Assessment of candidate immunohistochemical prognostic markers of meningioma recurrence

Type:

Original paper

Abstract:

Although tumour recurrence is an important and not infrequent event in meningiomas, predictive immunohistochemical markers have not been identified yet. The aim of this study was to address this clinically relevant problem by systematic retrospective analysis of surgically completely resected meningiomas with and without recurrence, including tumour samples from patients who underwent repeat surgeries. Three established immunohistochemical markers of routine pathological meningioma work-up have been assessed: the proliferative marker Ki-67 (clone Mib1), the tumour suppressor gene p53 and progesterone receptor (PR). All these proteins correlate with the tumour WHO Grade, however the predictive value regarding recurrence and progression in tumour grade is unknown.

Hundred and fourteen surgical specimens of 70 meningioma patients (16 male and 54 female) in a 16 years interval have been studied. All tumours had apparently complete surgical removal. On Mib1, PR and p53 immunostained sections the percentage of labelled tumour cells, the staining intensity and the multiplied values of these to parameters (the histoscore) was calculated. Results were statistically correlated with tumour WHO Grade, (sub)type, recurrence and progression in WHO Grade at subsequent biopsies.

Our results confirmed previous findings that the WHO Grade has strong forward proportion to Mib1 and p53 and an inverse proportion to the PR immunostain. We have demonstrated that Mib1 and p53 have significant correlation with and predictive value of relapse/recurrence irrespective of histological subtype of the same WHO grade. As a quantitative marker Mib1 has best correlation with percentage of labelled cells, whereas p53 with intensity & histoscore.

In conclusion, the immunohistochemical panel of PR, p53, Mib1 in parallel with applying standard diagnostic criteria based on H&E stained sections is sufficient and reliable to predict meningioma recurrence in surgically completely resected tumours.

Keywords:

immunohistochemistry; Ki-67; meningioma; p53; progesterone receptor; prognostic markers; tumour recurrence

Manuscript body

Download source file (33.48 kB)

Assessment of candidate immunohistochemical prognostic markers of meningioma recurrence.

Tamás Csonka[1], Balázs Murnyák[1], Rita Szepesi[2], János Bencze[1], László Bognár[3], Álmos Klekner[3], Tibor Hortobágyi[1]

[1]Division of Neuropathology, Institute of Pathology, [2]Department of Neurology, [3]Department of Neurosurgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Corresponding author: Tibor Hortobágyi, MD, PhD, FRCPath, EFN

Division of Neuropathology, Institute of Pathology, University of Debrecen, 98. Nagyerdei krt. Debrecen, H-4032, Hungary

Email: hortobagyi@med.unideb.hu; tibor.hortobagyi@kcl.ac.uk

Phone: +3652255248

Manuscript body

Download source file (33.48 kB)

Introduction

Meningioma is one of the most frequent brain tumours [9]. According to the World Health Organization (WHO) classification there are several subtypes, like meningothelial, fibrous, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacyte rich, metaplastic, choroid, clear cell, rhabdoid, papillary and other rare morphological phenotypes [6,21]. The assigned WHO Grade I-III. reflects the probable prognosis which is determined by the subtype and/or specified morphological features such as mitotic rate, presence or absence of small geographic necrosis, nucleus-cytoplasm ratio an others [21]. Although tumour recurrence is important and not infrequent event, our knowledge on predisposing factors is rather limited. The risk of recurrence increases with the WHO grade being 7-25% in WHO grade I, 30-50% in WHO grade II and 50-95% in WHO grade III [27,28,32,33], respectively. The extent of resection assessed by the Simpson Grading System influences recurrence rates which is one reason of the wide range of probability [30]. Simpson Grading System classify the completeness of removal in a 5-tier scale ranging from macroscopically complete removal (grade I) to simple decompression with or without biopsy (grade V). Skull irradiation, inherited mutation of the NF2 gene (Neurofibromatosis type 2) and epigenetic factors may also predispose to recurrence [22,23].

Another important phenomenon is tumour progression to higher WHO Grade. However, the risk and probability of progression remains rather unpredictable – even less so than tumour recurrence. Hence, there is growing clinical need to identify additional and better predictors for recurrence and tumour progression than the currently used histological grade and extent of resection. Because immunohistochemistry has been routinely used in the pathological diagnostic practice

for decades, the search for predictive immunohistochemical markers is of importance. In our study we focussed on 3 well known immunohistochemical markers in routine pathological work-up of meningioma: the proliferative marker Ki-67 (clone Mib1), the tumour suppressor gene p53 and progesterone receptor. All these proteins have been studied in meningioma and the correlation with tumour grade have been confirmed by several studies. However, the predictive value regarding recurrence and progression in tumour grade remains unknown. The aim of this study is to address these clinically relevant questions by a systematic retrospective analysis of meningiomas with and without recurrence, with special emphasis on tumour samples from patients who underwent repeat surgeries due to tumour recurrence.

p53 is one of the major tumour suppressor proteins. The physiological functions of p53 are cell cycle regulation and conserve the stability of the genome by preventing mutations, therefore it is called ‘the guardian of the genome’[17]. More than 50 percent of human tumours carries deletion or mutation of the p53 genes (TP53) [13]. p53 can be activated by DNA damage, oxidative stress, osmotic shock, ribonucleotide depletion or oncogene expression. The activation is marked by an increase in the half-life of p53 and a change of its conformation [16] therefore shows increased LI with immunohistochemistry with the polyclonal antibodies routinely used in tumour diagnostics. The anticancer activity of p53 works through several mechanisms: it activates DNA repair proteins, induces growth arrest at the G1/S regulation point through p21 or initiates apoptosis if the DNA damage is irreversible [12]. It has been investigated also in meningioma and several studies showed positive correlation with grade, and tumour recurrence [4,7,8,14,15,24,26], whereas authors reported the grade as an independent predictive factor of recurrences with high Mib1 and p53 LI being supportive marker helpful in borderline cases [31].

Ki-67 is necessary for cellular proliferation; it is present during all active phases of the cell cycle, and absent from the G0 phase. Mib1 is the usually applied clone of the Ki-67 antibody which is widely used as proliferative marker in the routine diagnostic work-up. The Mib1 LI shows a strong correlation with tumour growths, relapse/recurrence, length of in various tumours [2,3,34] including meningioma [18,19].

Progesterone receptor (PR) is a steroid hormone receptor. It has been demonstrated that meningioma cells show positivity for PRs; the ratio of the positive cells is inversely proportional to the WHO Grade [18,27]. Also described earlier the cellular biosynthesis of PR in meningioma is not oestrogen regulated as it is other sex steroid in tissues [5,7]. PR is encoded by the *PGR* gene on the long arm of the chromosome 11. In physiological situation after binding the progesterone hormone the receptor undertake a dimerization and is transported to de nucleus to binding to the DNA and inducing transcription. Both form (progesterone receptor A and progesterone receptor B) has a regulatory domain, DNA binding domain, a hinge section and a ligand binding domain, but only the PR-B form possess transcription activation function.

The Mib1 antibody, p53 and PR are widely used immunohistochemical markers in meningioma diagnosis. In high-grade meningioma the Mib1 labelling index (LI) is higher [1,4,28,29]. In our previous study we have reported a significant correlation between the frequency and intensity of p53 immunostaining and WHO Grade [10].The reduced of PR immunoreactivity is another known feature in the high grades of meningioma [18,20,25].

The aim of this study is to establish an easy-to-use immunohistochemical panel for the routine neuropathological use, which can predict meningioma

relapse/recurrence. For validation we analysed the changes in immunohistochemical characteristics and expression patterns during relapse/recurrence and examined their relation to tumour grade.

Materials and methods

Hundred and fourteen surgical specimens of 70 meningioma patients (16 male and 54 female) in a 16 years interval have been retrospectively studied. All cases were revised by a consultant neuropathologist (TH) and divided into three grades and histological subtypes according to the WHO classification [21].

We established two study groups: Patients with one or more recurrence/relapse(s) (R/R group) and patients with meningioma without any radiological or post mortem evidence of recurrence/relapse (non-R/R group). Only cases with apparently complete surgical removal and no evidence of residual tumour on post-operative MRI were included.

After the surgical removal tissue samples were processed to generate sections from formalin fixed and paraffin embedded (FFPE) blocks which were stained with haematoxylin-eosin (H&E). One representative tissue block was selected per case. From these blocks tissue micro arrays (TMAs) were build. Each TMA contained samples from 10 cases (three samples from each cases) plus 2 normal brain tissue sample in the left upper corner as reference to enable specimen identification in the TMA (Fig. 1.). In total 12 TMA were built, containing tissue samples from 114 neurosurgical interventions.

Immunohistochemistry (IHC) was performed according to standardized methods. In brief, 4 µm thick sections from TMA blocks were stained with p53 mouse monoclonal antibody (clone DO-7, M7001, DAKO, Glostrup, Denmark); PR antibody

(NCL-PGR-312, clone 16, Novocastra, Newcastle, UK) and anti-Ki-67 antibody (clone Mib1, M7240, DAKO, Glostrup, Denmark) according to the manufacturers' protocol with 1:100, 1:100 and 1:200 dilution for p53, PR and Ki-67, respectively. Sections were incubated with the primary antibody for 6 hours in room temperature; the visualization was performed with SuperSensitive™ One-step Polymer-HRP Detection System on Leica Bond Max™ fully automated IHC stainer with negative controls (omitting the primary antibody).

All of the H&E and immunostained TMA sections were scanned with a Panoramic Scanner (3DHistech, Budapest, Hungary). Two digital images were taken at 400x magnification from each tissue samples, in total 6 from each case. According to the intensity of nuclear staining of cells 4 semi quantitative scores were applied: 0 (none), 1+ (weak), 2+ (moderate) and 3+ (strong) (Fig. 2.).

Images in 10 reference cases were analysed quantitatively with ImageJ (NIH, Bethesda, USA) software Cell Counter function, to determine the exact percentage of immunopositive cells (Fig. 3.). These images were used as reference cases to aid accurate semi-quantitative assessment in all cases. This is a method easily and reliably applicable in the routine pathological diagnostic practice, similarly to the assessment of percentage of immunopositive cells in other tumours.

Not only the percentage value of immunopositive cells but also the average labelling intensity score (0-3+; for reference images see Fig. 2) of the staining were calculated in each picture. Similarly to the Histoscore of breast carcinoma i.e. the multiply of the percentage of the positive cells and the average intensity of the positive cell nuclei [11] were calculated.

Data were analysed with SPSS 22.0 for Windows (IBM, Armonk, NY, USA) statistical programme, using Kruskall-Wallis H-test, Mann-Whitney U-test and

Wilcoxon signed ranks test. The patient were grouped by the grades (65 WHO Grade I; 33 WHO grade II and 16 WHO Grade III) and also based on the recurrence or relapse (R/R group) showed up at least 5 years after resection: patients without recurrence or relapse (non-R/R group), patients with definitive relapse or recurrence (R/R group).

The non-R/R cases were WHO Grade I, while the R/R group have 18 WHO Grade I, 9 WHO grade II and 2 WHO Grade III tumour in the 1st histological sample.

Ethical approval has been obtained from the Institutional Research Ethics Committee (Number: DEOEC RKEB: 2437-2005).

Results

The 70 patients' average age was 56 years in the time of the first pathological examination. There were no significant differences between the R/R group and the non-R/R group. There were 16 patients (3 male and 13 female; average age 54 years) without recurrence or relapse (non-R/R group) with at least 5 years survival after surgery and 31 patients (8 male and 23 female; average age 53 years, overall time of recurrence 19.6 months) with definitive relapse or recurrence (R/R group). Further 23 patients (5 male and 18 female, average age 59 years) were operated within 5 years without recurrence/relapse; however the time window was too short to include them in the non-R/R group. There were 65 WHO Grade I cases, 33 WHO Grade II cases and 16 WHO Grade III cases. All of the non-R/R cases were WHO Grade I. The R/R group contained 19 WHO Grade I, 9 WHO Grade II and tree WHO Grade III cases according to the 1st neuropathological diagnosis of the first surgical specimen. There were 8 patients whose subsequent surgical specimens had higher WHO Grade than the first; 15 patients whose first and last cases both showed the

same grade; and 6 patient who only had 1 histological samples and the recurrence/relapse was diagnosed by imaging techniques.

The histological subtypes were not statistically different between the R/R group and non-R/R group: there were 6 meningothelial, 5 transitional, 3 fibrous and 2 psammomatous in the non-R/R group, while 9 meningothelial, 6 transitional, 3 fibrous, one psammomatous, one clear cell, 8 atypical and 3 anaplastic in the R/R group. There was no increased tendency for recurrence for any WHO grade 1 subtype. Among grade 2-3 meningiomas there was no specific subtype which had higher frequency of recurrence than the respective grade in general.

There is significant correlation between WHO tumour grade and Mib1 LI (%) ($p < 0.001$), Mib1 staining intensity ($p=0.001$), Mib1 histoscore ($p<0.001$), p53 staining intensity ($p<0.001$), p53 histoscore ($p=0.031$), PR LI (%) ($p<0.001$), PR intensity ($p<0.001$) and PR histoscore ($p<0.001$) respectively (Kruskall-Wallis test). Comparing only Grade I and Grade II tumours there is significant correlation with Mib1 LI (%) ($p<0.001$), Mib1 intensity ($p<0.001$), Mib1 histoscore ($p<0.001$), p53 intensity ($p=0.001$), PR LI (%) ($p=0.014$), PR intensity ($p=0.029$) and PR histoscore ($p=0.013$) respectively. Comparing Grade II and Grade III tumours there is significant correlation with p53 intensity ($p=0.049$), PR LI (%) ($p=0.008$), PR intensity ($p=0.008$) and PR histoscore ($p=0.009$) respectively. When comparing Grade I and Grade III tumours there is significant correlation of higher grade with increased Mib1 LI (%) ($p<0.001$), Mib1 histoscore ($p<0.001$), p53 intensity ($p<0.001$), p53 histoscore ($p=0.023$), PR LI (%) ($p<0.001$), PR intensity ($p<0.001$), PR histoscore ($p<0.001$) respectively (Mann-Whitney test) (Table 1., Fig. 4.).

Irrespective of the grades of the R/R group comparing the non-R/R and R/R groups there are significant correlation with the Mib1 LI (%) ($p<0.001$), Mib1 intensity

(p=0.004), Mib1 histoscore (p<0.001), p53 LI (%) (p=0.027), and the WHO grade (p=0.003) respectively (Fig. 5.). In WHO Grade I tumours in the R/R group there are significant correlation with the Mib1 LI (%) (p=0.009), Mib1 histoscore (p=0.029), p53 LI (%) (p=0.032), p53 histoscore (p=0.038) respectively (Table 2., Fig. 6.).

In the R/R groups compare the first case with the recurrent/relapsed cases there are significant difference between the Mib1 LI (%) (p=002), Mib1 histoscore (p=0.001), p53 intensity (p=0.006) and the grade (p=0.001) respectively; and with Wilcoxon signed rank test compared the first and last case of the same patient there are significant difference in the grade (p=0.007), Mib1 LI (%) (p=0.042), Mib1 histoscore (p=0.050), and p53 LI (%) (p=0.042) respectively (Table 3., Fig.7.).

According our data the WHO grade has strong forward proportion to Mib1 and p53 and an inverse proportion to the PR immunostain (as shown in several previous papers). As a quantitative marker the Mib1 has better correlation with percentage, whereas p53 with intensity & histoscore. Therefore, the panel of PR, p53, Mib1 is sufficient to characterize meningioma immunohistochemically regarding risk of recurrence possible as an integral part of the routine diagnostic histopathological practice.

Discussion

Meningioma is one of the most common intracranial tumour with high incidence in the neurosurgical practice. The histological subtypes are well characterised by the WHO, and the grading is based on these histological characteristics, morphological findings and the mitotic ratio. The Simpson Grading System also can provide further information of the probability of the recurrence [30].

The aim of this study was to establish an easy-to-use immunohistochemical panel for the routine neuropathological use, which can predict meningioma relapse/recurrence. This is particularly relevant for tumours in problematic localization (e. g. falx memingiomas).

For validation we analysed the changes in immunohistochemical characteristics and expression patterns during relapse/recurrence and their relation to tumour grade.

Meningiomas usually are non-infiltrative neoplasms therefore complete surgical resection is curative. However, tumour may spread laterally in small nests in the dura mater which could be source of recurrence. Hence no chemotherapy is effective even in high grade meningiomas – radiotherapy increases malignant transformation [33]– another argument for discovery of relatively simple predictive markers of tumour progression and recurrence.

However the Mib1 labelling index can be different according which laboratory performed the reaction [8], in a standardized methods can help with the data interpretation, and comparison both in routine and experimental purpose. Accordant to previous studies the higher initial Mib1 LI has a predictive value regarding increased probability of recurrence. In R/R cases during evolution in time (i.e. time between 1st and last surgical procedure) there was an increase in Mib1 LI consistent with the known fact that tumour progression may occur over time which is reflected by increased proliferative potential and higher WHO grade.

The routine used p53 antibody does not separate the wild type and the mutant protein. Interestingly, the p53 LI and histoscore (but not the labelling intensity) has an inverse correlation with chance of recurrence in the WHO Grade I tumours in our study, but if we examine all the WHO Grades, the higher staining in the higher grades, change to forward proportion, similarly with the prior studies [7,14,15]. This

may be explained by the fact the p53 immunoreactivity does not distinguish between wild type (WT) and mutant protein; in non-recurrent cases increased normal protein may have a beneficial effect as p53 is involved in DNA damage repair. In contrast, in recurrent cases p53 is more likely to be mutant and ineffective thereby contributing to tumour growth and recurrence. Mutation analysis could answer this problem, however, the focus of our study is on immunohistochemical markers, and therefore it is beyond the scope of the current project. Today the antibodies specific to mutated p53 are not routinely used therefore not applied in this study. The p53 LI and histoscore decreased during time to recurrence which may indicate decreased levels of WT protein.

PR has inverse relation with tumour grade in concert with previous reports [10,18,20,25] and no predictive value regarding recurrence.

Using p53 and Ki-67 molecular markers and the relatively simple and quick assessment method the increased risk of recurrence can be reliably predicted. However, it is foreseeable that the presented method has the potential for further improvement with the use of digitalized histological specimens, because this enables automated quantitative image analysis as an integral component of the diagnostic process.

In summary, we have demonstrated a rather simple immunohistochemistry-based method with routinely used molecular markers to identify patients with increased risk of recurrence. Further work is needed to validate our work in more patients, multiple centres and in a prospective manner with long follow-up. The combination of histological, surgical and imaging markers may be a more sensitive tool to predict recurrence and this can also be tested in future studies.

Manuscript body

Download source file (33.48 kB)

Acknowledgements

This work has been supported by the National Brain Research Program, Hungary (KTIA_13_NAP-A-II/7, and KTIA-NAP-13-1-2013-0001).

Conflict of interest

The authors have no competing interests.

References

1. Abramovich, C.M. and R.A. Prayson, Histopathologic features and MIB-1 labeling indices in recurrent and nonrecurrent meningiomas. Arch Pathol Lab Med, 1999. 123(9): p. 793-800.
2. Adamczyk, A., H. Jesko, and R.P. Strosznajder, Alzheimer's disease related peptides affected cholinergic receptor mediated poly (ADP-ribose) polymerase activity in the hippocampus. Folia Neuropathol, 2005. 43(3): p. 139.
3. Affar, E.B., et al., Role of poly (ADP-ribose) polymerase in rapid intracellular acidification induced by alkylating DNA damage. Proc Natl Acad Sci U S A, 2002. 99(1): p. 245-250.
4. Amatya, V.J., et al., Immunohistochemical study of Ki-67 (MIB-1), p53 protein, p21WAF1, and p27KIP1 expression in benign, atypical, and anaplastic meningiomas. Hum Pathol, 2001. 32(9): p. 970-5.
5. Blankenstein, M., et al., Presence of progesterone receptors and absence of oestrogen receptors in human intracranial meningioma cytosols. Eur J Cancer and Clin Oncol, 1983. 19(3): p. 365-370.
6. Bodi, I., T. Hortobagyi, and S. Buk, A 72-year-old woman with right frontal extra-axial mass. Brain Pathol, 2008. 18(2): p. 279-82.
7. Cho, H., et al., Role of p53 gene mutation in tumor aggressiveness of intracranial meningiomas. J Korean Med Sci, 1999. 14(2): p. 199-205.
8. Chozick, B.S., et al., Immunohistochemical evaluation of erbB-2 and p53 protein expression in benign and atypical human meningiomas. J Neurooncol, 1996. 27(2): p. 117-26.
9. Claus, E.B., et al., Epidemiology of intracranial meningioma. Neurosurgery, 2005. 57(6): p. 1088-95; discussion 1088-95.
10. Csonka, T., et al., Poly(ADP-ribose) polymerase-1 (PARP1) and p53 labelling index correlates with tumour grade in meningiomas. Folia Neuropathol, 2014. 52(2): p. 111-20.
11. De Vos, M., V. Schreiber, and F. Dantzer, The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art. Biochem pharmacol, 2012. 84(2): p. 137-146.
12. el-Deiry, W.S., et al., WAF1, a potential mediator of p53 tumor suppression. Cell, 1993. 75(4): p. 817-25.
13. Hollstein, M., et al., p53 mutations in human cancers. Science, 1991. 253(5015): p. 49-53.

Manuscript body

Download source file (33.48 kB)

14. Kamei, Y., et al., Prognostic significance of p53 and p21WAF1/CIP1 immunoreactivity and tumor micronecrosis for recurrence of meningiomas. J Neurooncol, 2000. 46(3): p. 205-13.
15. Karamitopoulou, E., et al., Prognostic significance of MIB-1, p53, and bcl-2 immunoreactivity in meningiomas. Hum Pathol, 1998. 29(2): p. 140-5.
16. Kastan, M.B. and S.J. Kuerbitz, Control of G1 arrest after DNA damage. Environ Health Perspect, 1993. 101 Suppl 5: p. 55-8.
17. Koshland, D.E., Jr., Molecule of the year. Science, 1993. 262(5142): p. 1953.
18. Kumar, S., et al., Evaluation of 1p and 14q status, MIB-1 labeling index and progesterone receptor immunoexpression in meningiomas: Adjuncts to histopathological grading and predictors of aggressive behavior. Neurol India, 2014. 62(4): p. 376-82.
19. Lanzafame, S., et al., Correlation between histological grade, MIB-1, p53, and recurrence in 69 completely resected primary intracranial meningiomas with a 6 year mean follow-up. Pathol Res Pract, 2000. 196(7): p. 483-8.
20. Longstreth, W., et al., Epidemiology of intracranial meningioma. Cancer, 1993. 72(3): p. 639-648.
21. Louis, D.N., et al., The 2007 WHO classification of tumours of the central nervous system. Acta neuropathol, 2007. 114(2): p. 97-109.
22. Murnyak, B., et al., Epigenetics of Meningiomas. Biomed Res Int, 2015. 2015: p. 532451.
23. Murnyak, B., R. Szepesi, and T. Hortobagyi, [Molecular genetics of familial tumour syndromes of the central nervous system]. Orv Hetil, 2015. 156(5): p. 171-7.
24. Ohkoudo, M., et al., Expression of p53, MDM2 protein and Ki-67 antigen in recurrent meningiomas. J Neurooncol, 1998. 38(1): p. 41-9.
25. Omulecka, A., et al., Immunohistochemical expression of progesterone and estrogen receptors in meningiomas. Folia Neuropathol, 2006. 44(2): p. 111-5.
26. Ozen, O., B. Demirhan, and N. Altinors, Correlation between histological grade and MIB-1 and p53 immunoreactivity in meningiomas. Clin Neuropathol, 2005. 24(5): p. 219-24.
27. Perry, A., et al., Merlin, DAL-1, and Progesterone Receptor Expression in Clinicopathologic Subsets of Meningioma: A Correlative Immunohistochemical Study of 175 Cases. J Neuropathol Exp Neurol, 2000. 59(10): p. 872-879.
28. Perry, A., et al., "Malignancy" in meningiomas: a clinicopathologic study of 116 patients, with grading implications. Cancer, 1999. 85(9): p. 2046-56.
29. Perry, A., et al., The prognostic significance of MIB-1, p53, and DNA flow cytometry in completely resected primary meningiomas. Cancer, 1998. 82(11): p. 2262-9.
30. Simpson, D., The recurrence of intracranial meningiomas after surgical treatment. J Neurol Neurosurg Psychiatry, 1957. 20(1): p. 22-39.
31. Terzi, A., et al., The significance of immunohistochemical expression of Ki-67, p53, p21, and p16 in meningiomas tissue arrays. Pathol Res Pract, 2008. 204(5): p. 305-14.
32. Touat, M., et al., Successful treatment of multiple intracranial meningiomas with the antiprogesterone receptor agent mifepristone (RU486). Acta neurochir, 2014. 156(10): p. 1831-1835.
33. Umansky, F., et al., Radiation-induced meningioma. Neurosurg Focus, 2008. 24(5): p. E7.
34. Wiemels, J., M. Wrensch, and E.B. Claus, Epidemiology and etiology of meningioma. J Neurooncol, 2010. 99(3): p. 307-314.

Table 1

Download source file (18.12 kB)

Table 1.:

	Mib1 Percentage	Mib1 Intensity	Mib1 Histoscore	p53 Percentage	p53 Intensity	p53 Histoscore	PR Percentage	PR Intensity	PR Histoscore
Kruskall-Wallis	,000	,001	,000	,316	,000	,031	,000	,000	,000
Mann-Whitney grade I-II	,000	,000	,000	,272	,001	,065	,014	,029	,013
Mann-Whitney grade II-III	,449	,320	,831	,654	,049	,376	,008	,008	,009
Mann-Whitney grade I-III	,000	,086	,000	,198	,000	,023	,000	,000	,000

Comparison of Grade I, Grade II and Grade III cases with Kruskall-Wallis test, and the WHO Grade pairs with Mann-Whitney test. Immunostain percentage, intensity (average instensity of cells: 0, 1, 2 or 3) and Histoscore (intensity x percentage), for Mib1, p53 and Progesteron receptor (PR).

Table 2

Download source file (17.37 kB)

Table 2.:

	Grade	Mib1 Percentage	Mib1 Intensity	Mib1 Histoscore	p53 Percentage	p53 Intensity	p53 Histoscore	PR Percentage	PR Intensity	PR Histoscore
Mann-Whitney any grades	,003	,000	,004	,000	,027	,955	,069	,207	,497	,215
Mann-Whitney grade I	1,000[b]	,009[b]	,126[b]	,029[b]	,032[b]	,195[b]	,038[b]	,708[b]	,708[b]	,858[b]

Comparison of the non-recurrance/relapse (non-R/R) cases and recurrence/relapse (R/R) cases first surgical specimens without regarding the grade (firs row) and only in WHO Grade I cases (second row).

Table 3

Download source file (17.51 kB)

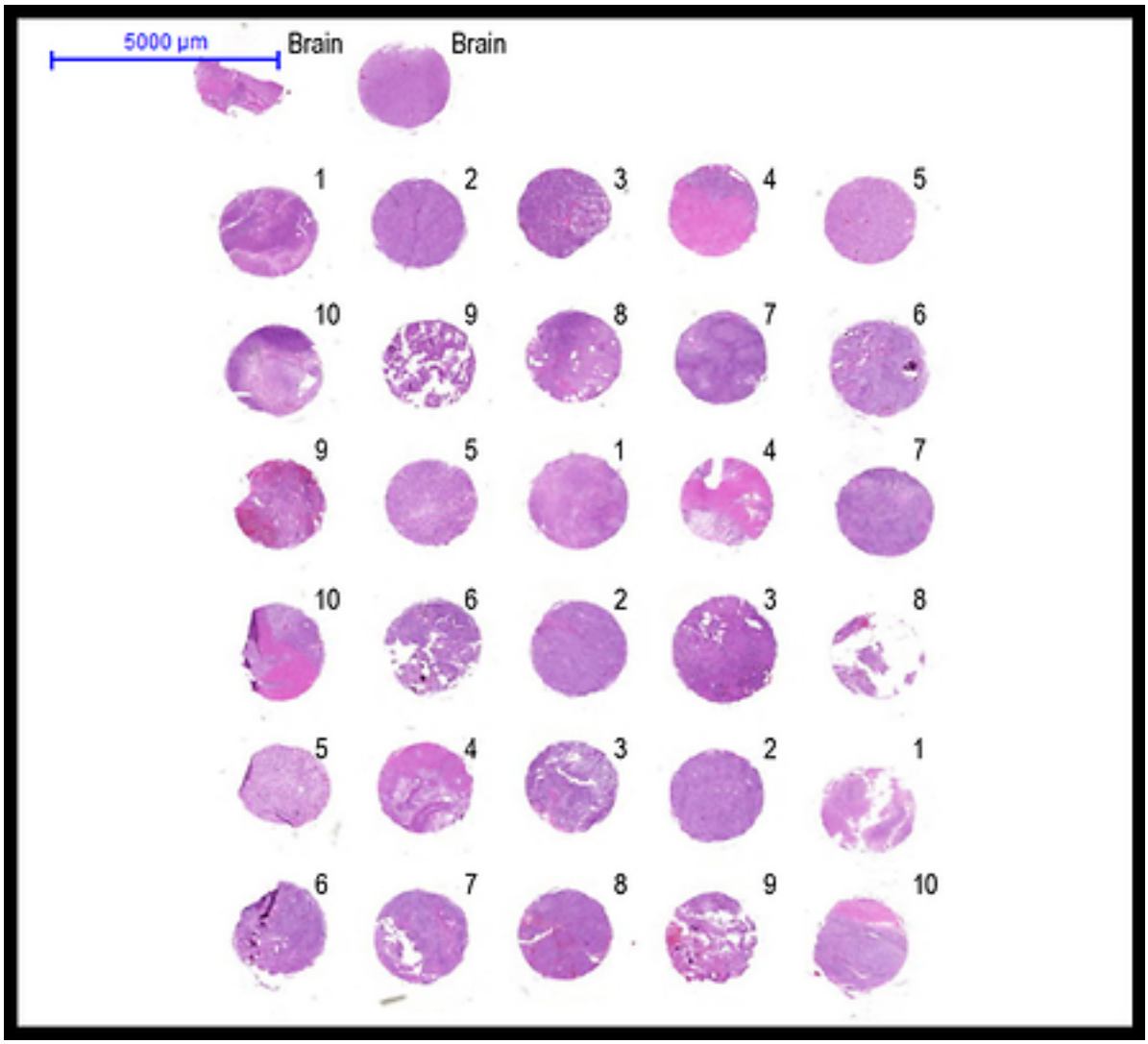
Table 3.:

	Grade	Mib1 Percentage	Mib1 Intensity	Mib1 Histoscore	p53 Percentage	p53 Intensity	p53 Histoscore	PR Percentage	PR Intensity	PR Histoscore
Mann-Whitney progression	0,001	0,002	0,098	0,001	0,861	0,006	0,553	0,154	0,154	0,159
Wilcoxon progression	0,007	0,042	0,237	0,050	0,042	0,484	0,559	1,000	0,545	0,876

Comparison of the first and last surgical specimens of the recurrence/relapsed (R/R) cases with Mann-Withney test (first row) and Wilcoxon signed rank test (second row). Immunostain percentage, intensity (average instensity of cells: 0, 1, 2 or 3) and Histoscore (intensity x percentage), for Mib1, p53 and Progesteron receptor (PR).

Figure 1

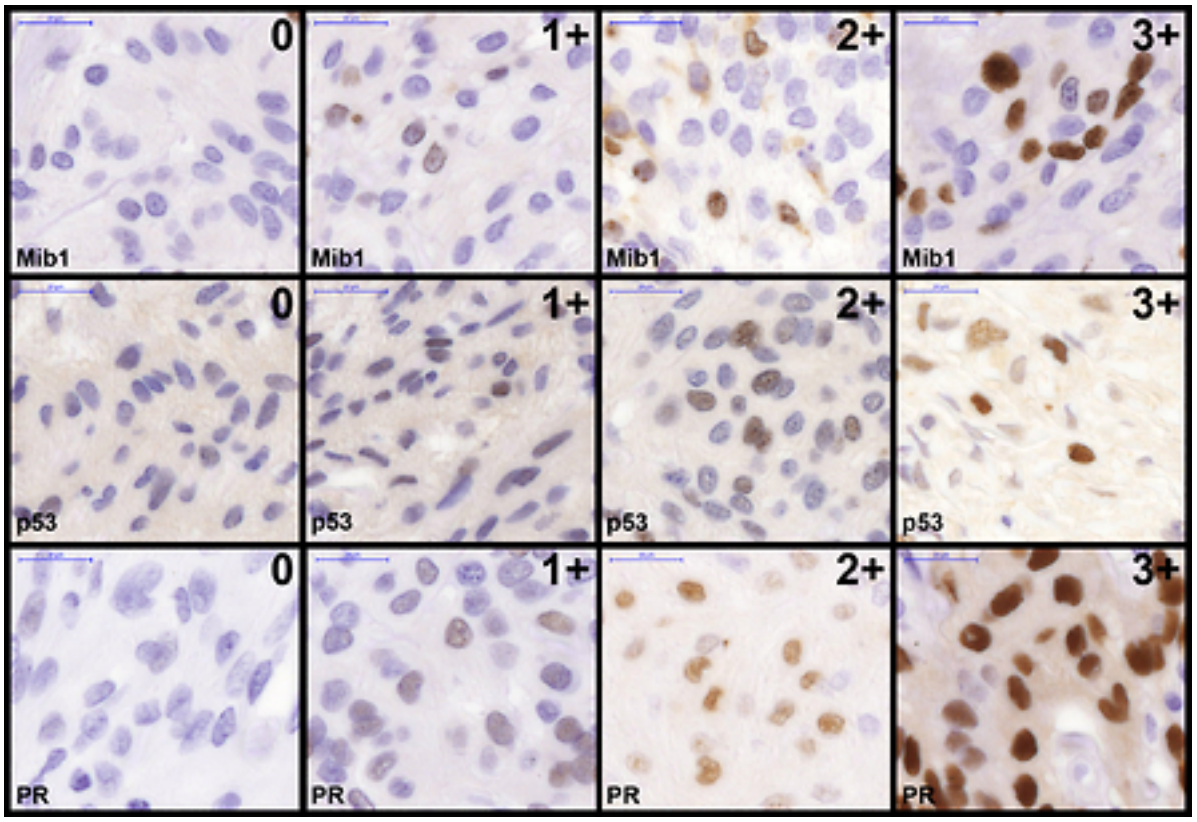
Download source file (147.74 kB)



Low magnification image of histological slide stained with haematoxylin-eosin, made from tissue microarray (TMA) paraffin block. In the upper left corner there are two tissue (brain) samples for guidance regarding localization. The numbers from 1 to 10 represent the individual cases. Every 'donor' case have 3 different samples in the 'recipient' block. Scale bar 5mm.

Figure 2

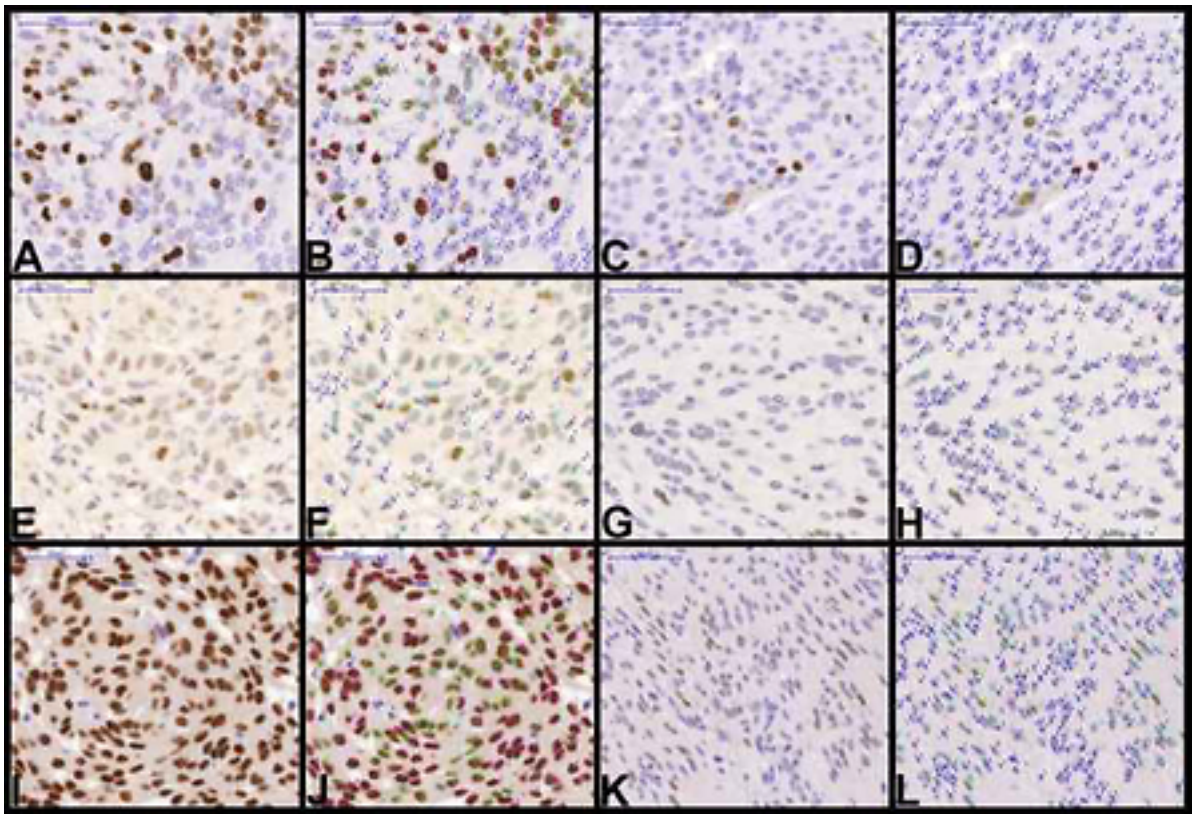
Download source file (510.34 kB)



Ki-67 (clone Mib1), p53, and progesterone receptor (PR) immunostain with representative images of the different immunolabelling intensities: negative (0), minimal positivity (1+), medium positivity (2+), to strong positivity (3+). Scale bar 20μm.

Figure 3

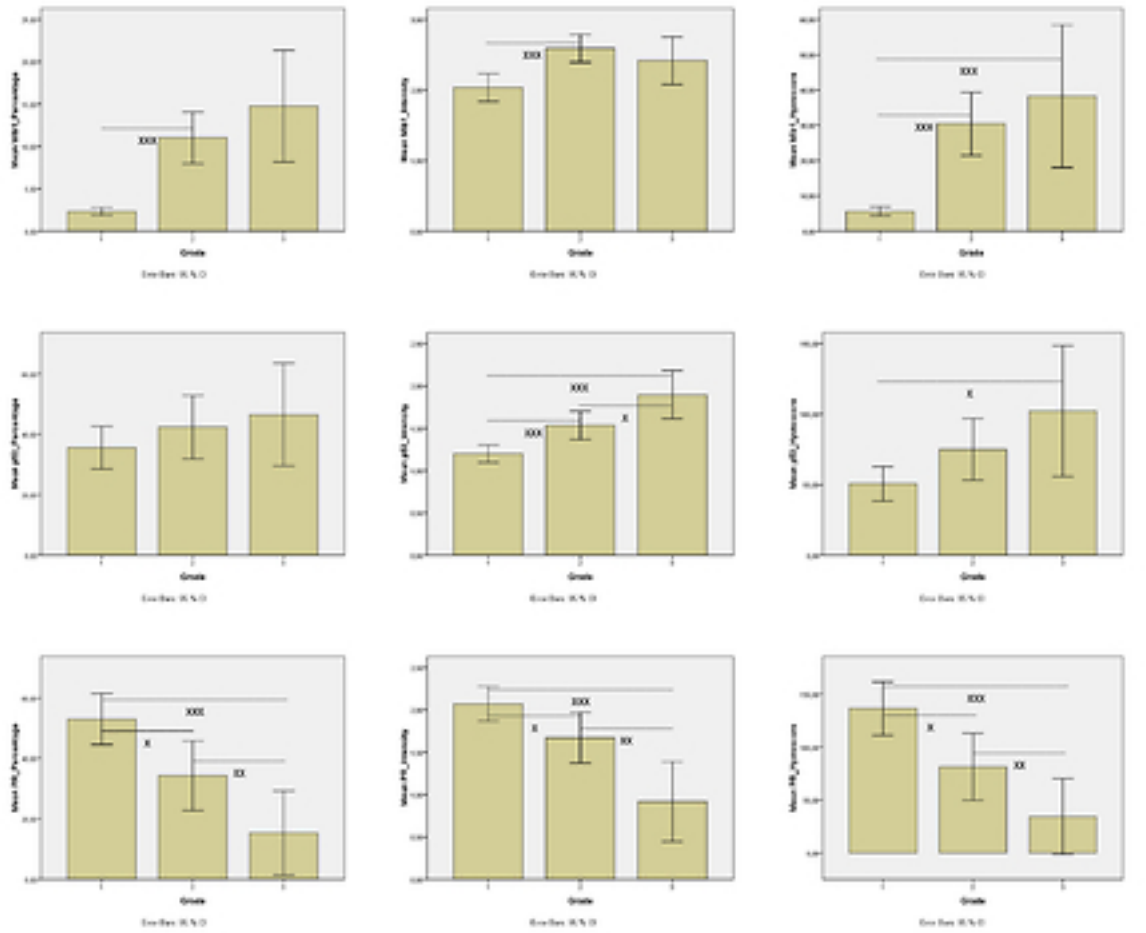
Download source file (46.45 kB)



Ki-67 (clone Mib1), p53 and progesterone receptor (PR) immunopositive cells counted using ImageJ programme. A, C, E, G, I, K pictures are the originals, whereas B, D, F, H, J L show cells numbered with ImageJ Cell Counter plug-in. The numbers from 1-4 stand for the negative, 1+, 2+ and 3+ cells, respectively. A-B pictures are immunostained for Mib1 (43,5% positivity). The C-D pictures are immunostained for Mib1 (6,9% positive). E-F pictures are stained for p53 (58,9% positive). G-H pictures immunostained for p53 (9,8% positive). I-J pictures stained for PR (93,9% positive). K-L pictures immunostained for PR (31,3% positive).

Figure 4

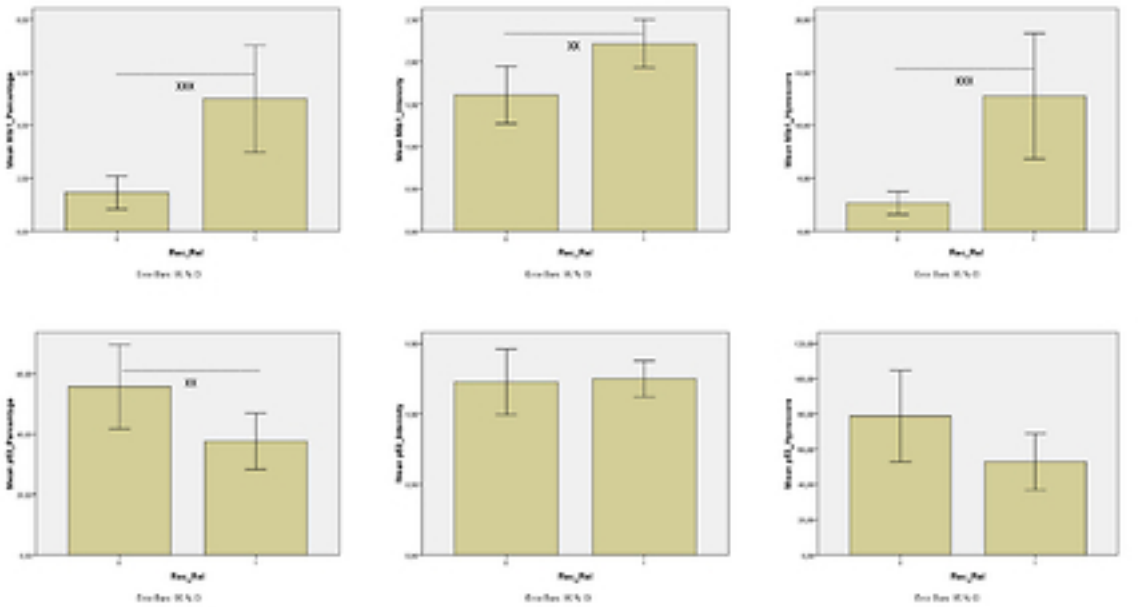
Download source file (279.53 kB)



Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), p53 and Progesteron receptor (PR) for WHO Grade I, II and III cases.

Figure 5

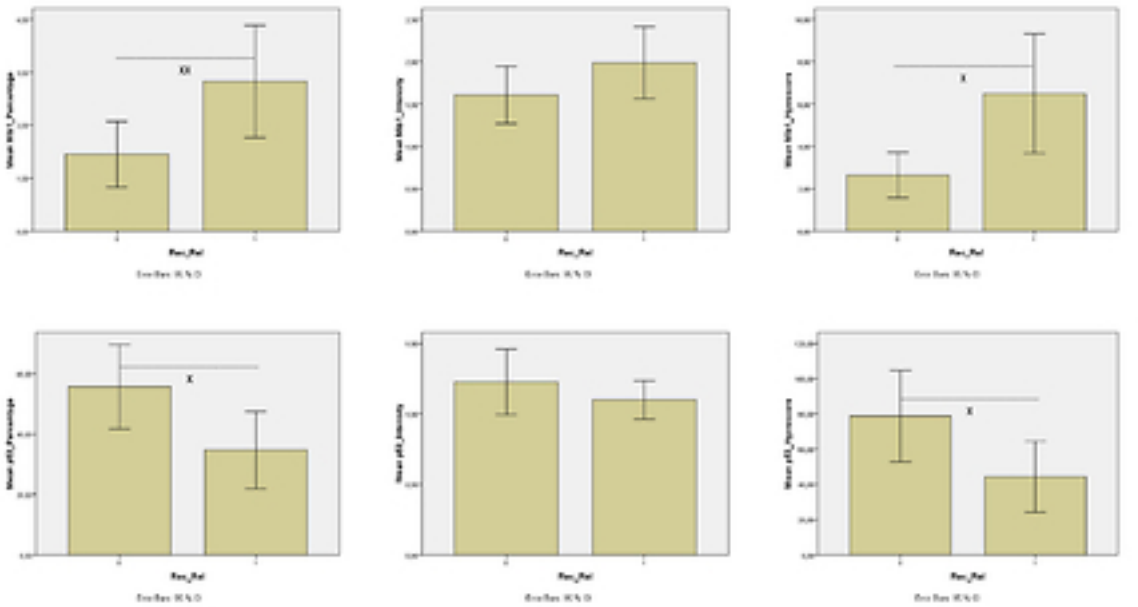
Download source file (176.59 kB)



Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for non-reccurence/relapse (non-R/R) and reccurence/relapsed (R/R) cases, without regarding the WHO grades.

Figure 6

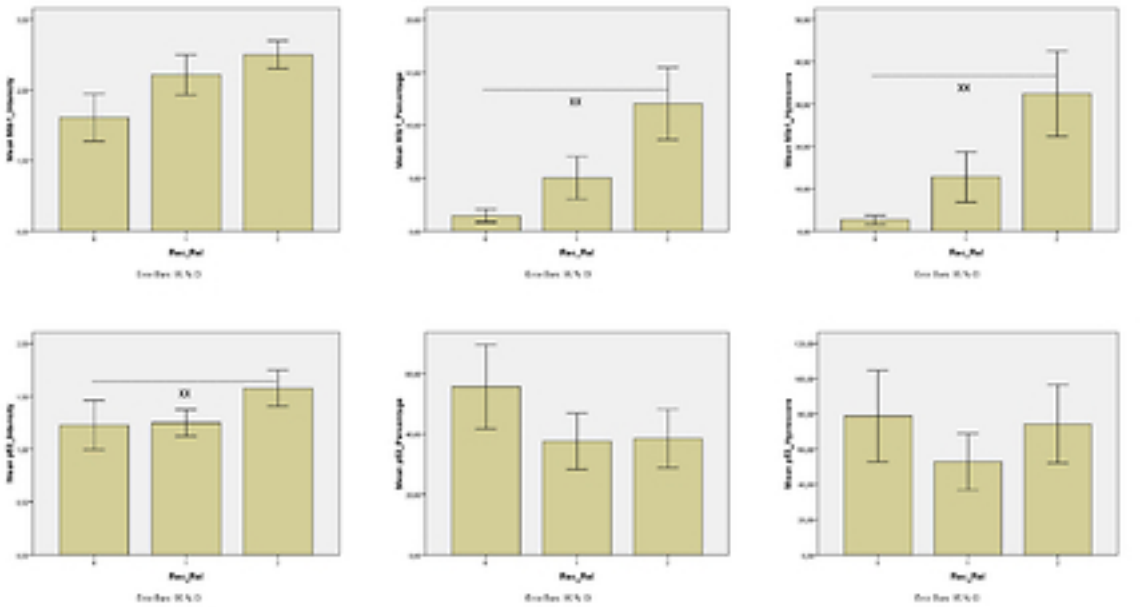
Download source file (177.08 kB)



Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for non-reccurence/relapse (non-R/R) and WHO Grade I. reccurence/relapsed (R/R) cases.

Figure 7

Download source file (185.55 kB)



Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for the 1st, 2nd and last surgical specimens of the recurrence/replase (R/R) cases.

Index

Manuscript body

Manuscript body 1 - Download source file (33.48 kB)

Tables

Table 1 - Download source file (18.12 kB)

Table 2 - Download source file (17.37 kB)

Table 3 - Download source file (17.51 kB)

Figures

Figure 1 - Download source file (147.74 kB)
Low magnification image of histological slide stained with haematoxylin-eosin, made from tissue microarray (TMA) paraffin block. In the upper left corner there are two tissue (brain) samples for guidance regarding localization. The numbers from 1 to 10 represent the individual cases. Every 'donor' case have 3 different samples in the 'recipient' block. Scale bar 5mm.

Figure 2 - Download source file (510.34 kB)
Ki-67 (clone Mib1), p53, and progesterone receptor (PR) immunostain with representative images of the different immunolabelling intensities: negative (0), minimal positivity (1+), medium positivity (2+), to strong positivity (3+). Scale bar 20µm.

Figure 3 - Download source file (46.45 kB)
Ki-67 (clone Mib1), p53 and progesterone receptor (PR) immunopositive cells counted using ImageJ programme. A, C, E, G, I, K pictures are the originals, whereas B, D, F, H, J L show cells numbered with ImageJ Cell Counter plug-in. The numbers from 1-4 stand for the negative, 1+, 2+ and 3+ cells, respectively. A-B pictures are immunostained for Mib1 (43,5% positivity). The C-D pictures are immunostained for Mib1 (6,9% positive). E-F pictures are stained for p53 (58,9% positive). G-H pictures immunostained for p53 (9,8% positive). I-J pictures stained for PR (93,9% positive). K-L pictures immunostained for PR (31,3% positive).

Figure 4 - Download source file (279.53 kB)
Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), p53 and Progesteron receptor (PR) for WHO Grade I, II and III cases.

Figure 5 - Download source file (176.59 kB)
Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for non-reccurence/relapse (non-R/R) and reccurence/relapsed (R/R) cases, without regarding the WHO grades.

Figure 6 - Download source file (177.08 kB)
Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for non-reccurence/relapse (non-R/R) and WHO Grade I. reccurence/relapsed (R/R) cases.

Figure 7 - Download source file (185.55 kB)
Percentage (%), Intensity (0, 1+, 2+, 3+) and Histoscore (intensity x percentage) of immunostain with Ki-67 (clone Mib1), and p53 for the 1st, 2nd and last surgical specimens of the recurrence/replase (R/R) cases.