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Elevated ratio of C-type lectin-like receptor 2 level and platelet count (C2PAC) aids in the diagnosis of post-operative venous thromboembolism in IDH-wildtype gliomas

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ABSTRACT

Introduction: Podoplanin (PDPN) is known to induce platelet aggregation via interacting with the C-type lectin-like receptor-2 on platelets and is involved in postoperative venous thromboembolism (VTE) formation. In this study, we investigate the correlation between soluble C-type lectin-like receptor (sCLEC-2) levels and PDPN expression in patients with high grade gliomas and the relationship between sCLEC-2 levels and the occurrence of VTE.

Materials and methods: Forty-four patients harboring high grade gliomas, treated surgically at the Department of Neurosurgery, Niigata University from April 2018 to August 2020, were included. Patients with high grade gliomas were divided into isocitrate dehydrogenase (IDH)- wildtype and mutant groups, and the presence or absence of VTE and the intensity of PDPN by immunohistochemistry were confirmed. Platelet counts, as well as plasma sCLEC-2 and PDPN were measured in these patients. Furthermore, the levels of sCLEC-2 concentration were divided by the platelet count (C2PAC index) for comparison.

Results: IDH-wildtype glioma patients highly expressed PDPN ($P < 0.001$) compared to IDH-mutant glioma patients. In total, 9 (20.5 %) patients were diagnosed with VTE during the follow-up period, of which 8 patients harbored IDH-wildtype gliomas, and one patient an IDH-mutant glioma. Mean sCLEC-2 levels and C2PAC index in patients with IDH-wildtype gliomas were significantly higher than that of low or no PDPN expression group, which included patients with IDH-mutant gliomas ($P = 0.0004$, $P = 0.0002$). In patients with IDH-wildtype gliomas, the C2PAC index in patients with VTE was significantly higher than in patients without VTE ($P = 0.0492$). The optimal cutoff point of C2PAC for predicting VTE in IDH-wildtype glioma patients was 3.7 with a sensitivity of 87.5 % and specificity of 51.9 %.

Conclusion: Platelet activation is strongly involved in the development of VTE in patients with IDH-wildtype high grade gliomas, and C2PAC index is a potential marker to detect VTE formation after surgery.

1. Introduction

Patients with cancer have an increased risk of venous thromboembolism (VTE), a life- threatening complication with a significant increase of morbidity and mortality [1]. The incidence of VTE has been reported to be 10 % to 20 % of patients with cancer [2]. The risk of VTE in patients with cancer differs widely and is mainly driven by the primary site of the tumor [1]. Especially, in patients with high grade gliomas, VTE is a frequent complication [3], and is known to be among those with the highest risk of malignancies [1,2]. Several risk factors of VTE in patients with high grade glioma have been identified, such as patient age, leg paresis, glioblastoma tumor subtype, or intraluminal tumor thrombosis, underlying mechanisms explaining the high thrombotic risk remain to be elucidated [4]. We and others [3,5,6] have suggested that podoplanin

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(PDPN) expression in brain tumors increases risk for VTE and that 2-hydroxyglutarate produced by isocitrate dehydrogenase 1 (IDH1)-mutant tumors decreases the risk for VTE. We previously reported that postoperative VTE occurred commonly in patients with IDH-wildtype glioma, which highly expressed PDPN [5]. PDPN is a sialomucin- like glycoprotein and has potential to induce platelet aggregation via interacting with the C-type lectin-like receptor (CLEC)-2 on platelets [2,3,7,8].

CLEC-2 has been identified as a platelet receptor for a platelet activating snake venom, rhodocytin. [9] CLEC-2 protein is highly and almost specifically expressed in platelets and megakaryocytes in humans [9]. CLEC-2 is thought to be a critical player in thrombus formation, as evidenced by the fact that the plasma CLEC-2 levels are high in patients with suspected platelet activation [10,11]. Furthermore, the PDPN-CLEC-2 axis might play a crucial role in venous thrombosis [1,12]. Soluble C-type lectin-like receptor 2 (sCLEC-2) is released upon platelet activation and it has been introduced as a new biomarker of platelet activation [13]. Elevated plasma levels of sCLEC-2 were reported in patients with thrombotic microangiopathy, disseminated intravascular coagulation and patients with acute coronary syndrome and acute ischemic stroke [11,14,15]. However, the clinical correlation between plasma levels of sCLEC-2 and PDPN expression, which is the CLEC-2 ligand, has not been reported. Therefore, the aim of this study was to investigate the correlation between sCLEC-2 levels and PDPN expression in patients with high grade gliomas. We further investigated the relationship between sCLEC-2 levels and the occurrence of VTE and discuss the unique pathophysiology of VTE in IDH-wildtype gliomas.

2. Material and methods

2.1. Patients

In this study, 47 patients harboring high grade gliomas, treated surgically at the Department of Neurosurgery, Niigata University from April 2018 to August 2020, were included after approval by the institutional review board of Niigata University (#2020-0025). Patients with a previous history of VTE were excluded to create a uniform patient population to understand the incidence of VTE among patients undergoing surgery for gliomas. The study variables assessed included the following: age, sex, history of diabetes mellitus, steroid use, preoperative body mass index, pre- and postoperative leg paresis, preoperative Karnofsky Performance Scale (KPS), length of surgery, World Health Organization (WHO) grade and IDH status.

Patients routinely had thigh-high anti-embolism stockings placed on both lower extremities upon entering the operating room. In addition, sequential compression devices were also placed. These anti- VTE modalities were used throughout their hospital stay, where the stockings were always on, and the sequential compression devices were used until patients become ambulatory. Pharmacologic prophylaxis of VTE was not employed following surgery in this study, as prophylactic use of low molecular weight heparin is only approved for use in Japan in patients undergoing leg or abdominal surgery [16].

In addition to high grade gliomas patients, 6 patients who underwent microvascular decompression (MVD) and 15 healthy volunteers were included. MVD is a neurosurgical operation to treat trigeminal neuralgia or hemifacial spasm. The surgery is designed to make space between the offending vessel and cranial nerves that cause trigeminal neuralgia (trigeminal nerve) or hemifacial spasm (facial nerve). Unlike resection of gliomas, this surgery does not involve resecting the brain parenchyma. These samples were added to the normal control subjects to exclude the possibility that sCLEC-2 is elevated because of surgical intervention and/or general anesthesia.

2.2. VTE diagnosis and blood samples

In patients with high grade glioma, D-dimer levels were checked at 3 and 7 days after surgery as previously described (Nanopia D-dimer: Sekisui Medical Co. Ltd., Tokyo, Japan) [17]. If D-dimer levels were high, but <5.0 μg/ml at 7 days after surgery, we continued to serially check D-dimer levels as deemed necessary. According to our previously published study [17] and that by Kawaguchi et al. [18], for patients with elevated D-dimer levels of ≥5.0 μg/ml, VTE was ruled out by spiral or helical computed tomography (CT) with administration of intravenous contrast or Doppler ultrasound. Almost all VTEs were detected by CT. Doppler ultrasound was performed only when VTE was not apparent on CT or the patient was allergic to contrast medium. Considering results from our previous study [17], we defined the group with serum D-dimer levels of <5 μg/ml as the non-VTE group in this study. For symptomatic cases of VTE, screening was performed even if serum D-dimer levels were <5 μg/ml. Most patients with VTE were started on heparin and switched to maintenance anticoagulation using an oral direct inhibitor of activated factor X.

Peripheral blood samples to detect plasma sCLEC-2 and PDPN were collected from all patients. In the VTE group, blood samples were taken at the time of VTE detection (7 to 30 days after surgery, mean 11.8 ± 10.6). For patients with elevated D-dimer levels of <5 μg/ml (non-VTE group), blood samples were usually taken within a week (mean 4.9 ± 2.9) of surgery. In the MVD group, blood samples were taken one week after surgery (mean 7.0 ± 0.0) and used for sCLEC-2 measurement. In all patients undergoing surgery, platelet counts were routinely measured before and after surgery.

2.3. Immunohistological analysis

Surgical specimens were fixed with 20 % buffered formalin and embedded in paraffin. Histopathologic diagnosis of the tumor was performed in accordance with the 2016 WHO classification system [19] by an experienced neuropathologist (A.K.). The paraffin-embedded sections were processed for immunohistochemistry with mouse monoclonal antibodies against human IDH1 R132H (clone H09; Dianova, Hamburg, Germany; dilution 1:100) in all cases using methods previously described. We also performed PDPN (clone D2–40; Cell Marque, Rocklin, California, USA; 1:100) immunostaining in all cases. PDPN staining intensity was semiquantitatively classified into the following degrees: (−) negative; (+) moderate expression (<50 % of cancer cell express PDPN) and (++) high expression (>50 % of cancer cells express PDPN at a strong intensity level).

2.4. Measurement of plasma sCLEC-2 and podoplanin

To detect sCLEC-2 in plasma, Kazama et al. established a sandwich enzyme-linked immunosorbent assay (ELISA) using F(ab')2 anti-CLEC-2 monoclonal antibodies [13]. Blood samples were collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant agent. Plasma was obtained by centrifugation for 10 min at 1500*g* and stored at −80 °C. Levels of sCLEC-2 were assessed using ELISA kits according to the manufacturer's instructions (LSI Medience, Tokyo, JAPAN). To ensure the reliability of the ELISA kit, sCLEC-2 levels were also evaluated with the automated immunoanlayzer STACIA (LSI Medience). When the values detected by ELISA was >2.5 time higher than those detected by STACIA, they were excluded from the analysis.

CLEC-2 is expressed on the surface of platelets. Platelets cannot be completely removed when plasma is separated using citrate. Therefore, in patients with high platelet counts, the effect of sCLEC-2 derived directly from platelets cannot be ignored. Therefore, dividing sCLEC-2 levels by platelet count (C2PAC index) serves as a reliable marker [20]. In this study, sCLEC-2 ELISA values, not STACIA values, were used for calculating the C2PAC index.

Plasma PDPN was measured using the human podoplanin ELISA kit (RayBiotech, Georgia, USA) in IDH-mutant and IDH-wildtype glioma cases. Plasma obtained at the same time as that for sCLEC-2 analysis was used. Two patients, 1 IDH-wildtype and 1 IDH-mutant, were excluded

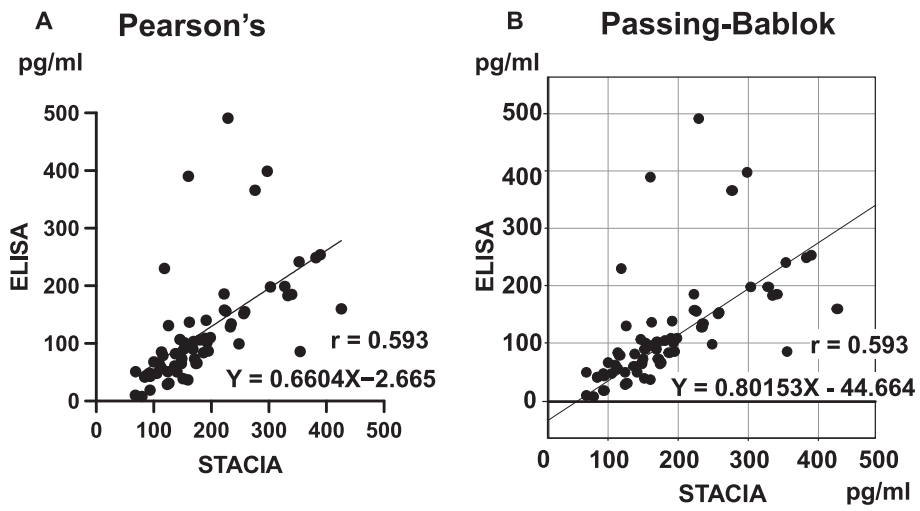

Fig. 1. The correlation between Soluble C-type lectin-like receptor 2 (sCLEC-2) measured by enzyme-linked immunosorbent assay (ELISA) and STACIA. (A) The Pearson's regression of sCLEC-2 measured by ELISA and STACIA had a moderate, positive correlation ($r = 0.593$, slope 0.6604). (B) The slope for Passing-Bablok regression, which minimizes the effects of outliers, was 0.80153.

Table 1
Demographics of the study patients.

	Total (44)	IDH-wildtype (35)	IDH-mutant (9)	*p* value
Age, yrs				<0.001
Mean (±SD)	61.7 ± 12.8	65.3 ± 10.3	47.3 ± 11.9	
Sex, n(%)				0.40
Male	25 (56.8)	21 (60.0)	4 (44.4)	
BMI				0.36
Mean (± SD)	23.1 ± 3.5	23.3 ± 3.7	22.1 ± 2.7	
DM, n(%)	3 (6.8)	3 (8.6)	0	0.36
Steroid, n(%)	19 (43.2)	17 (48.6)	2 (22.2)	0.16
KPS				0.39
Mean (± SD)	70.2 ± 20.7	68.9 ± 19.7	75.6 ± 25.1	
Paresis, n(%)	19 (43.2)	16 (45.7)	3 (33.3)	0.50
Grade, n(%)				<0.01
III	14 (31.8)	6 (17.1)	8 (88.9)	
IV	30 (68.2)	29 (82.9)	1 (11.1)	
PDPN, n(%)				<0.001
++	26 (59.1)	26 (74.3)	0	
+	9 (20.5)	8 (22.9)	1 (11.1)	
−	9 (20.5)	1 (2.9)	8 (88.9)	
p-PDPN, ng/ml Mean (± SD)	26.2 ± 105.0	30.4 ± 116.5	8.7 ± 12.2	0.47
VTE, n(%)	9 (20.5)	8 (22.9)	1 (11.1)	0.44

from PDPN analysis because of insufficient blood.

2.5. Statistical analysis

For comparison of two groups, paired *t*-test, Student t-test or Mann-Whitney *U* test was used, and for comparison of three or more groups, the ANOVA test with post-hoc Tukey-Kramer test. Theχ^2 test or Fisher exact probability method were used to evaluate the association of 2 categorical variables. The Prism 9 (GraphPad Software, San Diego, CA) software was used for statistical analyses. For Passing-Bablock analysis to compare different methods, StatFlex Ver 7 (Artec Co. Ltd., Osaka, Japan) software was used.

3. Results

3.1. Main characteristics of the study patients

A total of 47 patients with high grade glioma were studied. Of those, 3 were excluded because the results of sCLEC-2 measured by ELISA was >2.5 times higher than that measured by STACIA. The results of sCLEC-2 measured by ELISA had a moderate, positive correlation with the results measured by STACIA ($r = 0.593$, slope 0.6604, Fig. 1A) The slope for Passing-Bablock regression, which is used to compared different methods and minimizes the effects of distribution of errors and outliers [21], was 0.80153, Fig. 1B). Forty-four patients were included in the study with a mean age of 61.7 ± 12.8 years (range: 40–71 years) and 56.8 % were men. In total, 35 (79.5 %) patients were IDH-wildtype, and 9 (20.5 %) patients were IDH-mutant (Table 1). In terms of baseline characteristics, IDH-wildtype glioma patients were significant older (65.3 ± 10.3 vs 47.3 ± 11.9 years, $P < 0.001$), had higher WHO grade ($P < 0.01$) and highly expressed PDPN ($P < 0.001$).

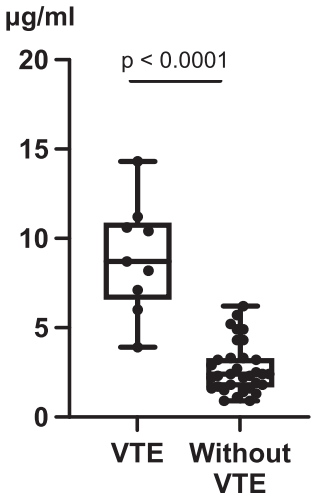

Fig. 2. The D-dimer levels in patients with venous thromboembolism (VTE) and without VTE. The D-dimer levels in patients with VTE was significantly higher than that in patients without VTE.

3.2. Incidence of VTE in this study

Of the patients included in this study, 9 (20.5 %) patients were diagnosed with VTE during the follow-up period. Eight patients harbored IDH-wildtype gliomas, and one patient an IDH-mutant glioma. The D-dimer levels in patients with VTE (8.9 ± 3.1 μg/ml) was

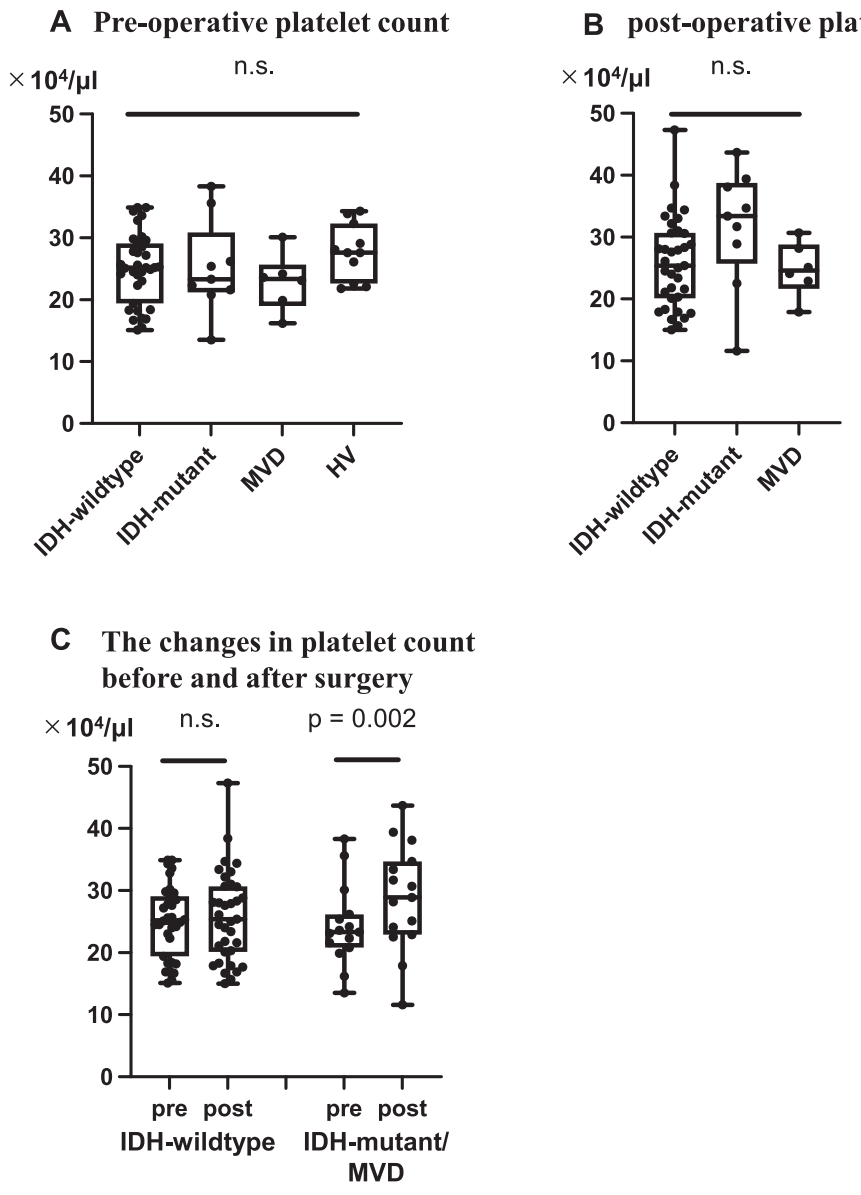

Fig. 3. Platelet counts pre- and post-surgery. There was no significant difference in pre-operative platelet count among isocitrate dehydrogenase (IDH)-wildtype gliomas, IDH-mutant gliomas, microvascular decompression (MVD) groups and healthy volunteer groups. (A). Post-operative platelet count was not significantly different in each 3 groups as they were before surgery (B). Comparing the changes in platelet count before and after surgery, IDH-wildtype gliomas showed a non-significant difference, however patients in the IDH-mutant glioma and MVD groups showed a significant increase in the number of platelets (C).

significantly higher than that in patients without VTE (2.8 ± 1.6 μg/ml) ($P < 0.0001$, Mann-Whitney test) (Fig. 2). VTE was diagnosed at a mean of 9 days (range: 4–30 days) after surgery. CT from chest to lower extremities and ultrasonography identified isolated deep venous thrombosis (DVT) in 5 (55.6 %) patients, and combined DVT and pulmonary embolism (PE) in 4 (44.4 %) patients. There were no patients with isolated PE in this study. All were asymptomatic and no fatal VTE occurred. Six patients were treated with therapeutic anticoagulant therapy; of them, 5 patients received edoxaban and one patient received apixaban. The remaining 3 patients were not treated with anticoagulation and rigorously monitored because anticoagulation was considered to be contraindicated due to postoperative intracranial hemorrhage in 1 patient, and the detected VTE was small in size and distal in 2 patients. None of the 3 patients who were untreated developed symptomatic VTE.

3.3. Pre and post-operative platelet count

There was no significant difference in pre-operative platelet count among the 4 groups (Fig. 3A, $P = 0.366$, One-way ANOVA, Tukey's multiple comparison test). Post-operative platelet count was not significantly different in each group as they were before surgery (Fig. 3B, $P = 0.110$, One-way ANOVA, Tukey's multiple comparison test). Comparing the changes in platelet count before and after surgery, IDH-wildtype gliomas showed a non-significant difference, however patients in the IDH-mutant glioma and MVD groups showed a significant increase in the number of platelets (Fig. 3C, $P = 0.430$ and $P = 0.002$, paired *t*-test).

3.4. The levels of plasma PDPN, sCLEC-2 and C2PAC index

Mean sCLEC-2 levels in patients with IDH-wildtype gliomas (157.7 ± 111.9 pg/ml) was significantly higher than healthy volunteers (52.3 ± 36.7 pg/ml) ($P = 0.0012$, One-way ANOVA, Tukey's multiple comparison test) but not IDH-mutant glioma patients (120.7 ± 43.2 pg/ml) or patients undergoing MVD (76.3 ± 23.4 pg/ml), in part due to small number of cases these groups (Fig. 4A). When grouping together the 3 groups with expected low or no PDPN expression, namely the IDH-mutant glioma, MVD and control groups, we found that the IDH-wildtype glioma group had a significantly higher (Fig. 4B, 157.7 ± 111.9 pg/ml vs 77.6 ± 46.6 pg/ml, $P = 0.0004$, Mann-Whitney test). The C2PAC index was highest in patients with IDH-wildtype gliomas, followed by the IDH-mutant glioma, MVD and control groups, although a significant difference was observed only between IDH-wildtype glioma

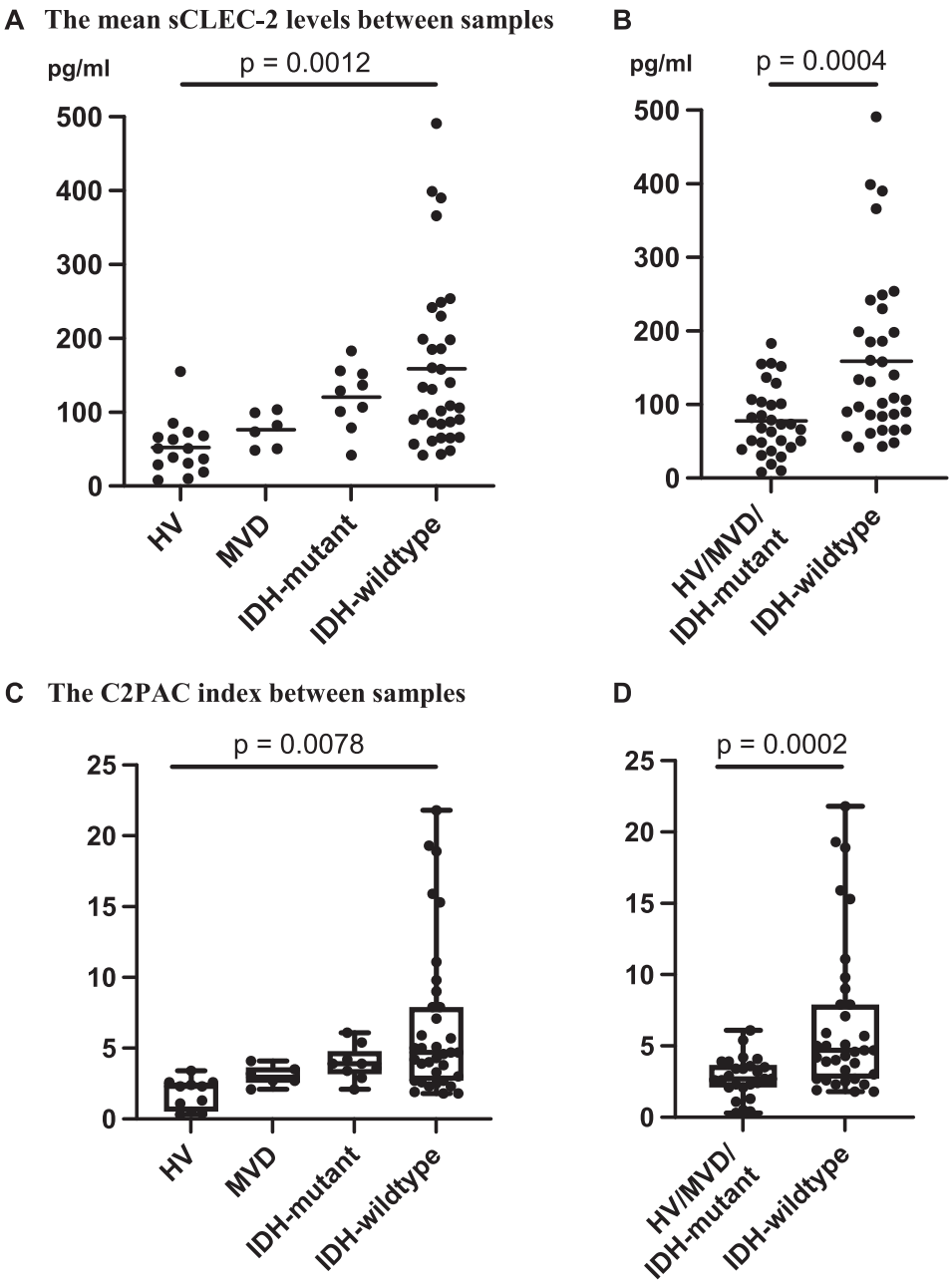

Fig. 4. Soluble C-type lectin-like receptor 2 (sCLEC-2) and C2PAC levels of healthy volunteers, patients undergoing microvascular decompression (MVD), patients with isocitrate dehydrogenase (IDH)-mutant gliomas and patients with IDH-wildtype gliomas. Mean sCLEC-2 levels in patients with IDH-wildtype gliomas was significantly higher than healthy volunteers, but not IDH-mutant glioma patients or patients undergoing MVD (A). There was significant difference in mean sCLEC-2 levels between the 3 groups and the IDH-wildtype glioma group (B). The C2PAC index was highest in patients with IDH-wildtype gliomas, followed by the IDH-mutant glioma, MVD and control groups, although a significant difference was observed only between IDH-wildtype glioma group and normal volunteers (C). There was significant difference in C2PAC index between the 3 groups and the IDH-wildtype glioma group (D).

group and normal volunteers (Fig. 4C, $P = 0.0078$, One-way ANOVA, Tukey's multiple comparison test). When comparing the IDH-wildtype glioma group with the other 3 groups, we found that the IDH-wildtype glioma group had a significantly higher C2PAC index (Fig. 4D, $P = 0.0002$, Mann-Whitney test). Plasma PDPN was also higher in the IDH-wildtype glioma group compared to IDH-mutant, although not statistically significant (Table 1, 30.4 ± 116.5 vs 8.7 ± 12.2 ng/ml, $P = 0.47$).

3.5. Incidence of VTE in IDH-wildtype gliomas

Having found that patients with PDPN-expressing, IDH-wildtype gliomas have higher C2PAC index, we next sought to find whether the C2PAC index could be used as a biomarker to detect VTE in these patients. The patients with IDH wildtype gliomas were classified into 2 groups: 8 patients with VTE, and the other 27 patients without VTE. There were not significant differences between the two groups in terms of age, sex, BMI, history of DM, steroid use, KPS, presence of leg paresis, length of surgery, WHO grade, and PDPN status (Table 2). As expected, D-dimer levels were significantly higher in the VTE group (9.0 ± 3.3 vs 2.7 ± 1.3 μg/ml, $P < 0.01$). Although not statistically significant, patients in the VTE group tended to have higher levels of plasma PDPN (Table 2, 90.7 ± 237.1 vs 11.8 ± 25.4 ng/ml, $P = 0.56$) and sCLEC-2 than patients without VTE (Fig. 5A, 173.9 ± 104.4 vs 116.6 ± 80.1 pg/ml, $P = 0.2399$, Mann-Whitney test). The C2PAC index in patients with VTE was significantly higher than in patients without VTE (median 5.15 vs. 3.60, $P = 0.0492$, Mann-Whitney test). The optimal cutoff point of C2PAC for predicting VTE was 3.7 with a sensitivity of 87.5 % and specificity of 51.9 % (Fig. 5B).

4. Discussion

In this study, we found that the sCLEC-2 level and C2PAC index tended to be higher in patients with IDH-wildtype high grade gliomas, which were suggested that the platelet activation was enhanced. In

Table 2
Demographics of the patients with IDH-wildtype gliomas.

	Total (35)	with VTE (8)	w/o VTE (27)	p value
Age, yrs				0.51
Mean (±SD)	65.3 ± 10.3	67.5 ± 8.7	64.7 ± 10.8	
Sex, n(%)				0.51
Male	21 (60.0)	4 (50.0)	17 (63.0)	
BMI				0.81
Mean (±SD)	23.3 ± 3.7	23.0 ± 5.1	23.4 ± 3.2	
DM, n(%)	3 (8.6)	1 (12.5)	2 (7.4)	0.65
Steroid, n(%)	17 (48.6)	6 (75.0)	11 (40.7)	0.09
KPS				0.83
Mean (±SD)	68.9 ± 19.7	67.5 ± 20.5	69.3 ± 19.8	
Paresis, n(%)	16 (45.7)	5 (62.5)	11 (40.7)	0.32
Grade, n(%)				0.69
III	6 (17.1)	1 (12.5)	5 (18.5)	
IV	29 (82.9)	7 (87.5)	22 (81.5)	
PDPN, n(%)				0.23
++	26 (74.3)	5 (62.5)	21 (77.8)	
+	8 (22.9)	3 (37.5)	5 (18.5)	
−	1 (2.9)	0	1 (3.7)	
Platelet, $\times 10^4/\mu l$ Mean (±SD)	25.8 ± 7.3	24.6 ± 6.8	28.9 ± 9.3	0.64
D-dimer, μg/ml Mean (±SD)	4.3 ± 3.4	9.0 ± 3.3	2.7 ± 1.3	<0.01
p-PDPN, ng/ml Mean (±SD)	30.4 ± 116.5	90.7 ± 237.1	11.8 ± 25.4	0.56

addition, we found that platelet activation is strongly involved in the development of VTE in patients with IDH-wildtype high grade gliomas as well as the coagulation system, which may be useful for elucidation the pathology of VTE and providing a new biomarker for VTE associated with IDH-wildtype high grade gliomas.

To our knowledge, this is the first study performed to evaluate the level of sCLEC-2 in patients with cancer associated VTE. In past report, a major role of CLEC-2 in arterial thrombosis has been reported, and the expression of recombinant CLEC-2 and PDPN was found in atherosclerotic lesions, especially in advanced atherosclerotic lesions, indicating the role of CLEC-2 in atherosclerotic lesion formation [11,14,15]. CLEC-2 has been shown to facilitate thromboinflammation, which may be an important pathogenesis for VTE [14]. Platelets play an important role in inducing thromboinflammation. Some cancer cells stimulate platelets through the expression of a membrane protein, PDPN [2]. PDPN is the only known physiological ligand for the platelet activation receptor CLEC-2 and the binding of PDPN to CLEC-2 induces platelet aggregation [2,7,9]. We previously reported that PDPN positivity and IDH-wildtype status are independent risk factors for VTE and that PDPN expression was strongly suppressed in IDH-mutant gliomas [5]. The inverse relation of IDH mutation status and VTE was reported by Unruh et al. [6] and Nazari et al. [1], who reported that VTEs did not occur in IDH-mutant glioma patients. In this study, the sCLEC-2 levels and C2PAC index in patients with IDH-wildtype gliomas were higher than that in patients with IDH-mutant gliomas, patients undergoing MVD and healthy volunteers. This result suggests that platelets were activated in patients with high expression of PDPN. Indeed, patients with IDH-wildtype glioma, which strongly expressed PDPN, showed high postoperative plasma PDPN levels. Furthermore, among patients with IDH-wildtype glioma, plasma PDPN levels were even higher in the VTE group. Taken together, the activation of PDPN and CLEC-2 axis enhances platelet activation in IDH-wildtype glioma patients, leading to the development of VTE.

Interestingly, changes in the platelet count before and after surgery in patients with IDH-wildtype gliomas were smaller than that of patients with IDH-mutant gliomas and patients undergoing MVD. Riedl et al. reported PDPN expression was associated with decreased blood platelet counts [4]. They suggested that determination of PDPN expression in brain tumor samples might help to identify patients with very high risk of VTE who might benefit from primary thromboprophylaxis. There were no previous reports in the literature on changes in platelets before and after surgery in patients with high grade glioma. Given the results of this study, in patients with IDH-wildtype glioma, binding of PDPN to CLEC-2 increased platelet activity, but during subsequent thrombus formation the platelets were consumed, counteracting the cytokine-induced increase of platelets due to surgical stress.

In IDH-wildtype gliomas patients, the C2PAC index was significantly higher in patients with complications of VTE than non-VTE patients. The optimal cutoff point of C2PAC index for predicting VTE was 3.7 with specificity of 87.5 %, sensitivity of 51.9 %. This finding suggested that high C2PAC index might be one of the predictors for VTE in patients with high grade glioma, especially with IDH-wildtype gliomas, in addition to D-dimer [11]. These results suggest that C2PAC index may be a new biomarker for early detection of VTE associated with IDH-wildtype gliomas, although further studies are warranted. Given the striking difference in the incidence of VTE in IDH-wildtype glioma compared to IDH-mutant glioma patients, to select candidates for rigorous evaluation of C2PAC index, prediction of IDH-status is important. As a non-invasive method for analyzing IDH-status before surgery, we have previous reported the usefulness of detecting 2-HG by magnetic resonance spectroscopy (MRS). IDH-mutant gliomas produce the

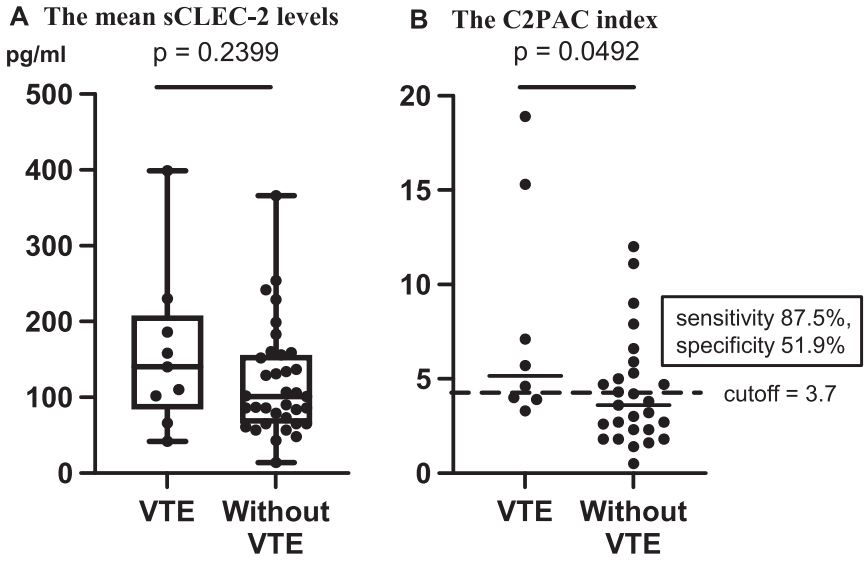

Fig. 5. Soluble C-type lectin-like receptor 2 (sCLEC-2) and C2PAC index in patients with venous thromboembolism (VTE) and without VTE in patients with isocitrate dehydrogenase (IDH)-wildtype gliomas. Patients in the VTE group tended to have higher levels of sCLEC-2 than patients without VTE (A). The C2PAC index in patients with VTE was significantly higher than in patients without VTE and the optimal cutoff point of C2PAC for predicting VTE was 3.7 with a sensitivity of 87.5 % and specificity of 51.9 % (B).

oncometabolite 2-HG from α-ketoglutarate, which accumulates in tumor cells [22,23]. 2-HG can be measured by MRS and can therefore be used to determine IDH-status before surgery.

In this study, we found that platelet activation as well as coagulation is strongly involved in the development of VTE, especially in patients with IDH-wildtype high grade gliomas. This finding may be useful for elucidating the pathophysiology of VTE in these patients. Platelet activation is the essential initial step in the development of VTE. Indeed, patients with lower KPS can be hypercoagulable or be in prethrombotic states, which are exacerbated after craniotomy, leading to a tendency for thrombosis. Taken together with the results of the present report, we speculate the following mechanisms for VTE in patients with IDH-wildtype, high grade gliomas. Surgical resection of PDPN expressing gliomas lead to the release of PDPN into the blood, resulting in the binding of CLEC-2 to PDPN which increased platelet activity, leading to thrombosis at distant sites such as the deep veins in the postoperative period. Therefore, plasma PDPN increases early after surgery, and plasma sCLEC-2 and C2PAC index increase with platelet activation. Platelet activation leads to thrombus formation, which causes elevation of D-dimer. Supplementary fig. 1 shows a representative case in which plasma PDPN, sCLEC2 and D-dimer were analyzed at multiple time-points after surgery. Although further studies are warranted, surgical factors such as the use of an ultrasonic suction devise, usage of the ultrasonic devise at low or high power, en bloc or piecemeal resection, total or non-total resection, may impact the amount of PDPN released into the bloodstream.

4.1. Limitations

As the study population was relatively small, the relationship between the level of sCLEC-2 levels and development of VTEs should prospectively be examined in a large-scale study. The present study is limited by retrospective nature at a single center. The number of recruited individuals is small for multi- variate analysis. Furthermore, patients with D-dimer levels of <5 mg/ml have not been screened for VTE. Whether aggressive screening and early treatment of VTE is justified in high grade glioma cases requires additional research both prospectively and preclinically. Elucidating the exact cause of VTE in patients with high grade glioma and these studies will ultimately lead to the understanding of glioma biology. More studies, including animal experiments, are needed to confirm this hypothesis.

4.2. Future perspectives

Early detection of postoperative VTE is essential for safe patient management. The present study indicates that C2PAC index may be useful for the early prediction of VTE. Assessment of D-dimer is effective for the screening for VTE. However, D-dimer is elevated as a result of VTE, and its levels can also be affected by surgical intervention. Elevation of PDPN and sCLEC-2 occur before the development of VTE and may be less affected by surgery. This study is a preliminary one, looking at plasma sCLEC-2 levels in glioma patients after surgery, and was designed to monitor the development of VTE by D-dimer. Thus, the serial assessment of changes in plasma PDPN and sCLEC-2 before the formation of VTE is insufficient. Further studies are needed to prove the impact of the PDPN-sCLEC-2 axis in VTE formation in glioma patients after surgery, and whether sCLEC-2 can be used to reliably predict VTE.

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Declaration of competing interest

Masahide Kawamura and Kamon Shirakawa are employees of LSI Medience, but were not involved in data analysis. The authors have no other conflicts of interest to declare.

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References

[1] P.Mir Seyed Nazari, J. Riedl, I. Pabinger, C. Ay, The role of podoplanin in cancer-associated thrombosis, Thromb. Res. 164 (Suppl 1) (2018) S34–S39.
[2] K. Suzuki-Inoue, Platelets and cancer-associated thrombosis: focusing on the platelet activation receptor CLEC-2 and podoplanin, Blood 134 (22) (2019) 1912–1918.
[3] P.Mir Seyed Nazari, J. Riedl, M. Preusser, F. Posch, J. Thaler, C. Marosi, P. Birner, G. Ricken, J.A. Hainfellner, I. Pabinger, C. Ay, Combination of isocitrate dehydrogenase 1 (IDH1) mutation and podoplanin expression in brain tumors identifies patients at high or low risk of venous thromboembolism, J. Thromb. Haemost. 16 (6) (2018) 1121–1127.
[4] J. Riedl, M. Preusser, P.M. Nazari, F. Posch, S. Panzer, C. Marosi, P. Birner, J. Thaler, C. Brostjan, D. Lotsch, W. Berger, J.A. Hainfellner, I. Pabinger, C. Ay, Podoplanin expression in primary brain tumors induces platelet aggregation and increases risk of venous thromboembolism, Blood 129 (13) (2017) 1831–1839.
[5] J. Watanabe, M. Natsumeda, M. Okada, Y. Kanemaru, Y. Tsukamoto, M. Oishi, A. Kakita, Y. Fujii, Podoplanin expression and IDH-wildtype status predict venous thromboembolism in patients with high-grade gliomas in the early postoperative period, World Neurosurg. 128 (2019) e982–e988.
[6] D. Unruh, S.R. Schwarze, L. Khoury, C. Thomas, M. Wu, L. Chen, R. Chen, Y. Liu, M.A. Schwartz, C. Amidei, P. Kumthekar, C.G. Benjamin, K. Song, C. Dawson, J. M. Rispoli, G. Fatterpekar, J.G. Golfinos, D. Kondziolka, M. Karajannis, D. Pacione, D. Zagzag, T. McIntyre, M. Snuderl, C. Horbinski, Mutant IDH1 and thrombosis in gliomas, Acta Neuropathol. 132 (6) (2016) 917–930.
[7] K. Suzuki-Inoue, Y. Kato, O. Inoue, M.K. Kaneko, K. Mishima, Y. Yatomi, Y. Yamazaki, H. Narimatsu, Y. Ozaki, Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells, J. Biol. Chem. 282 (36) (2007) 25993–26001.
[8] M.A. Cassatella, Human mature neutrophils as atypical APC, Blood 129 (14) (2017) 1895–1896.
[9] K. Suzuki-Inoue, G.L. Fuller, A. Garcia, J.A. Eble, S. Pohlmann, O. Inoue, T. K. Gartner, S.C. Hughan, A.C. Pearce, G.D. Laing, R.D. Theakston, E. Schweighoffer, N. Zitzmann, T. Morita, V.L. Tybulewicz, Y. Ozaki, S.P. Watson, A novel syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2, Blood 107 (2) (2006) 542–549.
[10] K. Suzuki-Inoue, M. Osada, Y. Ozaki, Physiologic and pathophysiologic roles of interaction between C-type lectin-like receptor 2 and podoplanin: partners from in utero to adulthood, J. Thromb. Haemost. 15 (2) (2017) 219–229.
[11] Y. Yamashita, K. Suzuki, T. Mastumoto, M. Ikejiri, K. Ohishi, N. Katayama, K. Suzuki-Inoue, H. Wada, Elevated plasma levels of soluble C-type lectin-like receptor 2 (CLEC2) in patients with thrombotic microangiopathy, Thromb. Res. 178 (2019) 54–58.
[12] H. Payne, T. Ponomaryov, S.P. Watson, A. Brill, Mice with a deficiency in CLEC-2 are protected against deep vein thrombosis, Blood 129 (14) (2017) 2013–2020.
[13] F. Kazama, J. Nakamura, M. Osada, O. Inoue, M. Oosawa, S. Tamura, N. Tsukiji, K. Aida, A. Kawaguchi, S. Takizawa, M. Kaneshige, S. Tanaka, K. Suzuki-Inoue, Y. Ozaki, Measurement of soluble C-type lectin-like receptor 2 in human plasma, Platelets 26 (8) (2015) 711–719.
[14] X. Zhang, W. Zhang, X. Wu, H. Li, C. Zhang, Z. Huang, R. Shi, T. You, J. Shi, Y. Cao, Prognostic significance of plasma CLEC-2 (C-type lectin-like receptor 2) in patients with acute ischemic stroke, Stroke 50 (2019) 45–52.
[15] A. Nishigaki, Y. Ichikawa, M. Ezaki, A. Yamamoto, K. Suzuki, K. Tachibana, T. Kamon, S. Horie, J. Masuda, K. Makino, K. Shiraki, H. Shimpo, M. Shimaoka, K. Suzuki-Inoue, H. Wada, Soluble C-type lectin-like receptor 2 elevation in patients with acute cerebral infarction, J. Clin. Med. 10 (15) (2021).
[16] M. Nakamura, N. Yamada, M. Ito, Current management of venous thromboembolism in Japan: current epidemiology and advances in anticoagulant therapy, J. Cardiol. 66 (6) (2015) 451–459.
[17] M. Natsumeda, T. Uzuka, J. Watanabe, M. Fukuda, Y. Akaiwa, K. Hanzawa, M. Okada, M. Oishi, Y. Fujii, High incidence of deep vein thrombosis in the perioperative period of neurosurgical patients, World Neurosurg 112 (2018) e103–e112.
[18] T. Kawaguchi, T. Kumabe, M. Kanamori, T. Nakamura, R. Saito, Y. Yamashita, Y. Sonoda, M. Watanabe, T. Tominaga, Early detection of venous thromboembolism in patients with neuroepithelial tumor: efficacy of screening with serum D-dimer measurements and doppler ultrasonography, J. Neuro-Oncol. 101 (3) (2011) 495–504.
[19] D.N. Louis, A. Perry, G. Reifenberger, A. von Deimling, D. Figarella-Branger, W. K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World

Health Organization classification of tumors of the central nervous system: a summary, Acta Neuropathol. 131 (6) (2016) 803–820.
[20] H. Ishikura, Y. Irie, M. Kawamura, K. Hoshino, Y. Nakamura, M. Mizunuma, J. Maruyama, M. Nakashio, K. Suzuki-Inoue, T. Kitamura, Early recognition of sepsis-induced coagulopathy using the C2PAC index: a ratio of soluble type C lectin-like receptor 2 (sCLEC-2) level and platelet count, Platelets 33 (6) (2022) 935–944.
[21] L. Bilic-Zulle, Comparison of methods: passing and bablok regression, Biochem Med (Zagreb) 21 (1) (2011) 49–52.
[22] M. Natsumeda, H. Igarashi, T. Nomura, R. Ogura, Y. Tsukamoto, T. Kobayashi, H. Aoki, K. Okamoto, A. Kakita, H. Takahashi, T. Nakada, Y. Fujii, Accumulation of 2-hydroxyglutarate in gliomas correlates with survival: a study by 3.0-tesla magnetic resonance spectroscopy, Acta Neuropathol. Commun. 2 (2014) 158.
[23] M. Natsumeda, K. Motohashi, H. Igarashi, T. Nozawa, H. Abe, Y. Tsukamoto, R. Ogura, M. Okada, T. Kobayashi, H. Aoki, H. Takahashi, A. Kakita, K. Okamoto, T. Nakada, Y. Fujii, Reliable diagnosis of IDH-mutant glioblastoma by 2-hydroxyglutarate detection: a study by 3-T magnetic resonance spectroscopy, Neurosurg. Rev. 41 (2) (2018) 641–647.