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CASE REPORT

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DNA methylation profiling from cerebrospinal fluid as a diagnostic tool for pineoblastoma

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Abstract

Pineoblastoma is a rare and aggressive malignancy that often affects pediatric populations. Accurate diagnosis is challenging due to histological overlap with other central nervous system tumors and limited molecular data. DNA methylation profiling and analysis of circulating tumor DNA (derived from both cell dissemination as well as cell-free– cfDNA) in cerebrospinal fluid (CSF) are emerging tools for precise tumor classification, in the field of pediatric central nervous system tumors. Here, we report a challenging case of a 17-year-old refugee girl with a previous diagnosis of a primitive neuroectodermal tumor. Formalin-fixed, paraffin-embedded tissue was not available for histopathological re-evaluation. However, the methylation profiling of low amount of CSF-derived DNA classified the tumor as "pineoblastoma, subtype miRNA processing altered 1, subclass A," enabling patient management. The diagnosis was later confirmed through tissue-based DNA methylation analysis of a secondary lesion, demonstrating that the epigenetic signature faithfully reflected tumor features. This case report highlights the potential of CSF-based DNA methylation profiling as a minimally invasive yet accurate diagnostic tool for pediatric CNS tumors. The concordance between CSF and tissue profiling supports the integration of liquid biopsy into diagnostic workflows, allowing for earlier diagnosis and personalized treatment strategies. However, more studies are needed to demonstrate the reliability of our approach in other CNS malignancies.

Keywords DNA methylation, Cerebrospinal fluid, Pineoblastoma, Liquid biopsy, Diagnosis

Introduction

Pineal parenchymal neoplasms are malignancies that mostly occur in childhood, representing 3–11% of all pediatric brain tumors compared to < 1% of brain tumors in adults [1].

The 2021 World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) has defined four types of pineal gland tumors, including pineocytoma,pineal parenchymal tumor of intermediate differentiation,pineoblastoma (PB), and papillary tumor of the pineal region [2].

Among these tumors, pineoblastomas are the most common, accounting for 40% of parenchymal pineal cancers, and the more aggressive ones [3] with frequent

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invasion and dissemination throughout the craniospinal axis [4].

Due to the rarity of these tumors, the current knowledge of the biology and the molecular features of PBs is limited thus impacting accurate diagnosis and treatment.

In the most recent years, genome-wide DNA methylation profiling has been recognized as a suitable or rather essential diagnostic tool auxiliary to conventional histopathology for many tumor types, especially CNS tumors. In addition, liquid biopsy based on the analysis of tumor DNA in body fluids, has emerged as a promising approach in molecular profiling, early detection, and response to treatments in CNS tumors. In these tumors, cerebrospinal fluid (CSF) is the most indicated substrate for DNA source, derived from both cell dissemination as well as cfDNA, due to its direct interaction with brain tumor cells [5–7]. On the contrary, its detection in other body fluids could be limited by the presence of the blood-brain barrier [8].

The relevance of DNA methylation profiling in improving differential diagnosis is quite clear in the context of primitive neuroectodermal tumors of the central nervous system (PNET). The diagnosis of these tumors is troublesome due to the limited knowledge of the molecular markers and the histological overlap with other neuroepithelial CNS tumors. Sturum and colleagues [9] have demonstrated that by performing DNA methylation profiling of PNET these tumors didn't generate one distinct cluster: the majority of them clustered with other CNS tumors while the remaining cases generated four different clusters that identify four new CNS tumor entities (CNS neuroblastoma with FOXR2 activation, CNS Ewing sarcoma family tumor with CIC alteration, CNS high-grade neuroepithelial tumor with MN1 alteration and CNS high-grade neuroepithelial tumor with BCOR alteration). Finally, a few cases were not classified into any cluster and have been defined as CNS Embryonal Tumors, Not Otherwise Specified.

DNA Methylation profiling of PB has allowed for the definition of the molecular identity of these tumors helping to discriminate them from other embryonal CNS tumors and to better clarify intertumoral heterogeneity among PB [9]. Indeed based on molecular features, pineoblastomas have been divided into four molecular subtypes with distinct age of onset and prognosis: pineoblastoma, microRNA processing-altered 1; pineoblastoma, microRNA processing-altered 2; pineoblastoma, RB1-altered (pineal retinoblastoma); and pineoblastoma, MYC- and/or FOXR2-activated [10].

Case presentation

A 17-year-old girl Ukrainian refugee was presented to our hospital with a history of PNET of the lumbar spinal cord metastatic to the brain diagnosed 6 months earlier in her country. However, formalin-fixed, paraffin-embedded (FFPE) tissue was unavailable for histological revision in our center. Initial imaging revealed a hypointense region on T1-weighted Magnetic Resonance Imaging (MRI) along the spinal cord (Fig. 1 A). The patient had previously undergone two cycles of vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) chemotherapy and intrathecal methotrexate therapy.

MRI of the brain and spine, performed at our institution, revealed leptomeningeal involvement localized to the left facial acoustic nerve bundle, medullary alterations, and root involvement of the cauda, which appeared reduced in extension (Fig. 1 B).

Cytological analysis of the CSF confirmed the presence of neoplastic cells exhibiting expression of synaptophysin (Fig. 1 C). Differential diagnoses at this stage included various embryonal and high-grade tumors comprised in the morphological spectrum of PNET. However, due to the lack of tumor tissue, a more precise characterization was not possible, thus we decided to use cerebrospinal fluid as a liquid biopsy source.

To achieve accurate tumor classification, DNA methylation profiling was conducted as previously described [11], using DNA extracted from the CSF. Specifically, 50 ng of DNA was utilized as input material for the analysis. Sample was analyzed using the Infinium Methylation EPIC v1 (850k) or v2 (930k) BeadChip (Illumina), according to the protocol issued by the manufacturer. We uploaded raw methylation intensity data files (IDATs) to v12.5 (for EPIC version 1) or v12.8 (for EPIC version 2) of the DKFZ/Heidelberg Brain Tumor Classifier (https://www.molecularneuropathology.org) [12]. The methylati on profile unequivocally classified the tumor as "pineoblastoma, subtype miRNA processing altered 1, subclass A" with a calibrate score of 0.85, enabling more precise treatment planning. We also analyzed the raw DNA methylation data of the collected cases with R (v4.3.1). First, we compared them with cases obtained from the literature [12, 13], choosing CNS entities that display histological similarities with PB. Specifically, we performed data loading and probe filtering using package ChAMP [14] and processing each array platform separately (HumanMethylation450, EPIC, or EPICv2) using method "minfi" [15] and filtering out probes located on known single nucleotide variants, sex chromosomes or with detection p-value > 0.01. Then, we merged the raw beta values according to probe name and genomic position and normalized the entire matrix using the BMIQ method [16]. Finally, we used the 10,000 probes with highest standard deviation to compute the 1-variance weighted Pearson correlation between the samples, and then used this correlation matrix to compute the distance matrix that became the input of Rtsne function from Rtsne package (https://CRAN.R-project.org/package=Rt

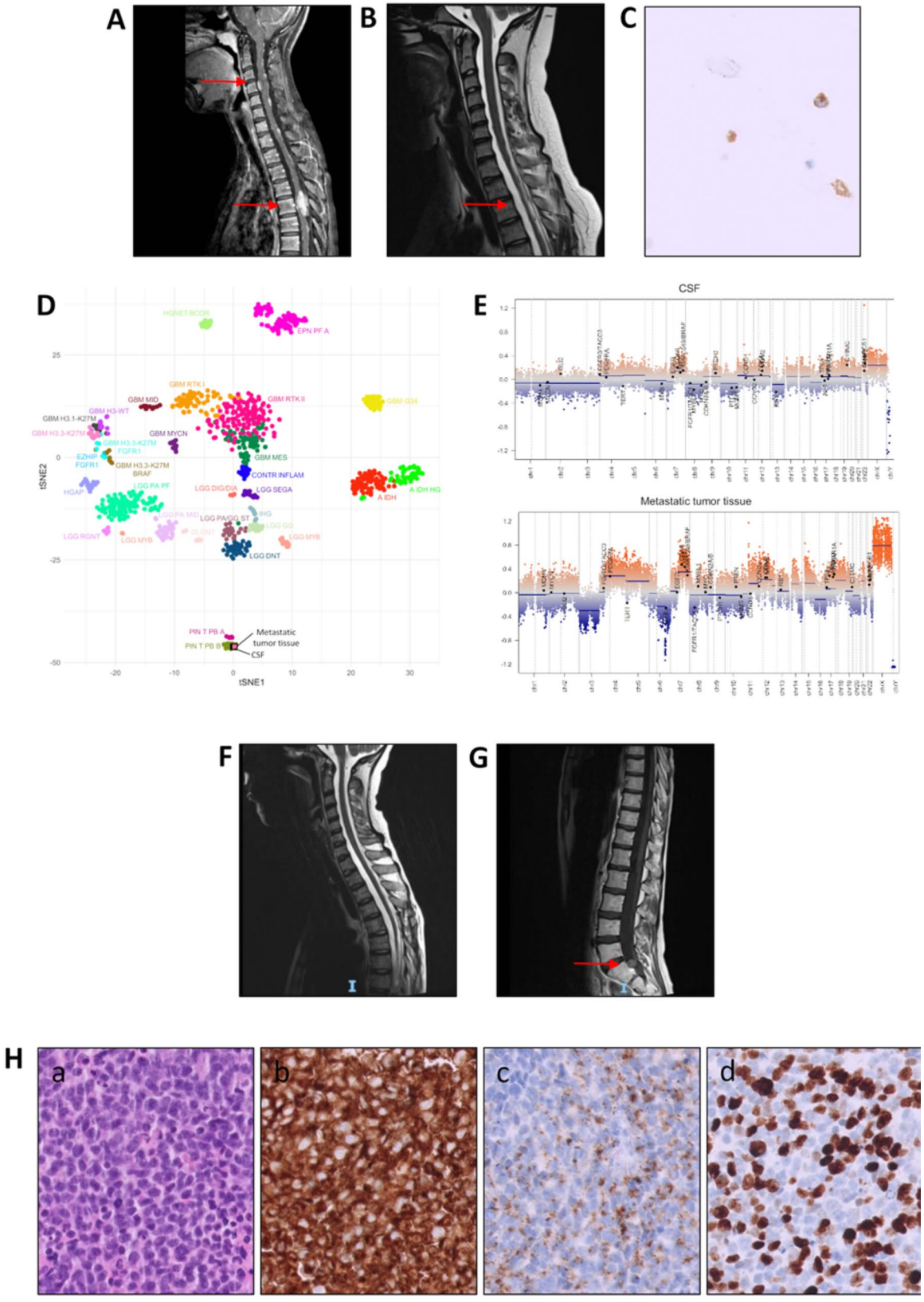

Fig. 1 (See legend on next page.)

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Fig. 1 **A**: T1-weighted MRI sagittal images of the patient pre-surgery, revealing a hypointense region along the spinal cord (red arrow). **B**: T2-weighted MRI sagittal images of the patient post-surgery and chemotherapy. **C**: Cytological analysis on CSF sample confirmed the presence of Synaptophysin positive cancer cells (Magnification 40×). **D**: t-distributed stochastic neighbor embedding plots of DNA methylation clustering patterns. The patient's samples, CSF and metastatic tumor tissue biopsy, are outlined in black, and clustered together with pineoblastoma group B (PIN T, PB B). Brain tumor classifier was determined by version 11b4. **E**: CNV analysis of CSF (upper panel) and tumor tissue (bottom panel) from the same patient, demonstrated multiple and complex chromosome gains (red) and losses (blue). **F**: T1-weighted MRI sagittal images of the patient in follow-up, showing marked improvement in leptomeningeal disease. **G**: T1-weighted MRI sagittal images of the patient pre-surgery, revealing a solid lesion in the sacral canal (red arrow). **H**: Immunohistochemical features. (**a**) haematoxylin and eosin staining; immunopositivity for (**b**) Synaptophysin, (**c**) Chromogranin and (**d**) KI-67 proliferation index more than 50% of tumoral cells (Magnification 40×).

sne). This analysis classified the CSF methylation profile in the subgroup of pineoblastoma group B (PIN T, PB B (v11.4, corresponding to the subtype miRNA processing altered 1)) (Fig. 1 D).

We also used R package Conumee (https://bioconductor.org/packages/devel/bioc/vignettes/conumee/inst/doc/conumee.html) to perform Copy Number Variation (CNV) analysis of the sample. CNV profile suggested gain of chromosome (c.) X and 7q (Fig. 1 E, upper panel).

The patient first received the first two courses of ICE-based chemotherapy (Ifosfamide, Carboplatin, and Etoposide). Then she underwent a cycle of high-dose thiotepa followed by tandem autologous hematopoietic cell transplantation, a strategy to delay radiation therapy in pediatric high-risk brain tumors [17]. The latter treatment resulted in significant toxicity, characterized by grade 4 mucositis with weight loss, delayed bone marrow recovery and prolonged hospitalization.

A follow-up brain-cord MRI, six months later, showed marked improvement in leptomeningeal disease and partial response at the encephalic level (Fig. 1 F). Craniospinal irradiation was complicated by hematological toxicity and malnutrition, requiring temporary suspension.

Approximately 18 months following initial admission to our institution, a surveillance MRI revealed disease recurrence, characterized by contrast enhancement in the floor of the fourth ventricle and a solid lesion in the sacral canal extending to the right sacral foramen (Fig. 1 G).

Subsequently, the sacral lesion was surgically resected to achieve nerve decompression. The tumor specimen was formalin-fixed and paraffin-embedded for subsequent histopathological evaluation, immunohistochemistry, and cytogenetic and molecular analyses. Microscopic examination revealed a neoplasm consisting of sheets of cells with a high nuclear-cytoplasmic ratio, numerous mitoses (8 mitoses/mm square), and large areas of necrosis. Tumor cells express synaptophysin diffusely, and Chromogranin focally, while Neurofilaments were present in isolated elements. The lesion was negative for GFAP, OLIG2 and LIN28. Moreover, limited expression of p53 (5% of neoplastic cells) was observed and the Ki67 labeling index was 50% (Fig. 1 H). The lesion also showed a moderate diffuse positivity for mTOR.

Overall the pathological features were in keeping with the diagnosis of PB. Moreover, DNA methylation profiling confirmed the diagnosis of "pineoblastoma, subtype miRNA processing altered 1, subclass A", matching the results on CSF. Furthermore, at tSNE analysis the tumor clustered in the subgroup of pineoblastoma group b, consistent with CSF sample (Fig. 1 D bottom panel).

CNV profile of the tumor tissue showed a more pronounced gain of chromosome (chr.) X and 7q, and highlighted additional alterations, such as gain of chr. 4, loss of chr. 3p and a portion of chr. 3q and loss of chr. 6q (Fig. 1 E, bottom panel). This divergence was likely related to the different source of biological materials, timing (sacral biopsy was performed almost two years later) and the pharmacological treatments received between the two procedures.

A DNA-targeted panel investigating more than 500 genes (TruSight Oncology 500, Illumina) identified a pathogenic inactivating mutation in *DICER1* (p. Arg676*; variant allele frequency: 98.6%), in line with the diagnosis of "pineoblastoma, subtype miRNA processing altered 1, subclass A". Moreover, the patient carried *CTNNB1* (p. Gln193_Val195delinsLeu; variant allele frequency: 47%) and *EGFR* (p. Lys80Thr; variant allele frequency: 28%) mutations. The lesion was also analyzed with an RNA-targeted panel (Custom Archer Fusion Panel) which yielded a negative result.

Three months after surgery, MRI revealed regular post-surgical evolution and reduction of the pseudonodular lesion. For mTOR overexpression, in consideration of the toxicity of previous therapies, oral therapy with everolimus was started. However, eight months later, the patient passed away in a palliative care center for the progression of the disease.

Discussion and conclusions

This case report highlights the integration of two advanced diagnostic approaches—DNA methylation profiling and liquid biopsy—in the clinical evaluation of a pediatric brain tumor. DNA methylation profiling has become an indispensable tool in neuro-oncology, increasingly incorporated into the diagnostic workflow for CNS tumors. It offers a robust alternative or complement to traditional histological and molecular diagnostic methods [12, 18].

Liquid biopsy, particularly the analysis of circulating tumor DNA and cell-free DNA, has also emerged as a promising non-invasive diagnostic strategy. Circulating DNA captures both genetic and epigenetic alterations of the primary tumor, making it a powerful tool for clinical applications. This is especially critical in pediatric brain tumors, where the anatomical location of the tumor often poses challenges for surgical access and obtaining sufficient tissue for biopsy. In cases like the one described here, where FFPE tissue is unavailable, conventional diagnostic approaches are further constrained.

Recent research has demonstrated the potential of combining DNA methylation profiling with liquid biopsy to enhance tumor characterization. However, evidence specifically supporting its application in pediatric CNS tumors remains limited. This report underscores the utility of this integrated approach, particularly in addressing the diagnostic challenges posed by pediatric brain tumors, and highlights the need for further studies to validate and expand its clinical applicability [5, 19].

In our patient initially presenting with a descriptive diagnosis of PNET, DNA methylation analysis of CSF played a pivotal role in achieving an accurate diagnosis of pineoblastoma, effectively overcoming the limitations posed by the absence of FFPE tissue.

As previously reported for central nervous system tumors, CSF—despite requiring the more invasive procedure of lumbar puncture compared to a blood draw—is likely the most reliable source for tumor DNA collection due to its proximity to the tumor microenvironment [20]. Using a minimal amount of input DNA, methylation profiling classified the tumor within the methylation class family of pineoblastoma with an optimal calibrated score. This finding was subsequently validated through DNA methylation analysis of tumor tissue obtained during a second surgical resection, reinforcing the robustness of the CSF-based approach.

Our results demonstrate that CSF-based DNA methylation profiling provides diagnostic accuracy comparable to conventional tissue-based methods while significantly reducing patient risk. However, we noted discrepancies in the CNV profiles between liquid biopsy and tissue samples. These differences likely arise from unique characteristics of liquid biopsy, including: (i) contamination with non-tumor cell-free DNA, (ii) the tumor's proximity to the body fluid sampled, and (iii) therapy-induced genetic alterations acquired during treatment cycles or tumor evolution.

Prior to CSF-based DNA methylation profiling, treatment planning was based on the presumptive diagnosis of PNET, following a regimen of VIDE chemotherapy and intrathecal methotrexate. However, the identification of a distinct methylation profile confirming the diagnosis of pineoblastoma, influenced clinical decisions and guided therapy adjustments. Specifically, the refinement of the diagnosis reinforced the choice of an intensified treatment strategy (high-risk protocol), including high-dose chemotherapy with autologous hematopoietic cell transplantation, followed by radiotherapy.

This study underscores the potential of CSF-based DNA methylation profiling as a valuable diagnostic tool, particularly in cases where tissue samples are unavailable, while highlighting the need to account for the specific nuances of liquid biopsy in the diagnostic process.

Abbreviations

PIN T, PB B	Pineoblastoma tumor, pineoblastoma B
WHO	World health organization
CNS	Central nervous system
PNET	Primitive neuroectodermal tumors of the central nervous system
FFPE	Formalin-fixed, paraffin-embedded
MRI	Magnetic resonance imaging
VIDE	Vincristine, ifosfamide, doxorubicin, and etoposide
ICE	Ifosfamide, Carboplatin, and Etoposide
CSF	Cerebrospinal fluid
RT	Radiotherapy treatment
cfDNA	Cell-free DNA
chr	Chromosome
CNV	Copy number variation

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Author contributions
E.M., C.A., L.A. designed of the study; E.M., A.C., G.M., A.M., A.C., S.R. compiled the radiological, surgical and clinical data; E.M., A.M., C.A, L.A., L.P., S.P. acquisition, analysis, and interpretation of data; F.V., S.B., I.G., C.T. conducted the molecular studies; E.M., C.A., L.A. drafted the manuscript; S.R., A.M., F.L. revised the manuscript; all the authors reviewed the manuscript.C.A. and L.A. have contributed equally to this work and share the co-first authorship. E.M. and A.M. have contributed equally to this work and share the co-senior authorship. E.M. and L.A. share the corresponding position.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Appropriate intuitional approval and written consent was obtained from the patient's family for this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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