from microwave processing for antigen retrieval (69). Numerous studies have demonstrated the potential utility of the Ki-67/MIB-1 antibodies. Evaluation of a wide variety of tumors of the central nervous system have demonstrated correlations with histologic grade and outcome (Fig. 2a–c) (36, 70–94).

A variety of factors are important in the evaluation of MIB-1 immunostaining. Many CNS tumors, most notably gliomas, are heterogeneous in nature (Fig. 2d, e). Regional heterogeneity has been demonstrated with regard to cell proliferation (94–97). This has implications in specimen sampling. In a partially resected tumor, the portion of the neoplasm that has been removed does not necessarily contain the most proliferative area of the tumor. Likewise, selection of a block of “representative” tumor for immunostaining does not necessarily guarantee that the most proliferative area of the neoplasm is present in that block. The sampling issue becomes particularly important in the setting of stereotactic biopsies, where the amount of surgically sampled tissue is small. This underscores the importance of intraoperative consultation in the assessment of these cases to ensure that there is, at least, a correlation between the radiographic impression of what the lesion represents and the histology.

A number of technical aspects can effect staining with many of these antibodies. The source of antibody, i.e. manufacturer, may result in differences. This has been demonstrated in studies examining different sources of Ki-67 antibody, resulting in differences in labeling indices (98). Whereas delays in fixation are known to impact on the identification of mitotic figures, this does not seem to be a factor in the evaluation of cell proliferation using the Ki-67 antibody (98). Dilution of antibody used can certainly alter the staining observed. There is even a suggestion that differences in buffers can affect the results of immunostaining.

Variation in methodology with regard to determination of the labeling index may also result in apparent differences. There are several issues that potentially affect the determination of Ki-67/MIB-1 labeling indices, including how many cells should be evaluated, how one definitively excludes nontumor cells from evaluation, what area of the tumor should be evaluated, where counting should be performed, and whether counts should be manually determined or evaluated utilizing an image analysis system. By convention, and similar to mitosis counting, counts are usually performed in the area with the most immunostaining. An attempt is made to exclude nontumor cells in the determination of the labeling index; this may be variably easy to do. Counts should be performed at high magnification and only nuclear staining interpreted as positive. Use of an image analysis system in the evaluation of immunostaining has the advantage of being more objective since greater numbers of microscopic fields can be assessed, it reduces observer bias with regard to selection of fields to be evaluated, and it aids in the elimination of interpretation of some nontumor cells from analysis (81). The main benefit of this system is that the threshold of positivity can be set and is consistent in the evaluation, preventing an over-interpretation of background immunostaining as representing positivity. Unfortunately, such analysis requires equipment, which adds cost, and is generally more time consuming. From a practical standpoint, the relative rate of cell proliferation, which can be readily assessed by visual inspection, is more important than the precise number that is generated from a count.

Another factor that is potentially important in viewing the literature on immunohistochemical based methods of assessing cell proliferation are issues related to intraobserver and interobserver variability. Grzybicki et al, in analyzing interobserver variability associated with the MIB-1 antibody in 50 astrocytic gliomas of various grades, noted that there was a high level of interobserver variability (100). Conversely, in a series of 30 oligodendrogliomas that were evaluated by a group of 6 pathologists, the overall agreement was generally good, albeit
Fig. 2. a: Syncytial meningioma (WHO grade I) stained with MIB-1 antibody; the tumor had a labeling index of 0.8. b: Atypical meningioma (WHO grade II) stained with MIB-1 antibody; the tumor had a labeling index of 3.9. c: Anaplastic meningioma (WHO grade III) stained with MIB-1 antibody; the tumor had a labeling index of 21.2. d, e: Two adjacent microscopic fields of a glioblastoma multiforme stained with MIB-1 antibody highlighting tumor heterogeneity with regard to cell proliferation. f: Atypical meningioma (WHO grade II) stained with MIB-1 antibody; differences in the intensity of nuclear staining may account, in part, for interobserver variability in manually determining labeling indices. Original magnification: ×400.

not perfect (101). Differences among observers are generally related to varying thresholds for positivity (Fig. 2f) and selection of area to assess.

As with most of the immunohistochemical stains, evaluation of Ki-67 or MIB-1 antibody is potentially useful in select circumstances; although in many cases it probably does not add much over a careful evaluation of mitotic activity. It is particularly helpful in cases that are histologically graded as “borderline.” In this situation, a high labeling index may be helpful in suggesting that the tumor may be potentially more aggressive in terms of behavior. A low labeling index is less helpful because
one is not certain whether the labeling index is low because the tumor is really not very proliferative or whether the low labeling index represents a sampling phenomenon. Currently, there are no indications for actually changing tumor grade or basing grading on immunostain-related labeling indices. The establishment of precise cutoffs or guidelines with regard to labeling indices also needs to be approached with caution. Extrapolating another laboratory’s “cutoffs” to one’s own laboratory is problematic for many of the previously discussed reasons that can account for considerable variability among laboratories. If one is predisposed to establishing cutoffs, it is best done within the context of one’s own laboratory and experience. In general, one should be careful about how much significance one attaches to the precise labeling index value generated. In other words, the difference between a labeling index of 5.0 and 5.5 is probably not significant from a clinical standpoint; an index in the 5.0 to 5.5 range does indicate a moderate degree of cell proliferation, as most characteristically encountered in intermediate grade tumors. One should certainly not conclude that the tumor with an apparent index of 5.5 is necessarily going to behave worse than the tumor with an index of 5.0.

Although imperfect, ancillary means for evaluating cell proliferation can be useful, particularly if one gives careful consideration to the limitations associated with the particular methodology being utilized.

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REFERENCES


31. Heintz N, Sive HL, Roeder RG. Regulation of human histone gene expression: Kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. Mol Cell Biol 1993;3:539–50


75. Matsumoto T, Fuji T, Yabe M, Oka K, Hoshi T, Sato K. MIB-1 and p53 immunocytochemistry for differentiating pilocytic astrocytomas and astrocytomas from anaplastic astrocytomas and glioblastomas in children and young adults. Histopathology 1998;33:446–52
84. Coons SW, Johnson PC, Pearl DK. The prognostic significance of Ki-67 labeling indices for oligodendrogliomas. Neurosurgery 1997;41:878–85
90. Abramovich CM, Prayson RA. Histopathologic features and MIB-1 labeling indices in recurrent and nonrecurrent meningiomas. Arch Pathol Lab Med 1999;123:793–800
92. Abramovich CM, Prayson RA. MIB-1 indices in benign, aggres-