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INSTRUCTIONAL LECTURE

Practical procedures for the integrated diagnosis of astrocytic and oligodendroglial tumors

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Abstract

The publication of the 2016 World Health Organization Classification of Tumors of the Central Nervous System (2016 WHO CNS) represented a major change in the classification of brain tumors. However, many pathologists in Japan cannot diagnose astrocytic or oligodendroglial tumors according to the 2016 WHO CNS due to financial or technical problems. Therefore, the Japan Society of Brain Tumor Pathology established a committee for molecular diagnosis to facilitate the integrated diagnosis of astrocytic and oligodendroglial tumors in Japan. We created three levels of diagnoses: Level 1 was defined as simple histopathological diagnosis using hematoxylin and eosin staining and routine cell lineage-based immunostaining. Level 2 was defined as immunohistochemical diagnosis using immunohistochemical examinations using R132H mutation-specific IDH1, ATRX, and/or p53 antibodies. Level 3 was defined as molecular diagnosis, such as diagnosis based on 1p/19q status or the mutation status of the *IDH1* and *IDH2* genes. In principle, astrocytic and oligodendroglial tumors should be diagnosed based on the 2016 WHO CNS and/or cIMPACT-NOW criteria; however, the findings obtained through our diagnostic flowchart can be added to the histological diagnosis in parentheses. This classification system would be helpful for pathologists with limited resources.

Keywords Glioma · *IDH* mutation · 1p/19q-codeletion · WHO classification

Introduction

The publication of the 2016 World Health Organization Classification of Tumors of the Central Nervous System (2016 WHO CNS) represented a major change in the classification of brain tumors [1]. The 2016 WHO CNS defines diagnostic entities by combining molecular and histological information. While the classification promotes more precise diagnosis of glial tumors, many pathologists in Japan have found it hard to adapt to the new requirements. In addition, analyses of the isocitrate dehydrogenase (*IDH*) genes or 1p/19q status are not covered by the Japanese health insurance system as of 2018. Therefore, many pathologists cannot diagnose astrocytic or oligodendroglial tumors according to the 2016 WHO CNS.

Recently, the International Society of Neuropathology sponsored an initiative to evaluate and recommend proposed changes to a future CNS tumor classification; i.e., the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW). The cIMPACT-NOW subsequently published three reports, which updated

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the 2016 WHO CNS [2–4]. It is very important to resolve the problems and contradictions in the 2016 WHO CNS. However, at many hospitals in Japan numerous brain tumors are still being diagnosed as "not otherwise specified" (NOS), even after the cIMPACT updates.

In response to this problem, the Japan Society of Brain Tumor Pathology has established a committee for molecular diagnosis to facilitate the integrated diagnosis of astrocytic and oligodendroglial tumors in Japan.

First, we created three diagnostic levels: Level 1 was defined as simple histopathological diagnosis using hematoxylin and eosin staining and routine immunostaining. Level 2 was defined as immunohistochemical diagnosis using mutation-specific antibodies, such as antibodies against the IDH1 R132H protein or the products of the *ATRX* and *TP53* genes. Level 3 was defined as molecular diagnosis, such as diagnosis based on 1p/19q status or the mutation status of the *IDH1* and *2* genes. In principle, astrocytic and oligodendroglial tumors should be diagnosed based on the 2016 WHO CNS and/or cIMPACT-NOW criteria; however, the findings obtained through our diagnostic flowchart can be added to the histological diagnosis in parentheses (Fig. 1).

Level 1 diagnosis

All level 1 diagnoses are classified as NOS diagnoses under the 2016 WHO CNS. Therefore, as shown in Figs. 1a and 2a, when a diffuse glial tumor is diagnosed as a diffuse astrocytoma based on its histology alone, a diagnosis of diffuse astrocytoma, NOS (level 1) is made. The committee does not recommend level 1 diagnoses for astrocytic or oligodendroglial tumors under any circumstances.

Level 2 diagnosis

The analyses conducted at level 2 include immunohistochemical examinations using R132H mutation-specific IDH1, ATRX, and/or p53 antibodies. The committee recommends that the routine immunohistochemical and level 2 analyses should be undertaken at the same time (Fig. 1). Although the sensitivity of the R132H mutation-specific IDH1 antibody has been reported to be very high [5], the frequency of R132H mutations among all *IDH* mutations in Japanese patients ranges about 90–95% [6, 7]. Therefore, while positive staining with the R132H mutation-specific IDH1 antibody indicates the presence of a mutant form of IDH, negative staining does not definitively prove that the IDH-wildtype is present (Fig. 2c). We recommend that sequencing analysis of the *IDH1/2* genes (level 3B) should be performed in cases in which staining with the IDH1 R132H mutation-specific antibody produces negative results (Fig. 1). As an exception, the 2016 WHO CNS states that negative R132H mutation-specific IDH1 antibody staining should be considered to indicate the presence of IDH wildtype in glioblastoma patients aged ≥ 55 years (Fig. 1d) [1]. *TP53* gene mutations are a typical finding of diffuse astrocytomas with *IDH* mutations [8–11]. In addition, *TP53* gene mutations and 1p/19q codeletion are mutually exclusive in IDH-mutant gliomas [9–11]. Takami et al. reported that p53 immunohistochemistry exhibited 78.8% sensitivity and 96.7% selectivity for diagnosing diffuse astrocytoma [12]. Therefore, positive findings during p53 immunostaining can provide useful information for diagnosing diffuse astrocytomas with *IDH* mutations, but negative p53 antibody staining does not guarantee that the TP53-wildtype is present. *ATRX* gene mutations in gliomas are closely associated with *IDH* and *TP53* gene mutations. Some studies have reported that *ATRX* gene mutations are present in about 80–90% of IDH-mutant, 1p/19q-intact tumors [9–11]. The loss of nuclear ATRX protein staining during immunohistochemistry has been used as a surrogate marker of *ATRX* gene mutations [13]. However, there are no standard criteria for what constitutes loss of ATRX staining in gliomas. As reported previously, the loss of nuclear ATRX protein expression should only be interpreted as a specific finding in the presence of clear positive internal controls. The use of ATRX mutant tumors that exhibit ATRX negativity as a control is also recommended [14]. According to the diagnostic requirements of the 2016 WHO CNS, it is necessary to analyze the 1p/19q status of all diffuse astrocytomas and oligodendrogliomas. In cIMPACT-NOW update 2, it was stated that in cases involving mutant IDH-positive diffuse astrocytic cells that display strong, diffuse p53 immunopositivity but lack nuclear ATRX expression a diagnosis of diffuse astrocytoma, IDH-mutant can be rendered in the absence of 1p/19q testing (Fig. 1a) [3]. When the *IDH1* R132H mutation is detected in a diffuse astrocytoma and the absence of ATRX and/or the accumulation of TP53 proteins is noted, the diagnosis based on the 2016 WHO CNS would be diffuse astrocytoma, NOS. However, according to the cIMPACT-NOW criteria, the diagnosis should be diffuse astrocytoma, IDH-mutant. According to our proposal, the diagnosis should be diffuse astrocytoma, IDH-mutant (IDH1-positive, ATRX-negative, and/or TP53-positive, level 2) (Figs. 1a, 2a). Except for this situation, all astrocytic and oligodendroglial tumors that are analyzed using level 2 methods should be diagnosed as NOS. Therefore, level 3 analysis is required for a definitive diagnosis (Fig. 1).

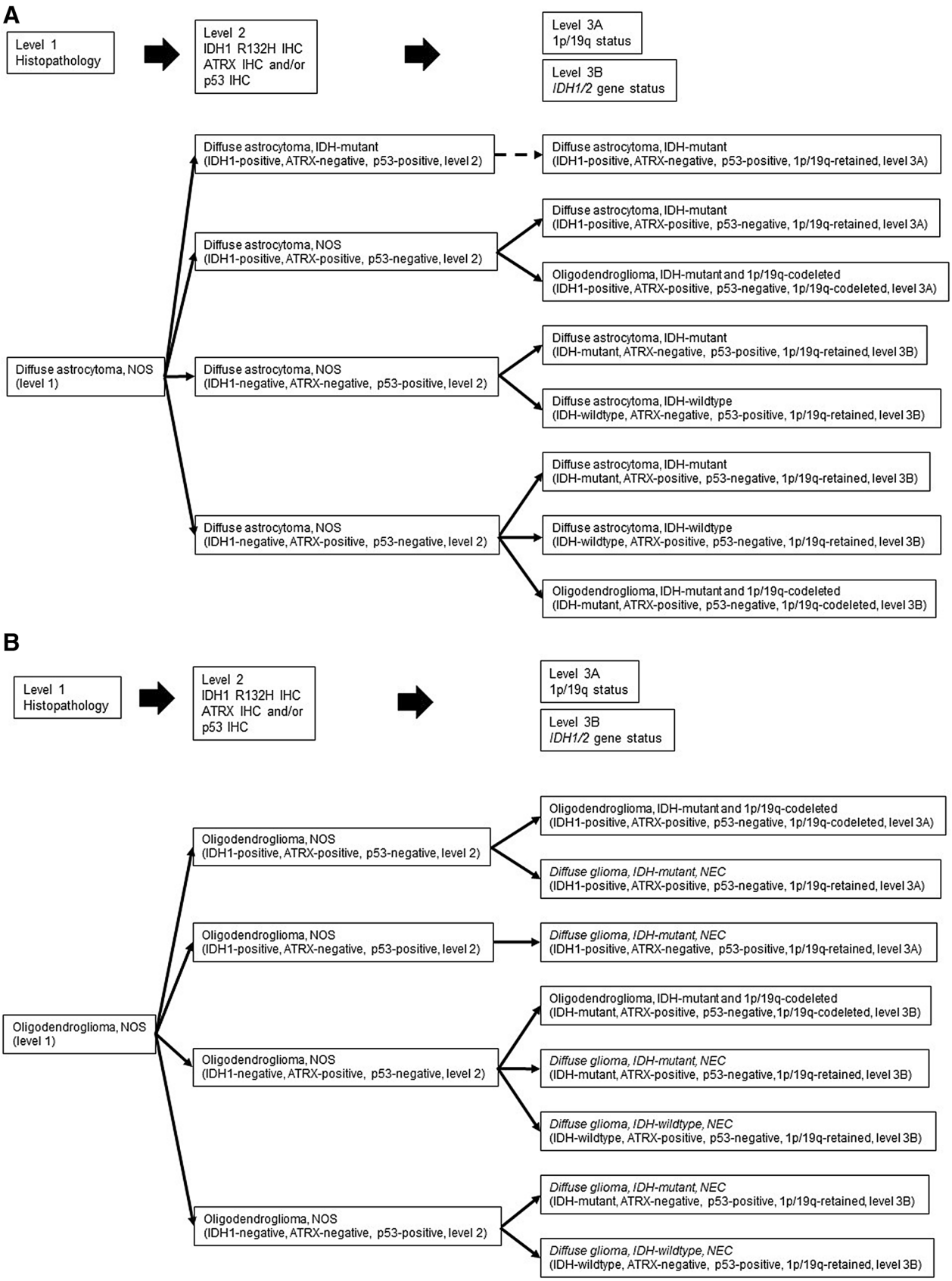

Fig. 1 Flowcharts of the classification of diffuse astrocytomas (**a**), oligodendrogliomas (**b**), oligoastrocytomas (**c**), and glioblastomas (**d**) based on their histological and molecular features

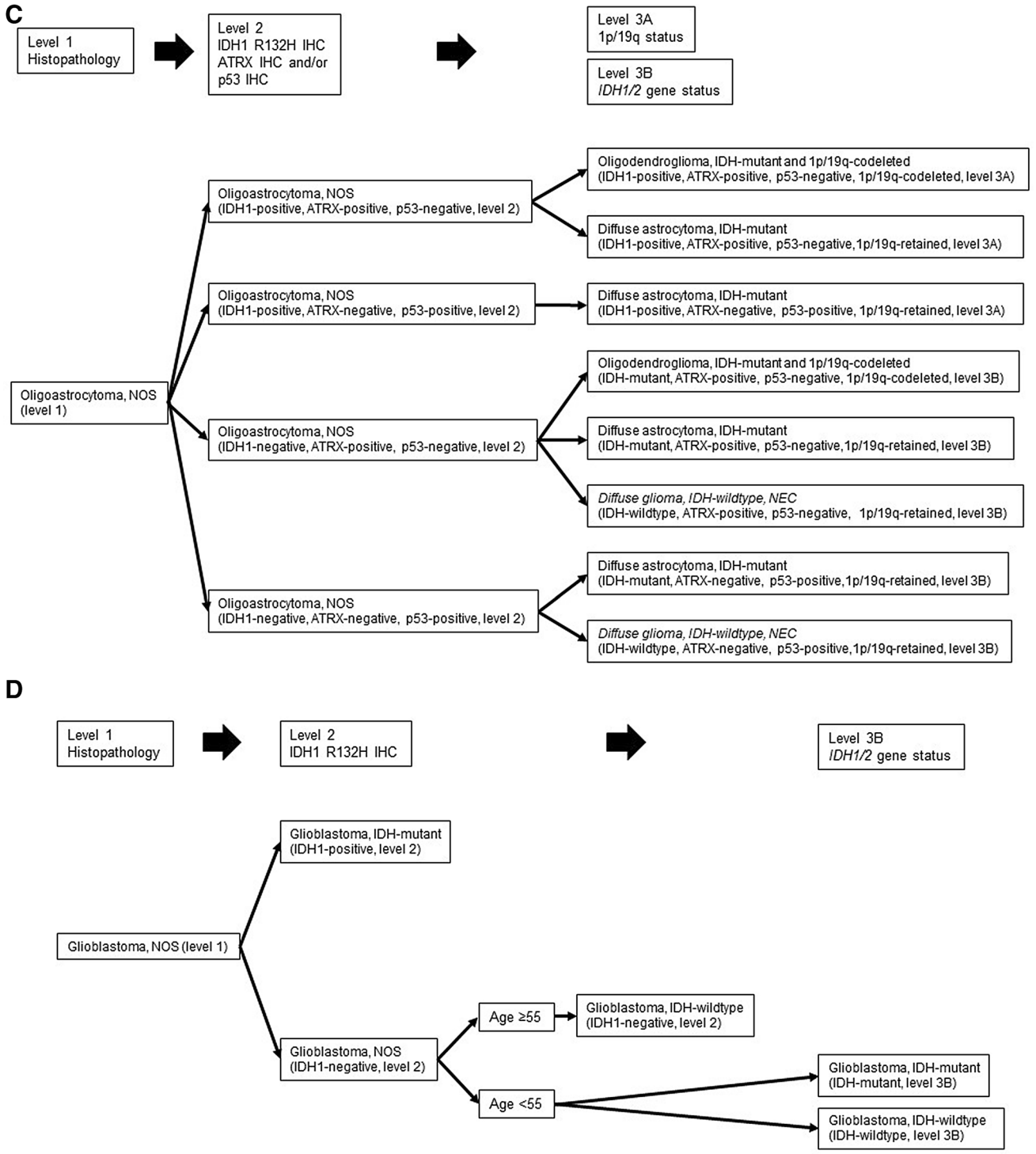

Fig. 1 (continued)

Level 3 diagnosis

Level 3 analysis is based on 1p/19q status (level 3A) and the mutation status of the *IDH1/2* genes (level 3B). 1p/19q-codeletion status can be analyzed using various molecular or genetic methods including fluorescence in situ hybridization (FISH), comparative genomic hybridization, chromogenic in situ hybridization, polymerase chain reaction (PCR)-based microsatellite analysis, real-time comparative quantitative PCR, and multiplex ligation-dependent probe

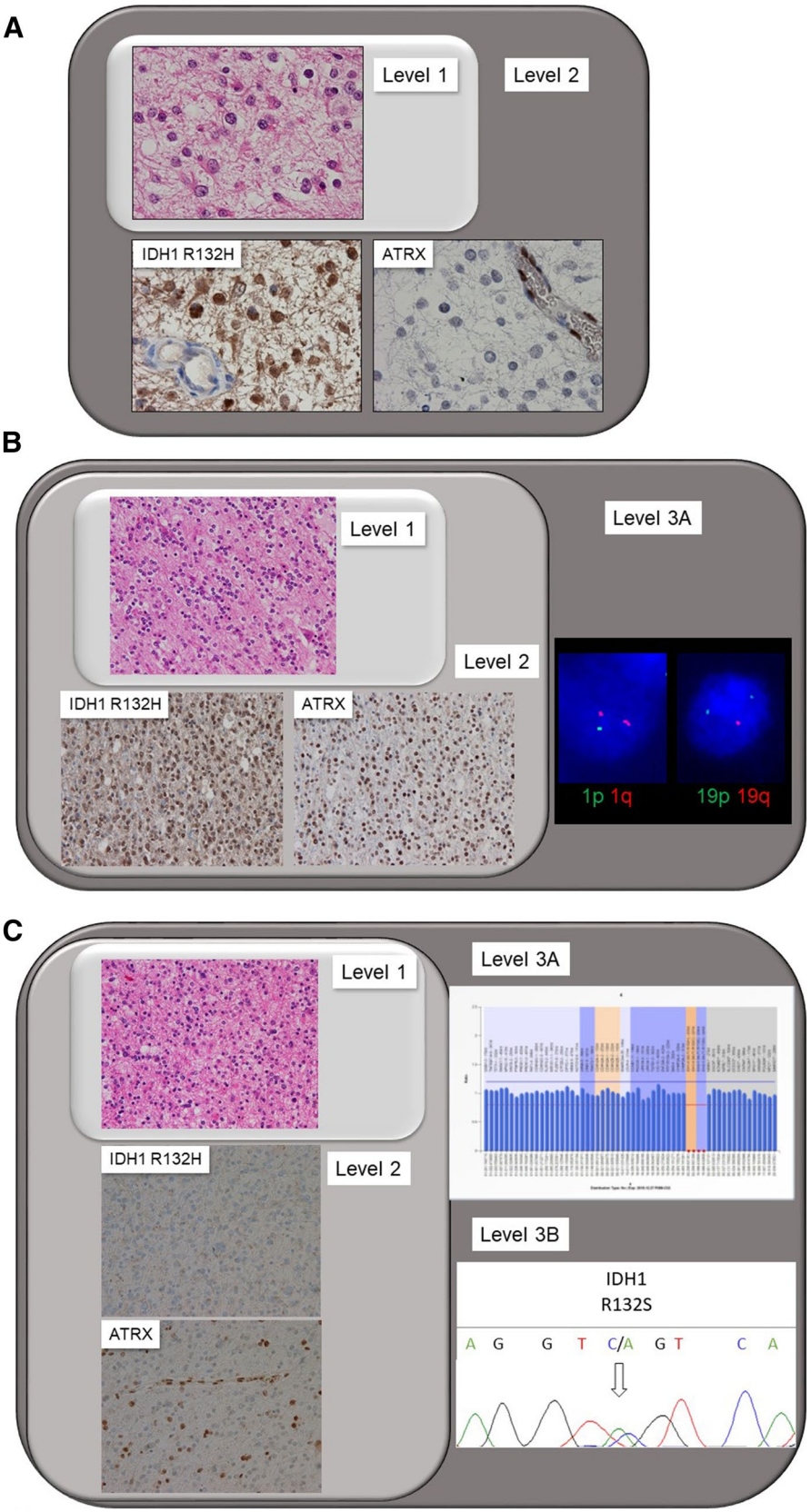

Fig. 2 **a** The tumor was composed of uniform neoplastic fibrillary astrocytic cells (level 1). Immunostaining revealed the marked accumulation of the IDH1 R132H protein (level 2) and the absence of the ATRX protein (level 2) in the tumor cells. The diagnosis was diffuse astrocytoma, IDH-mutant (IDH1 positive, ATRX negative, level 2). **b** A histological examination showed the honeycomb or fried-egg pattern that is typically seen in oligodendrogliomas (level 1). Immunostaining revealed the marked accumulation of the IDH1 R132H protein (level 2) and the presence of the ATRX protein (level 2) in the tumor cells. Fluorescent in situ hybridization analysis revealed the codeletion of 1p and 19q (level 3A). The definitive diagnosis was oligodendroglioma, IDH mutant and 1p/19q codeleted (IDH1 positive, ATRX positive, 1p/19q codeleted, level 3A). **c** A histological examination showed oligodendroglial-like and astrocytic cells (level 1). Immunostaining revealed the absence of both the IDH1 R132H protein and the ATRX protein (level 2) from the tumor cells. MLPA analysis revealed the retention of 1p and 19q (level 3A). Somatic mutations, which resulted in base pair changes from CGT (Arg) to AGT (Ser) in codon 132 of the IDH1 gene, were detected. The definitive diagnosis was diffuse astrocytoma, IDH mutant (IDH1 mutant, ATRX negative, 1p/19q retained, level 3B)

amplification (MLPA). Although FISH analysis using target-specific probes that hybridize to the subtelomeric regions of 1p36 and 19q13 might be the easiest method, it is important to be aware that partial deletion of the 1p36 region, which is completely different from the 1p/19q codeletion seen in oligodendrogliomas, frequently occurs in anaplastic astrocytomas and glioblastomas (Fig. 2b) [15]. Horbinski et al. reported that over 70% of oligodendrogliomas in which 1p/19q codeletion was detected by FISH and loss of heterozygosity was seen at 10q were subsequently found to be negative for 1p/19q codeletion in PCR-based analyses [16]. It is expected that a new FISH probe that hybridizes to the centromeric region of 1p will be developed. Other methods might be useful for avoiding false-positives for 1p/19q codeletion; however, PCR-based microsatellite analysis requires autologous non-tumor DNA as a control, and there is no consensus about the MLPA criteria for 1p/19q deletion status. The results of level 2 analyses can be helpful for interpreting information regarding 1p19q status (Fig. 2c). The cIMPACT-NOW proposed the term "NEC" (not elsewhere classified), which differs from "NOS". NEC reflects situations in which the necessary assays were performed and results were obtained, but the results do not indicate a specific WHO diagnosis [3]. In our opinion, although NEC diagnoses are not officially approved by the WHO, NEC diagnoses (level 3 diagnoses) should be considered more appropriate than NOS diagnoses (level 1 and 2 diagnoses) (Fig. 1). We would like to emphasize that the possibility of technical errors should be ruled out before NEC diagnoses are made. DNA sequencing of the *IDH1/2* genes is necessary to determine the *IDH* status of all astrocytoma and oligodendroglioma patients as well as young glioblastoma patients (aged < 55) in whom R132H mutation-specific IDH1 antibody staining produces negative results. R132L, R132S, R132G, and R132C mutations have been detected in the *IDH1* gene and R172G, R172K, R172S, R172M, and R172W mutations have been detected in the *IDH2* gene (Fig. 2c) [6–8].

The 2016 WHO CNS had a big impact on the classification of brain tumors, as it enabled much more precise diagnoses to be obtained compared with classical pathological diagnostic methods. However, lots of pathologists in Japan have found it difficult to adjust to the changes in the 2016 WHO CNS. Therefore, we propose that the results of immunohistochemical and/or molecular analyses and the relevant diagnostic level should be described in parentheses after the histological diagnosis according to the 2016 WHO CNS and/or cIMPACT-NOW criteria. By doing so, clinicians would able to get more precise information from pathologists.

This proposal would help to avoid confusion during the transition period. As of 2018, decisions regarding the treatment of gliomas have not depended on the results of molecular analyses, such as of *IDH* and/or 1p/19q status. However, it will be necessary to create a molecular testing system that is compatible with the 2016 WHO CNS in preparation for molecular-based therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest associated with this manuscript.

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