Serum amyloid beta-42 as a noninvasive biomarker for the prognosis and histologic features of glioma

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Abstract

PURPOSE

Glioma is often refractory. Histopathologic examination is essential to establish an initial diagnosis, and multiple imaging studies are conducted to assess the treatment response. However, these conventional approaches are usually accompanied by high risks and costs during treatment. The purpose of this study was to identify a novel, noninvasive, candidate biomarker for the histological prediction and prognostic assessment of glioma.

METHODS

Serum was prepared from blood samples collected preoperatively from 65 patients with WHO grade II–IV glioma between October 2004 and December 2017 in a single tertiary-level institution. The concentration of amyloid beta-42 (Aβ42) was measured by SMCxPRO (Merck) immunoassay. The clinical characteristics and histologic features of the patients, including the molecular subtype, were reviewed. Progression-free survival was evaluated as the primary outcome.

RESULTS

The mean age of the patients was 53.7 ± 12.2 years. Thirty-seven (56.9%) patients were male, and 21 (32.3%) patients had primary tumors. In Kaplan-Meier survival analysis, the group with higher serum Aβ42 (> 5.7 pg/ml) showed a poorer outcome ($p = 0.014$). In multivariate regression analysis, the serum Aβ42 concentration showed a significant association with EGFR expression and the Ki-67 labeling index. A higher serum Aβ42 concentration was associated with wild-type EGFR expression (odds ratio 0.237, $p = 0.022$), increased cell proliferation ($\beta = 0.339$, $p = 0.007$) and a poor outcome (hazard ratio 0.339, $p = 0.046$).

CONCLUSION

The serum Aβ42 level would be a good, noninvasive, candidate biomarker for the prediction of histological features and prognosis in glioma patients. Further studies with large cohorts might be required for its clinical use.

Introduction

Glioma is a tumor of glial cell origin responsible for 30% of central nervous system neoplasms and 80% of malignant tumors in the brain. [1] High-grade glioma exhibits highly aggressive behavior and a dismal prognosis. Initial diagnosis is made by magnetic resonance imaging (MRI) scans, followed by invasive neurosurgical procedures for histopathological and molecular confirmation. Patients are prone to

repetitive MRI follow-up with gadolinium-based contrasts to evaluate the response to the treatment, such as radiation therapy or chemotherapy. However, based on current clinical guidelines, the use of MRI to predict prognosis has several problems due to treatment-related changes, like pseudoprogression or pseudoresponse, requiring further examination by positron emission tomography with radioisotopes and sometimes reoperation for biopsy. [2] To the best of our knowledge and experience, these conventional approaches are not only invasive and inconvenient but also highly expensive.

On the other hand, recent studies have suggested novel liquid biopsy-based approaches to improve the limitations of previous methods. Blood-based liquid biopsy has become part of routine clinical care for the detection of therapeutically targetable mutations in many solid tumors outside the brain. [3] Identifying noninvasive biomarkers of glioma is also important. Several new candidates from among circulating DNAs, microRNAs, metabolites and proteins have been reported. [4, 5] However, no circulating biomarker is currently applicable in the clinical setting.

Amyloid beta-42 (Aβ42) is a peptide consisting of 42 amino acids that is derived from amyloid precursor protein (APP). Previous studies have shown that pan-neural expression of Aβ42 is related to increased glial cell proliferation [6] and reduced epidermal growth factor receptor (EGFR) levels in the brain in *in vivo* models [7]. In human research, it has been reported that Aβ accumulates in the brain tissue of glioma patients [8] and is detected in CSF and serum [9]. This finding suggests the potential of Aβ42 as a noninvasive biomarker for glioma.

The purpose of this study was to determine the potential of Aβ42 as a noninvasive biomarker of glioma. To evaluate serum Aβ42 levels, immunoassays were performed with serum sample s from glioma patients.

Methods

Study population

Blood samples from glioma patients who agreed and provided informed consent were collected preoperatively between October 2004 and December 2017 at our institution. We also collected demographic and tumor-related clinical data, including age at diagnosis, sex, etiology (primary or recurrent), histopathological grade and tumor subtype based on the World Health Organization (WHO) classification, Ki-67 labeling index, EGFR expression, extent of resection (EOR), and progression-free survival (PFS). This study was approved by the institutional review board of our institution.

Immunoassay for amyloid-beta42

Samples were used immediately after collection or stored at -20°C before they were subjected to more than two freeze-thaw cycles before use. Reagents from the SMCxPRO (Merck) kit were stored at 4°C and thawed at room temperature before the experiment. The antibody used for detection was kept in complete darkness before use.

The blood sample was centrifuged for 10 minutes at 13000 xg using a microcentrifuge. Only the supernatant was moved to a microcentrifuge tube using a pipette and diluted with a standard diluent reagent at a 1:4 ratio. One hundred microliters of standards and a 1:4 diluted sample were dispensed on each well of the assay plate.

Anti-Aβ42 antibody-coated beads were sufficiently resuspended for at least 20 minutes by using a spin rotator or by manual inversion. The anti-Aβ42 antibody-coated beads (0.45 ml) were then diluted with 11.55 ml of assay buffer. One hundred microliters of the bead solution was dispensed onto each well of the assay plate. The plate was completely sealed and incubated in a microplate shaker at 25°C and 500 rpm for 2 hours. After centrifugation with 20X detection antibody for 5 minutes at 14000 xg, 250 μl of the antibody supernatant was mixed with 4750 μl of assay buffer. The diluted antibody was moved to a clean tube using a 0.2 μm filter. When incubation of the assay plate was complete, the plate was centrifuged at 1100 xg for one minute, and the seal was opened. The assay plate was placed on a handheld magnet, and the supernatant was removed using a pipette. After the assay plate was displaced from the handheld magnet, 20 μl of antibody was added to each well. The plate was covered and placed in a shaker at 25°C for 30 minutes.

The plate was placed on the handheld magnet, and the supernatant was removed. The beads were washed four times. At the end of washing, 200 μl of 1X wash buffer was dispensed, after which the plate was displaced from the handheld magnet. After the plate was sealed, it was agitated at 750 rpm for 90 seconds, and the supernatant was carefully removed. Ten microliters of buffer D was dispensed in each well, and the plate was placed on a Sphere mag plate for two minutes. After 10 μl of the eluate had been moved from the assay plate to the V-bottom plate for reading, the plate was sealed, agitated at 25°C at 1500 rpm for one minute and centrifuged at room temperature at 1100 xg for one minute. The plate was sealed with an aluminum seal and placed in an SMCxPRO for analysis.

Statical analyses

All data were analyzed using Statistical Package for Social Sciences (SPSS) software version 26.0.0 (IBM, Armonk, NY, USA).

Subjects were divided into two groups by serum Aβ42 concentration. In our study, a healthy control serum Aβ42 concentration, which is needed to determine the threshold for Kaplan-Meier analysis, was not available. Instead, we performed the Kaplan-Meier analysis several times and compared the p-value from the log-rank tests in Kaplan-Meier analysis to determine the threshold for high or low serum Aβ42 concentration; the threshold was determined to be 5.7 pg/ml, for which the lowest p-value was obtained by log-rank test.

To compare PFS between the high Aβ42 (> 5.7 pg/ml) and low Aβ42 (≤ 5.7 pg/ml) groups, we used Kaplan-Meier analysis and the log-rank test. Cox proportional hazards regression analysis was used to assess the hazard ratios (HR) for PFS according to the level of serum Aβ42. To adjust for the effects of potential confounding factors on PFS, age, sex, tumor subtype, WHO grade and EOR were included as

covariates in multivariate analysis. The assumption of proportionality for Cox proportional hazards analysis was confirmed by ensuring that the two Kaplan-Meier curves for the high Aβ42 and low Aβ42 groups did not intersect.

To measure associations between histologic features and serum Aβ42 concentration, we calculated standardized coefficients (β) and odds ratios (ORs) using linear and logistic regression. A univariate regression model was used to determine variables associated with the Ki-67 labeling index and EGFR expression. A multivariate regression model was used to calculate β and the OR after adjusting for age, sex, and other variables that were found significant by univariate analysis ($p < 0.05$).

Results

Demographics and clinical characteristics of the patients

A total of 65 patients were included in the final analysis: 43 cases with glioblastoma (WHO grade IV), 11 cases with anaplastic oligodendroglioma (WHO grade III), 5 cases with anaplastic astrocytoma (WHO grade III), 5 cases with oligodendroglioma (WHO grade II), and 1 case with diffuse astrocytoma (WHO grade II) (summarized in Table 1).

Table 1
Basic characteristics of the subjects studied

Characteristic	Study population (n = 65)
Male sex, no. (%)	37 (56.9)
Age in years, mean (± SD)	53.7 (± 12.2)
Amyloid beta in pg/ml, mean (± SD)	6.26 (± 4.49)
Primary tumor, no. (%)	21 (32.3)
Diagnosis, no. (%)	
Glioblastoma multiforme	43 (66.2)
Anaplastic oligodendroglioma	11 (16.9)
Anaplastic astrocytoma	5 (7.7)
Oligodendroglioma	5 (7.7)
Diffuse astrocytoma	1 (1.5)
WHO grade, no. (%)	
Grade IV	43 (66.2)
Grade III	16 (24.6)
Grade II	6 (9.2)
EGFR mutation, no. (%)	46 (70.8)
Ki-67†, mean (± SD), %	14.71 (± 11.41)
Extent of resection, no. (%)	
Gross total resection	34 (52.3)
Subtotal resection	16 (24.6)
Partial resection	14 (21.5)
Biopsy	1 (1.5)
Data are shown as the median (range) or number (%). †n = 62	

The mean (± SD) age of the patients was 53.7 ± 12.2 years. The mean serum concentration of Aβ42 was 6.26 ± 4.49 pg/ml. Twenty-one (32.3%) patients had primary tumors, and the other 44 (67.7%) patients had recurrent tumors. The mean Ki-67 labeling index was 14.71 ± 11.41%.

Association between serum Aβ42 concentration and glioma patient prognosis

Kaplan–Meier analysis demonstrated that patients with high serum Aβ42 (> 5.7 pg/ml, n = 30) had significantly shorter PFS (p = 0.014; Fig. 1). Twenty-four out of 30 patients in the high Aβ42 group experienced progression, while 18 out of 35 patients in the low Aβ42 group did. The mean and median PFS of the high Aβ42 group were 29.5 ± 7.7 and 13.0 ± 3.90 months, respectively, while those of the low Aβ42 group were 67.1 ± 14.7 and 38.0 ± 18.0 months, respectively.

Univariate and multivariate Cox regression analyses were used to determine independent prognostic factors for PFS. In univariate Cox regression, the level of serum Aβ42, tumor subtype, WHO grade and EOR were significant factors for PFS (Table 2). In multivariate Cox regression, a high serum Aβ42 level (HR 2.021, p = 0.046) and grade II glioma (unlike grade IV glioma) (HR 0.241, p = 0.047) were independent prognostic factors for PFS after adjusting for age, sex, and other factors that were deemed significant by univariate analysis.

Table 2
Univariate and multivariate Cox regression analyses of PFS

Variable	Univariate Cox Regression		Multivariate Cox Regression	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex, female	0.675 (0.365–1.248)	0.210	0.730 (0.379–1.442)	0.365
Age, > 54 years	1.414 (0.750–2.665)	0.284	0.940 (0.460–1.918)	0.864
Amyloid beta (> 5.7 pg/ml)	2.111 (1.141–3.908)	0.017*	2.021 (1.011–4.038)	0.046*
Primary tumor	1.209 (0.641–2.283)	0.558		
Diagnosis				
GBM	5.197 (1.197–22.56)	0.028*	†	
AO	3.349 (0.675–16.60)	0.139	2.366 (0.305–18.37)	0.410
AA	Reference		Reference	
ODG	2.061 (0.376–11.31)	0.405	†	
DA	3.442 (0.303–39.13)	0.319	3.169 (0.267–37.63)	0.361
WHO grade				
Grade IV	Reference		Reference	
Grade III	0.432 (0.200–0.937)	0.034*	0.185 (0.026–1.340)	0.095
Grade II	0.445 (0.166–1.189)	0.106	0.241 (0.059–0.984)	0.047*
EGFR mutation	0.925 (0.485–1.763)	0.813		
Ki-67, > 15%	1.094 (0.517–2.316)	0.814		
EOR, no. (%)				
GTR	3.750 (1.484–9.477)	0.005*	1.881 (0.579–6.106)	0.293
STR	Reference		Reference	
PR	3.522 (1.295–9.578)	0.014*	2.614 (0.779–8.772)	0.120
Bx	0.000 (0.000)	0.980	0.000 (0.000)	0.979

*p-value < 0.05; †The degree of freedom is zero.

AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; Bx, biopsy; DA, diffuse astrocytoma; EOR, extent of resection; GBM, glioblastoma multiforme; GTR, gross total resection; ODG, oligodendroglioma; PR, partial resection; STR, subtotal resection

Unlike PFS, which was strongly associated with serum Aβ42 levels, the overall survival (OS) of glioma patients showed no association with serum Aβ42 levels in Kaplan-Meier analysis ($p = 0.409$). Over the observation period, six out of 30 patients in the high Aβ42 group expired, and 4 out of 35 patients in the low Aβ42 group did.

Association between serum Aβ42 concentration and histologic features of the glioma patients

Linear and logistic regression analyses were performed to determine whether the Aβ42 concentration in the serum of glioma patients was a significant predictor of the Ki-67 labeling index and EGFR expression.

In univariate linear regression analysis for the Ki-67 labeling index, age was a significant factor (Table 3). However, after adjusting for sex and age, the serum Aβ42 level was a significant factor for the Ki-67 labeling index ($\beta = 0.339$, $p = 0.007$). The Ki-67 labeling index, which is related to cell proliferation, was higher in the high Aβ42 group than in the low Aβ42 group.

Table 3
Univariate and multivariate linear regression analyses of Ki-67 (%)

Variable	Univariate Regression		Multivariate Regression		
	β ± SE	p-value	β ± SE	p-value	R^2 (adj. R^2)
Sex, female	-0.136 ± 2.953	0.293	-0.170 ± 2.711	0.153	0.208 (0.168)
Age (years)	0.288 ± 0.116	0.023*	0.373 ± 0.114	0.003*	
Amyloid beta (> 5.7 pg/ml)	0.230 ± 0.315	0.072	0.339 ± 0.304	0.007*	
Primary tumor	-0.075 ± 3.212	0.579			
Diagnosis					
AA	-0.020 ± 0.020	0.913			
DA	-0.070 ± 12.84	0.628			
GBM	0.211 ± 6.015	0.405			
AO	0.246 ± 6.704	0.281			
ODG	Reference				
WHO grade					
Grade II	-0.151 ± 5.438	0.252			
Grade III	-0.011 ± 3.384	0.931			
Grade IV	Reference				
EGFR mutation	-0.067 ± 3.334	0.604			

*p-value < 0.05; SE, standard error

AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; DA, diffuse astrocytoma; GBM, glioblastoma multiforme; ODG, oligodendroglioma

In univariate logistic regression analysis for EGFR mutation, the serum Aβ42 level and etiology were significant factors (Table 4). After adjusting for age, sex and the etiology of the tumor, the serum Aβ42 level was still a significant factor for EGFR mutation (OR 0.237, p = 0.022). This means that wild-type EGFR was expressed at greater levels when the serum Aβ42 level was high.

Table 4
Univariate and multivariate logistic regression analyses of EGFR mutation

Variable	Univariate Regression		Multivariate Regression	
	OR	P-value	OR (95%CI)	P-value
Sex, female	0.782 (0.267–2.291)	0.654	0.653 (0.197–2.163)	0.495
Age, > 54 years	2.364 (0.766–7.296)	0.135	1.953 (0.566–6.743)	0.290
Amyloid beta (> 5.7 pg/ml)	0.271 (0.087–0.844)	0.024*	0.237 (0.069–0.812)	0.022*
Primary tumor	0.283 (0.092–0.873)	0.028*	0.272 (0.079–0.943)	0.040*
Diagnosis				
AA	6.000 (0.354–101.6)	0.214		
DA	2423212264 (0.000)	1.000		
GBM	4.95 (0.723–33.90)	0.103		
AO	6.000 (0.210–15.41)	0.214		
ODG	Reference			
WHO grade				
Grade II	0.303 (0.053–1.743)	0.181		
Grade III	0.505 (0.147–1.736)	0.278		
Grade IV	Reference			
Ki-67, > 15%	1.059 (0.286–3.920)	0.932		

*P-value < 0.05; SE, standard error

AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; DA, diffuse astrocytoma; GBM, glioblastoma multiforme; ODG, oligodendroglioma; OR, odds ratio

Discussion

In this study, serum Aβ42 levels were investigated as a candidate noninvasive biomarker of glioma. The results demonstrated that a high concentration of serum Aβ42 was associated with wild-type EGFR expression, a high Ki-67 index and poor PFS.

Glial cell proliferation and EGFR expression are important factors for the evaluation of glioma. The level of proliferative activity, represented by the Ki-67 labeling index, is an ancillary tool for the grading of gliomas. [10, 11] The Ki-67 labeling index, the fraction of Ki-67-positive tumor cells, is often correlated

with the clinical course of disease. [12] Ki-67 expression might be a predictive factor for poor prognosis in glioma. [12, 13] EGFR is a transmembrane tyrosine kinase, and EGFR signaling leads to DNA synthesis and cellular proliferation. [14] EGFR is modified in several ways in glioma through overexpression, amplification, deletion, and other effects. Alterations in EGFR promote proliferation, survival, angiogenesis and invasion. [15] Although the clinical relevance of alterations in EGFR in glioma remains controversial, our study shows a possible association between the expression of EGFR and prognosis of glioma patients. [15, 16] Our research is consistent with previous studies showing that neural expression of Aβ42 reduces EGFR expression in the brain and induces glial cell proliferation. [6, 7]

Pan-neural expression of Aβ42 reduced total EGFR expression in the brains of 25-day-old *Drosophila* and 8-month-old mice, although the mechanism by which EGFR expression was suppressed by Aβ42 is still unclear. [7] However, a previous study showed that the intracellular domain of APP is important for the regulation of brain EGFR expression. Overexpression of the intracellular domain of APP reduced EGFR levels in the brains of APP-knockout mice, but overexpression of Aβ did not. [17] How, then, can we explain our finding that Aβ42 is associated with suppression of EGFR expression? Although Aβ42 alone cannot regulate EGFR expression, Aβ42 might affect EGFR expression by inducing APP production. [18]

Additionally, pan-neural expression of Aβ42 in *Drosophila* increased the number of glial cells in the larval brain. [6] In the same study, researchers showed the consumption of PD98059, a specific ERK inhibitor, did not affect glial cell proliferation throughout the neurons of Aβ42-expressing *Drosophila*. This result suggests that the increased glial cell proliferation induced by Aβ42 was not associated with the ERK/EGFR signaling pathway. This can explain our finding that a high serum Aβ42 concentration was associated with increased glial cell proliferation and reduced EGFR expression. Mechanisms other than ERK/EGFR signaling may be involved in the induction of glial cell proliferation by Aβ42.

Although we found that serum Aβ42 levels were associated with the histologic features and prognosis of glioma, our current study is limited for several reasons. First, it is a retrospective study. Based on our results, we could not assess the effect of tumor resection on serum Aβ42 levels. Thus, we could not determine whether serum Aβ42 levels were affected by the tumor itself. Second, all patients were Korean; hence, the results cannot be applied generally to other ethnic groups. Third, there is a possibility of selection bias because our sample size was small, and the patients were enrolled from a single institution. Finally, we used the 2007 WHO classification of CNS tumors, and patients had various tumor subtypes from grade II to IV. The heterogeneity of tumor subtypes and use of an old classification system are limitations of this study. Prospective studies with larger cohorts from multiple centers and the use of an updated WHO classification of CNS tumors are future aims to reinforce the results of our study.

In summary, our results suggest that serum Aβ42 levels are a noninvasive biomarker for the histologic features and prognosis of glioma. Further prospective studies with larger cohorts from multiple institutions might be required for the clinical use of Aβ42 as a biomarker for glioma.

Abbreviations

Amyloid beta-42, Aβ42

Amyloid precursor protein, APP

Epidermal growth factor receptor, EGFR

Extent of resection, EOR

Hazard ratio, HR

Magnetic resonance imaging, MRI

World Health Organization, WHO

Odds ratio, OR

Progression-free survival, PFS

Declarations

Author contributions

Conception and design: K.-S.A. and C.-Y.K.

Acquisition of data: All authors.

Analysis and interpretation of data: K.H. and M.N.

Drafting and revision of the manuscript: All authors.

Final approval: All authors.

Data availability

Raw data that supports the findings of this study are available from the corresponding author, upon reasonable request.

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Conflict of interest

All the authors declare that they have no conflict of interest.

Ethical Approval

Approved by institutional review board.

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Figures

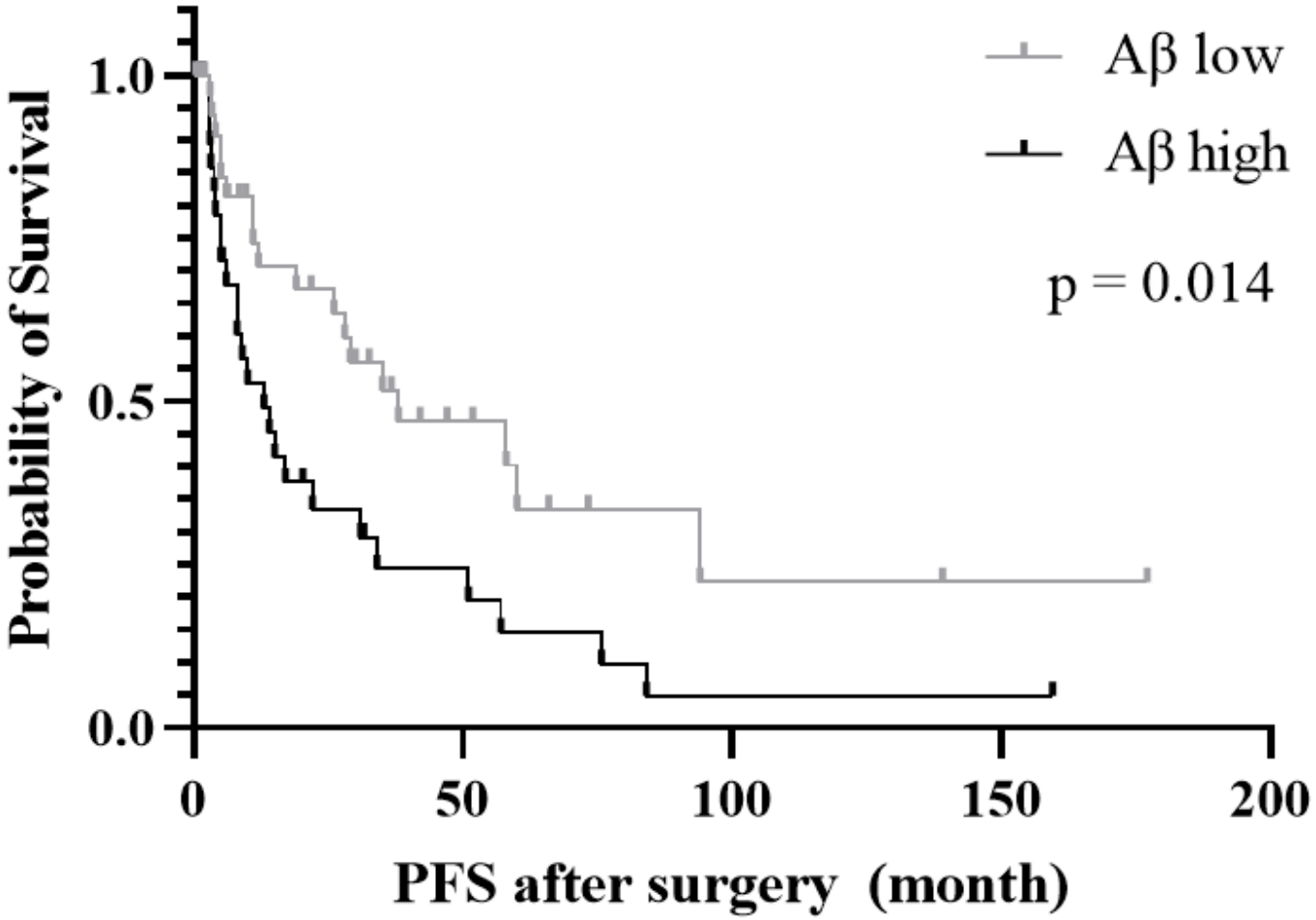

Figure 1

Kaplan-Meier curves for glioma patient PFS based on serum Aβ42 level. Kaplan–Meier curves display the PFS of patients with high Aβ42 levels (n = 30) and those with low Aβ42 levels (n = 35).