Pathology - Research and Practice 216 (2020) 152728

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

High density DNA methylation array is a reliable alternative for PCR-based analysis of the MGMT promoter methylation status in glioblastoma

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ARTICLE INFO

Keywords:
MGMT promoter methylation
MS-PCR
Illumina methylome bead chip array
High density DNA methylation array
Glioma
Glioblastoma

ABSTRACT

Aim: MGMT promoter methylation status is an important biomarker predicting survival and response to chemotherapy in patients suffering from glioblastoma. Since new diagnostic methods such as methylome-based classification of brain tumors are more and more frequently performed, we aimed at comparing the suitability of calculating the MGMT promoter methylation status in a quantitative manner from the methylome profiling as compared to the classic gold standard assessment by PCR.

Methods: Our cohort consisted of 39 cases diagnosed as "glioblastoma, IDH-wildtype" of which the MGMT promoter methylation status was analyzed with both methylation-specific PCR and high density DNA methylation array using the STP-27 algorithm. Contradictory results were validated by pyrosequencing.

Results: The inter-method reliability reached 77% (kappa-coefficient: 0.58) when also cases with an inconclusive result in one or the other method were taken into account. When only cases with conclusive results in both methods were considered, a very high inter-method reliability of 91% (kappa-coefficient: 0.86) could be achieved. For "methylated" cases, no contradictory results were obtained. For the remaining two cases with discrepant results subsequent pyrosequencing analyses spoke in favor of each previously applied method once.

Conclusion: In addition to its benefits for molecular subgrouping and copy number analysis of brain tumors, DNA-methylation based classification is a highly reliable tool for the assessment of MGMT promoter methylation status in glioblastoma patients.

Abbreviations: FFPE, formalin fixed paraffin embedded; GBM, glioblastoma; GBM_MES, methylation class glioblastoma, IDH wild type, subclass mesenchymal; GBM_RTK1, methylation class glioblastoma, IDH wild type, subclass RTK I; GBM_RTK2, methylation class glioblastoma, IDH wild type, subclass RTK II; GFAP, glial fibrillary acidic protein; HM450K, Illumina Infinium Human Methylation 450 Bead Chip Array; HRM, high resolution melt analysis; IDH, isocytrate dehydrogenase; IDH1_R132, Hisocitrate dehydrogenase one R132H; MAP2, microtubule associated protein 2; MCF_GBM, methylation class family glioblastoma; MGMT, O6-methylguanine methyltransferase; MS-MLPA, methylation specific Multiplex Ligation-dependent Probe Amplification; MS-PCR, methylation specific polymerase chain reaction; PCR, polymerase chain reaction; STP-27, algorithm to predict MGMT promoter methylation status using high density DNA methylation array data [2]

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https://doi.org/10.1016/j.prp.2019.152728
Received 19 August 2019; Received in revised form 8 November 2019; Accepted 10 November 2019

1. Introduction

High-grade gliomas account for up to 70% of adult malignant primary brain tumours. Glioblastoma (GBM) is the most malignant and also most frequent entity. In population-based studies, patient survival reaches approximately one year only [12,20]. The O6-methylguanine methyltransferase (MGMT) promoter methylation status is an independent prognostic factor in GBM patients, and predicts better response to therapy with alkylating agents [8]. The NOA-08 study has demonstrated the value of treatment decisions based on MGMT status [21]. The CeTeg/NOA-09 trial also showed improved efficacy of combined alkylating therapy, with prolonged overall survival in GBM patients with a methylated MGMT promoter [10].

MGMT promoter methylation is currently one of the most commonly requested molecular diagnostic analyses in neurooncology. Nevertheless, a variety of different methods to analyse this marker is available and currently in use [7].

Different tissue preparations, whether it might be native, cryoconserved, or formalin fixed, can be assessed with various analytical techniques. These include enzyme activity, immunohistochemistry, or DNA tests to assess MGMT promoter methylation status. Tissue quality and tumour cell content as well as DNA quality have to be assured before performing molecular diagnostics. Formalin fixation and paraffin embedding (FFPE) is the most widely used technique to conserve tissue for diagnostic purposes. Methods which use FFPE tissue are therefore most suitable in clinical settings.“

DNA methylation-specific assays can be categorized whether they produce qualitative or (semi-) quantitative results. All DNA-based diagnostic assays require a bisulphite conversion step in which non-methylated cytosine is converted to uracil by bisulphite, while 5-methylcytosine is protected from this conversion.

Methylation specific polymerase chain reaction (MS-PCR) uses a methylation specific primer set. The PCR products are separated on an agarose gel resulting in bands at differing heights for the methylated and non-methylated template. These have to be qualitatively interpreted by the investigator as methylated, non-methylated, or inconclusive. The method of interpretation includes the possibility of inter-observer variability. MS-PCR was the method of choice in most important patient cohort studies [8,19], nevertheless there are draw-backs in the method itself (see discussion).

DNA methylation analysis by pyro-sequencing gives quantitative results for the MGMT promoter methylation status [16]. The exact CpG-sites vary between different primer sets and cut-off values for interpretation have to be established from assay to assay. Some authors discuss the necessity of different cut-off values [3].

As an alternative approach, bead array-based techniques are entering the diagnostic field. Epigenome-wide analysis of DNA methylation patterns with high density DNA methylation arrays via the Illumina Infinium Human Methylation 450 Bead Chip Array (HM450 K), and more recently with the updated EPIC array, have received increasing attention in brain tumour research [5]. Hybridization of tumour DNA from FFPE tissue with these arrays allows for a methylation profile of > 450,000 (or > 850,000 for the EPIC) CpG sites distributed across the genome. Additional helpful diagnostic information can be obtained from methylation based techniques by the calculation of the copy number profile, or the classification into diagnostic DNA methylation classes [5]. It further allows for the analysis of the MGMT promoter methylation status, for example by the STP-27 algorithm designed by Bady et al. [1,2]. The algorithm is based on a stepwise logistic regression model [2] and analyses two different CpG sites within the MGMT gene region resulting in a classification of methylated, non-methylated, or unsure. For an overview about the different CpG sites analysed by the methods presented here, see Fig. 1 **(Figure 1)**. Still, there are various methods available that are currently in use in different laboratories. Thus, establishing standard methods and cut-off values is important for reliable patient treatment stratification. Several studies compared single methods with each other. Methylation specific PCR was compared to closed-tube high-resolution melt analysis (HRM) and pyro sequencing in 83 high-grade glioma patient samples [15]. The authors preferred HRM over MS-PCR and pyro-sequencing due to its simplicity, cost efficiency, accuracy, and speed. Furthermore, HRM was superior in predicting overall survival in glioma patients as compared to MS-PCR [15]. Another group compared pyro-sequencing and semi-quantitative MS-PCR in 139 glioblastoma patients of both frozen and FFPE-fixed specimen in eight centres [13]. Interlaboratory concordance was highest for pyro-sequencing. An additional group could demonstrate that quantitative MS-PCR was a rapid and effective detection method for routine use in pathology laboratories [18]. They compared the results of 350 glioma and ganglioglioma specimens using pyro-sequencing, immunohistochemistry, quantitative MS-PCR, methylation sensitive HRM, and next generation sequencing. One study compared the results of STP-27 via HM450 K to methylation specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) and immunohistochemistry in a cohort of 55 patients [17]. They conclude that both methods are of equal value.

In this study, we evaluated the validity of MGMT promoter methylation analysis resulting from STP-27 obtained from HM450 K as compared to the classic MS-PCR. Discrepant results were validated by pyro-sequencing. Our cohort consisted of 39 patients with IDH-wild type glioblastoma. The diagnosis was confirmed molecularly via the DNA methylation based classification. We also analysed whether the frequent loss of chromosome 10 in GBM samples influenced the accuracy of both methods.

The aim of our study was to analyse if the increasingly used and less biased high-density DNA methylation array technique would be able to substitute MS-PCR in the future.

2. Material and methods

2.1. Patient data and specimen collection

FFPE samples from patients with IDH-wild type glioblastoma, WHO grade IV, were obtained from the Neurological Institute (Edinger Institut), University Hospital Frankfurt am Main, Germany and the Institute of Pathology and Neuropathology, Eberhardt-Karls University and Comprehensive Cancer Center Tübingen-Stuttgart, Tuebingen, Germany. Neuropathological diagnostics was performed by at least two experienced neuropathologists in accordance to the diagnostic categories proposed by Capper et al. [5]. A total of 39 patients with a confirmed methylation profile of adult GBM, IDH-wild type, entered the study (MCF_GBM, GBM_RTK1, GBM_RTK2, GBM_MES). The cohort consisted of 14 female and 25 male patients. The median age was 61 years; the youngest patient was 39 years of age, the oldest 86. 34 newly diagnosed GBM patients and five recurrent GBM patients entered the study. 33 samples were collected with an open surgery, while six samples were taken via stereotactic biopsy. For summary information, see Table 1 and Supplementary Table 1. The use of patient material was approved by the ethical committee of the Goethe University Frankfurt, Germany (GS 04/09 and SNO-06-2014). The neuropathological assessment was based on HE stainings as well as immunohistochemical stainings against glial fibrillary acidic protein (GFAP), mutation specific isocitrate dehydrogenase 1 R132H (IDH1_R132 H) [6], the proliferation marker Ki67, and microtubule associated protein two (MAP2). All cases were IDH1_R132H negative, GFAP was positive to varying extents, and MAP2 was mainly expressed on cell processes to a heterogenous extent. Ki67 proliferation index ranged between 3–15% (data not shown).

2.2. MGMT promoter methylation status assessed by MS-PCR

For each case, the paraffin block showing the largest amount of vital tumour tissue (more than 70% vital tumour tissue at best) was selected for methylation-specific polymerase chain reaction. If areas of CNS

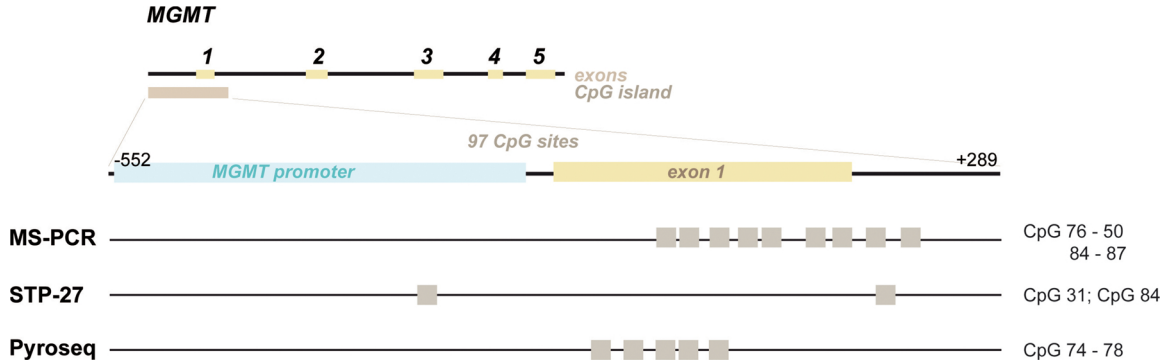

Fig. 1. Diagram of the MGMT gene and the promoter region with CpG sites analysed by MS-PCR, STP-27, and MS-specific pyro-sequencing. The analysed area contains a CpG region stretching out between base -552 and +289 containing 97 CpG sites that cover both the promoter region as well as parts of exon 1. MS-PCR analyses CpG 76–80 and CpG 84–87; STP-27 analyses CpG 31 and CpG 84; methylation specific pyro-sequencing analyses CpG 74-78. Figure adapted and modified from [13,17].

Table 1
Patient cohort.

Glioblastoma, IDH wild type, WHO °IV	
Sex (M/F)	25/14
Mean Age (range; years)	61 (39–86)
Sample Type (open surgery/stereotactic biopsy)	33/6
Disease state (primary/recurrence)	34/5
MS-PCR (methylated/non-methylated/inconclusive	12/24/3
STP-27 (methylated/non-methylated/unsure)	14/21/4

tissue or extensive necrosis were prominent, we improved the sample quality by micro-dissection (Identifying viable tumour tissue in the HE staining and harvesting these areas for DNA extraction). Four slides of ten µm thickness were cut from each paraffin block for whole mount specimen. Ten or more patient samples of approx. 1 mm^3 were collected via a stereotactic biopsy. In these cases, we pooled three to four biopsy specimens from each patient. Slides were deparaffinised by repeated washing in xylene and in 96% alcohol. In Frankfurt, DNA-Isolation was performed using EpiTect Fast Lyse All Bisulfite Kit (Quiagen, Hilden, Germany). PCR was performed on the Thermocycler T3000 (Biometra). For PCR, two µl of sodium bisulphite-pretreated DNA was amplified by the following primer sets:

(I) *MGMT*-methylated forward primer: GTT TTT AGA ACG TTT TGC GTT TCG AC; (II) *MGMT*-methylated reverse primer: CAC CGT CCC GAA AAA AAA CTC CG;

(III) *MGMT*-non-methylated forward primer: TGT GTT TTT AGA ATG TTT TGT GTT TTG AT;

(IV) *MGMT*-non-methylated reverse primer: CTA CCA CCA TCC CAA AAA AAA ACT CCA.

For methylated *MGMT* sequence, a 122-bp fragment is expected, while the amplification of the non-methylated *MGMT* sequence results in a fragment of 129-bp. In each MSP run, DNA from the glioma cell line LNT229 was used as positive control for a methylated *MGMT* promoter, DNA from healthy volunteer donors were used as positive control for a non-methylated *MGMT* promoter status, and H_2O was used as negative control. After MS-PCR, 20 µl of each sample was loaded on a 2% agarose gel at 120 V for 35 min.

2.3. Illumina Infinium Human Methylation 450 bead chip array analysis

The HM450 K array was used to obtain the DNA methylation status of 482,421 CpG sites (Illumina, San Diego, USA), according to the manufacturer's instructions at the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ) Heidelberg, Germany [5]. Methylation of the CpG islands within the MGMT gene were calculated with the STP-27 algorithm considering cg 12434587 and cg 12981137 as described by Bady et al. [2].

2.4. Pyro-sequencing

Pyro-sequencing was performed according to the manufacturers instructions using the commercially available pyro-sequencing kit Pyro Mark Q24 (Quiagen, Hilden, Germany), which allowed for quantification of methylation level in region +17 to +39 of exon 1 of the MGMT gene. Pyro-sequencing was carried out at the Department of Neuropathology, University of Heidelberg, Germany. The same DNA extraction that underwent STP-27 analysis was examined using pyro-sequencing. An overview of the different CpG sites analysed by the three methods is given in Fig. 1.

2.5. Statistical analysis

For correlation analyses of ordinal-scaled variables, we used Cohen´s kappa correlation analysis. A significance level of $\alpha = 0.05$ was chosen for all tests. Statistical analysis was performed using JMP 11.0 software (SAS, Cary, NC, USA) and Prism 6 (GraphPad, La Jolla, CA, USA).

3. Results

3.1. DNA-methylation based classification supports the neuropathologic diagnosis

The histopathologic diagnosis of all cases was confirmed by at least two experienced neuropathologists and validated by the high-density-methylation profile using the DNA methylation based classification as published by Capper et al. [5]. The results are given in Table 2. Only cases of a confirmed methylation profile of an adult GBM, IDH-wild type, entered the study. Split to subgroups, we saw 10% (4 out of 39) GBM_MES, 15% (6 out of 39) GBM_RTK1, 20 % (8 out of 39) GMB_RTK2 and 54% (21 out of 39) MCF_GBM. Samples were classified into the group of MCF_GBM, if their classifier values belonged into various GBM subgroups not reaching the threshold alone.

Table 2
DNA methylation based classification (v.11.2).

Methylation Classifier v.11.2	
GBM_RTK1	6
GBM_RTK2	8
GBM_MES	4
MCF_GBM	21

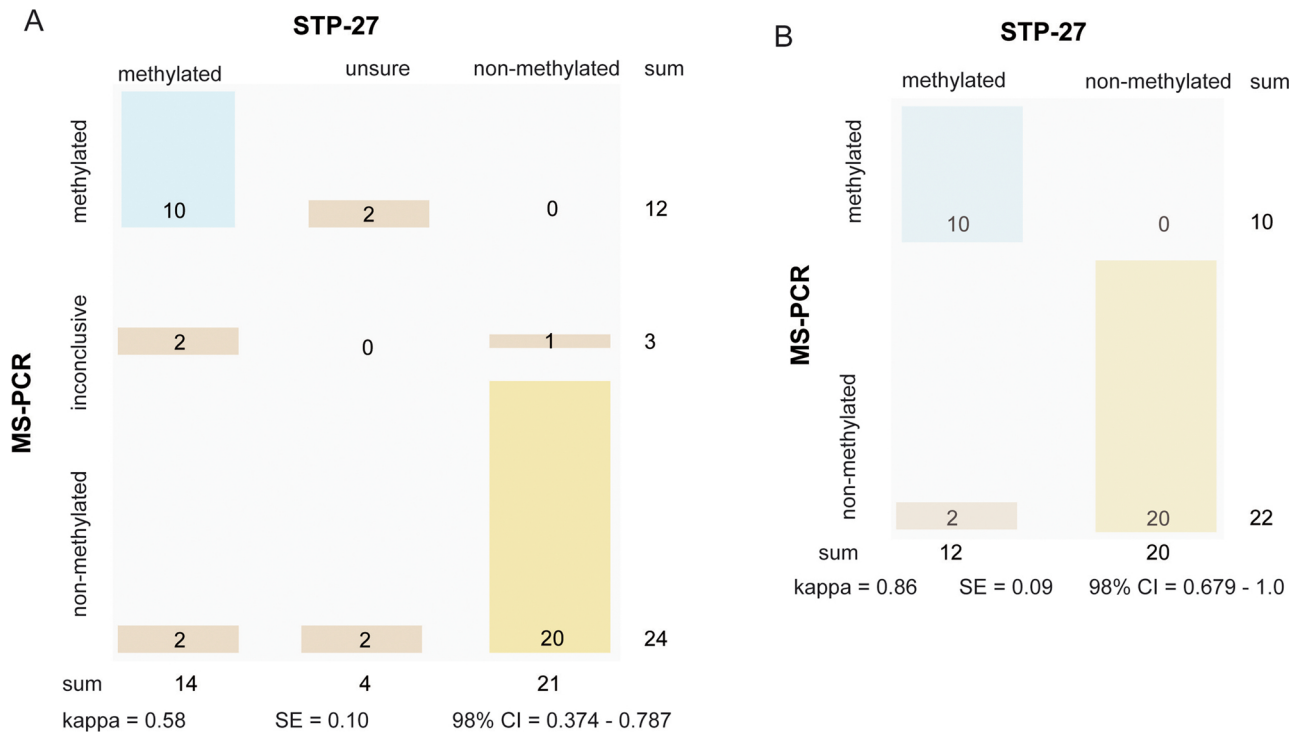

Fig. 2. Association plots for MS-PCR and STP-27. (A) Association plot of STP-27 and MS-PCR of all cases (N = 39) (Blue = methylated, brown = inconclusive/unsure, yellow = non-methylated). Cohen´s kappa = 0.58 with a standard error (SE) of 0.1 and a 98% confidence interval between 0.374 and 0.787. (B) Association plot of STP-27 Analysis and MS-PCR of the subgroup of conclusive cases. Cases with an inconclusive (MS-PCR) or an unsure (STP-27) were censored (N = 32) (Blue = methylated, yellow = non-methylated). Cohen´s kappa = 0.86 with a standard error (SE) of 0.09 and a 98% confidence interval (CI) between 0.679 and 1.0.

3.2. STP-27 and MS-PCR are highly concordant

We compared the results of these two independent and methodologically unrelated methods. Out of all 39 patient samples tested, MS-PCR classified 12 cases as “methylated”, 24 cases as “non-methylated”, and three cases as “inconclusive”. STP-27 classified 14 cases as “methylated”, 21 cases as “non-methylated”, and four cases as “unsure”. Taking a closer look at the data, out of 12 “methylated, MS-PCR” cases 10 cases were classified as methylated by STP-27 while two cases were classified as “unsure” by STP-27, with no case being contradictory. MS-PCR classified three cases as “inconclusive”, of which two were classified as “methylated”, and one was classified as “non-methylated” by the STP-27. All cases deemed “inconclusive” by MS-PCR were unambiguously classified by STP-27. Within the 24 “non-methylated” cases in MS-PCR, STP-27 classified 20 as “non-methylated”, however two as “methylated”, and another two as “unsure” **(**Fig. 2A**)**. Two cases therefore remained contradictory (non-methylated by MS-PCR but methylated by STP-27). The overall concordance rate ranges from 77 % for all congruent cases (30 out of 39 cases), to 83 % for the methylated cases (10 out of 12 cases) and 86 % for the non-methylated cases (20 out of 24 cases). The kappa-coefficient is 0.58 (Cohen´s kappa).

Furthermore, we performed a subgroup analysis of all cases that showed a conclusive prediction in both methods. Therefore, we censored all cases with an “inconclusive” or “unsure” result in either of the two methods. After censoring, 32 cases remained for further analyses. MS-PCR classified 10 cases as “methylated”, of which all cases (100%) were also classified as “methylated” by STP-27. MS-PCR classified 22 cases as “non-methylated”, of which 20 were classified as “non-methylated” and two were classified as “methylated” by STP-27 (Fig. 2B**).** In case of “non-methylated” results according to MS-PCR, 91% cases were congruent with STP-27 results leading to a kappa-coefficient of 0.86 (Cohen´s kappa), underlining the very high inter-method reliability.

In two cases, we received contradictory results between MS-PCR and STP-27. We rechecked tissue quality and tumour cell content as well as procedure protocols with no detection of technical failures or concerns. As a further independent and clinically relevant method, we performed methylation-specific pyro-sequencing to evaluate the methylation status of the MGMT promoter. Three cut-off groups for pyro-sequencing were proposed by Quillien [13]: The first group below 8% (“non-methylated”) corresponds to a low level of methylation and poor survival; the second group higher than 12 % corresponds to “methylated” and higher overall survival. They propose a third group in the range of 9–12% as a grey zone with no clear benefit of temozolomide treatment of “unsure” significance. One case was “non-methylated” (peak values in %: 7, 8, 9, 6; mean value 7.5 %, case ID 32); the other case was “methylated” (peak values in %: 37, 72, 76, 75; mean value 65 %, case ID 34) according to pyro-sequencing **(**Fig. 3**)**. In case 32, pyro-sequencing favoured MS-PCR, in case 34 pyro-sequencing favoured STP27.

3.3. Copy number of chromosome 10 has no impact on MGMT promoter methylation testing

Chromosomal aberrations that frequently occur in cancer cells could affect the accuracy of genetic diagnostics. The MGMT gene resides on chromosome 10 and is frequently monozygous in GBM cancer cells. This may affect testing methods differently (potentially array-based techniques more and PCR-based techniques less). In our cohort, we saw only one copy of chromosome 10 in 85% of all cases (33 out of 39). With respect to small group sizes, there was no significant association of the result “unsure” or “inconclusive” with a loss of chromosome 10 with MS-PCR or STP-27 (data not shown). We compared whether the numeric values of STP-27 were related to the chromosome 10 statuses. The mean in difference of the groups “methylated” and “non-methylated” was rearranged into the groups “one copy of chromosome 10″ and “two copies of chromosome 10″. As tested with Fisher´s exact *t*-test with Welch´s correction for differing group sizes, there was no

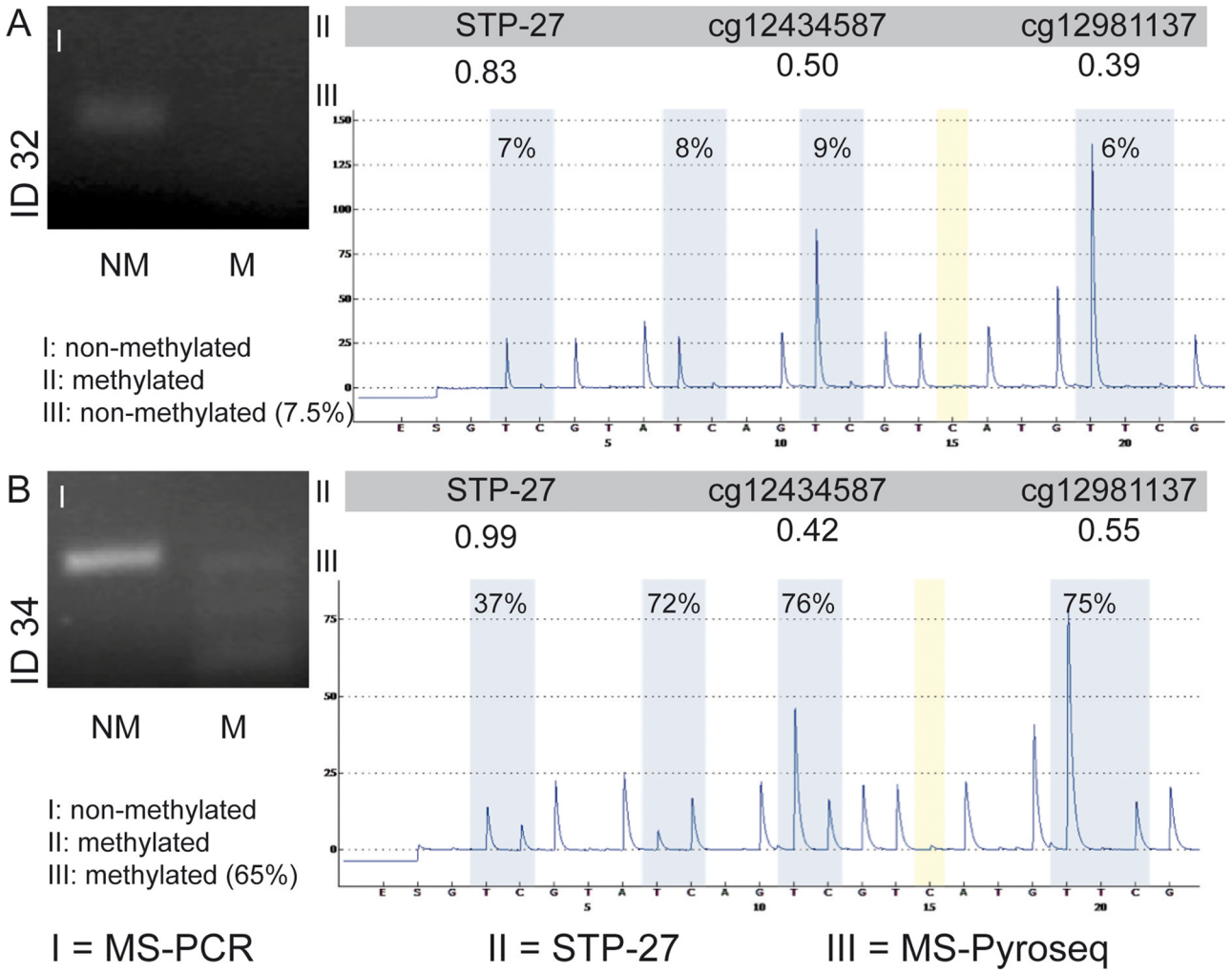

Fig. 3. Discrepant cases in MS-PCR and STP-27. Overview of the raw data of MS-PCR, STP-27, and MS-specific pyro sequencing of the MGMT promoter. Each panel represents one discrepant cases (A, B). I illustrates the respective area of agarose gel of the MS-PCR, II shows the raw-values of STP-27 (STP-27, cut-off 0.3582) and the raw values of the respective CpG - island of the MGMT-promoter (cg12434587 and cg12981137). The results of MS-pyro-sequencing of the MGMT promoter are depicted in III. Case 32: peak values in %: 7, 8, 9, 6; mean value 7.5 %; case 34: peak values in %: 37, 72, 76, 75; mean value 65 %, Cut-offs for pyro-sequencing as given in [13]. For the case ID see supplementary Table 1 (Supplementary Table 1).

statistically significant difference between numeric values of STP-27 and the chromosome 10 status (Fig. 4). In our cohort, the chromosomal loss did not affect the MGMT promoter methylation results.

4. Discussion

In this study, we compared the results of predicting the MGMT promoter methylation status by STP-27 to MS-PCR. Both methods require tumour tissue with a relevant amount of tumour cells. Our study cut-off was 70% tumour cell content. MS-PCR requires several manual work steps, which can usually be performed in one to two consecutive days. The final read out of the PCR product lanes requires visual interpretation of the agarose gel that may suffer from subjective bias. In most centres that perform MS-PCR, the MGMT-promoter methylation assay is done on a weekly basis. Besides the technician performing the assay, costs are low, and the equipment necessary is part of the standard infrastructure of most neuropathological laboratories.

In contrast, STP-27 requires high-resolution DNA-methylation arrays and specialized read-out analysis instrument. The bioinformatic analysis tools can be assessed online open-source via https://www.molecularneuropathology.org. The currently available DNA array (EPIC) offers eight sample slots, and cost increases if not all slots can be used. The manual part of the analysis takes at least four days. Due to the design of the assay and the robust method, HM450 K or EPIC provides very high reproducibility of its results. Costs for a single MGMT promoter methylation analysis are definitively higher than those generated by MS-PCR, but the array-based profiling also provides additional information about distinct molecular brain tumour classes and genome-wide copy number information. The use of EPIC seems feasible to answer important diagnostic questions, and then can be utilized to answer the MGMT promoter methylation status as a secondary result.

Overall, both methods provide highly concordant results. After censoring all cases being non-conclusive in one of both methods, in the setting of a “methylated” MGMT promoter both methods fully matched (100%). In the setting of a “non-methylated” MGMT promoter, the overlap was 91%. Impaired DNA quality may still be one explanation for the discrepancy as seen in two cases, despite proper DNA purity measurements by spectrometry. These two cases were either methylated or non-methylated according to pyro-sequencing, supporting the value of both methods – or the need for further improvement of both.

If STP-27 results in “unsure” for the MGMT promoter methylation status, regularly two of our centres perform pyro-sequencing and rely on the pyro-sequencing result. One centre diagnoses “non-conclusive”, especially if the DNA methylation based classification assigns the tissue to be non-tumour brain tissue (e.g. white matter, different areas of cortex or inflammatory tumour environment), which seems connected

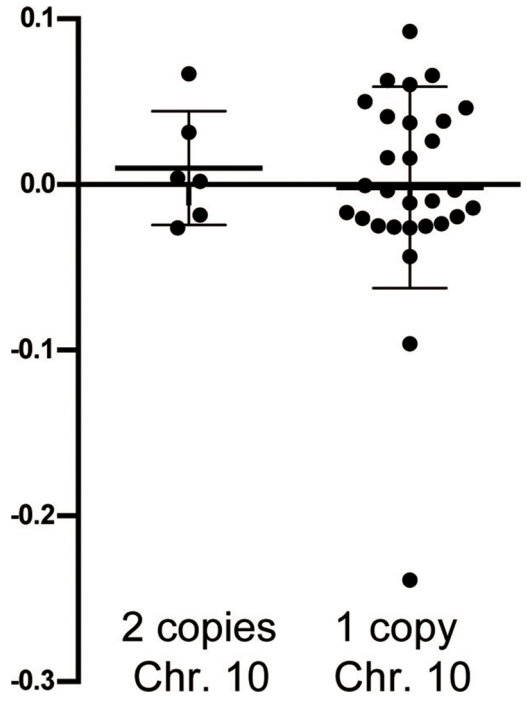

Fig. 4. STP-27 values in relation to the chromosome 10 status. All cases unambigiously classified into "methylated" or "non-methylated" by STP-27 underwent the analysis. Unsure cases were censored. The difference in mean values in the groups "methylated" and "non-methylated" were calculated and plotted against the chromosome 10 status ("1 copy Chr. 10″ or "2 copies Chr.10″). This method adjusted the values to comparable ranges ("non-methylated" with very low raw values as compared to "methylated" with high raw values) and still yielded reasonable group sizes. Statistical analysis was done with Fisher´s exact *t*-test with Welch´s correction for differing group sizes (29 with "one copy Chr. 10″ and 6 with "2 copies Chr. 10″).

in their hands. The treatment decision is made interdisciplinary in the tumour board; usually these patients are treated as "not methylated". Whether this pathway is correct, has to be determined by further studies and neuro-oncology task forces especially as there are some authors that report a grey zone of low MGMT promoter methylation with some sensitivity to temozolomide [9].

As compared to MS-PCR, STP-27 leads to a higher probability for a methylated and to a lower probability for a non-methylated result. STP-27 classifies results more often as "unsure", probably recognizing the diagnostic uncertainty and the existence of "intermediate" methylation to better extent, as proposed by Quillien [13].

Some studies deal with the problem of inconclusive MGMT promoter methylation status, varying results after repeated measurement and/or varying results after measurement with different methods. Xia et al. analysed 58 cases of glioblastoma with inconclusive MGMT promoter methylation status after MS-PCR in a cohort of 465 GBM patients [22]. They report a poor overall survival of these patients and postulate a dose-response relationship between patient survival and the extent of methylation in inconsistently methylated GBMs. Furthermore, they discuss technical factors as possible reasons for inconsistent results in repeated MGMT MS-PCR testing. Brigliadori et al. also report a correlation between extent of methylation and overall survival [4]. Recently, Hegi described a "grey zone" of low MGMT promoter methylation – these patients seem to confer some sensitivity to temozolomide treatment [9].

Both MS-PCR and STP-27 provide qualitative results. Pyro-sequencing results in a percentage of methylated DNA, which have to be interpreted with the help of cut-off values. These have to be chosen wisely. In our setting, the cut-off for methylation when using pyro-sequencing was below eight per cent for "non-methylated", above twelve per cent for "methylated" and an intermediate range (nine to twelve per cent) for "unsure" results [13]. These cut-off values were approximated to model overall survival predictions [13,14]. Comparison of cut-off values for methylated MGMT-promoter using pyro-sequencing cannot be done without taking into account the effect of different CpG-islands, which vary between different assays (see Fig. 1 for the methods used in this study).

We further analysed whether the status of chromosome 10 might influence the frequency of an "unsure" or" inconclusive" result. In our cohort, the copy number of chromosome 10 did not have an independent effect on the result of either assay, which is in line with the results of Bady [1]. Nevertheless, our study reflects the problems of diagnostic material with varying sample quality, tumour content, and DNA quality, all influencing analysis results.

In our hands, the prediction of the MGMT promoter methylation status via STP-27/HM450 K seems to have slight advantages over MS-PCR. STP-27/HM450 K is as a highly reliable and reproducible method to determine MGMT promoter methylation status in glioblastoma patients.

While the overall concordance is high (86% in the subgroup analysis), the rate of falsely classified cases is equal (2.5% as analysed via MS-specific pyro-sequencing), but STP-27 marked "unsure" results to a higher extent.

5. Conclusion

Despite the clearly higher costs of the STP-27 approach, this method extracts much more information from tumour tissue. Due to the many other markers and results which can be deduced from array-based epigenetic profiling, this method seems to be promising, also following the increasingly molecular classification system for brain tumours that was initiated by the 2016 WHO classification for brain tumours [11].

Authors' contributions

The paper is the result of the intellectual collaboration of all listed authors. In particular, AKB, PNH, and MM participated in the conception and design of the study, drafted the manuscript and performed the neuropathological analysis. DC and DJ performed HM450 K analysis and pyro-sequencing and helped interpreting the findings. JS performed the neuropathological analysis and MSP-PCR of the Tuebingen cases and helped interpreting the findings. AD, DS and MM supervised the study and helped interpreting the findings. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The use of patient material was approved by the ethical committee of the Goethe University Frankfurt, Germany (GS 04/09 and SNO-06-2014).

Availability of data and materials

Not applicable. Data sharing is not applicable for this article as no datasets were generated or analysed during the current study.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

We thank Maika Dunst, Tatjana Starzetz and Cornelia Penski for excellent technical assistance. We thank Christoph Bernhard Hoog Antink for language editing.

Funding

AKB received funding of the START program (grant 29/17) and the rotation program of the Medical Faculty of RWTH Aachen University. MM would like to thank the Luxembourg National Research Fond (FNR) for the support (FNR PEARL P16/BM/11192868 grant).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152728.

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