Outcome and molecular characteristics of adolescent and young adult patients with newly diagnosed primary glioblastoma: a study of the Society of Austrian Neurooncology (SANO)

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Background. Young age is a favorable prognostic factor for patients with glioblastoma multiforme (GBM). We reviewed the outcomes and molecular tumor characteristics of adolescent and young adult patients with GBM treated in 2 Austrian centers.

Patients and Methods. Data on patients with histologically proven primary GBM diagnosed from 18 through 40 years of age were retrospectively analyzed. All patients were treated with standard first-line therapy. The primary end points were overall survival (OS) and time to progression (TTP). IDH1-R132H mutation status was analyzed using immunohistochemistry, and MGMT promoter methylation was assessed using methylation-specific polymerase chain reaction.

Results. We included 70 patients (36 men and 34 women) with a median age of 33 years. IDH1-R132H mutations were detected in 22 (39.3%) of 56 cases and MGMT promoter methylation in 33 (61.1%) of 54 cases with available tissue samples. In patients with wild-type IDH, median TTP was 8.2 months and median OS was 24 months, compared with 18 months and 44 months, respectively, observed in patients with mutated IDH. Neither IDH1 nor MGMT status showed a statistically significant association with TTP or OS. Of note, the social and economical situation of the young patients with GBM was alarming, because only 17% succeeded in staying employed after receiving the diagnosis.

Conclusions. We found a high frequency of IDH1 mutations and MGMT promoter methylation among young adult patients with primary GBM that may contribute to the generally favorable outcome associated with young age. The social and economic coverage of patients with glioma remains an unsolved socio-ethical problem.

Keywords: adolescents and young adults, glioblastoma, IDH1 mutation status, MGMT promoter methylation, outcome.

Receiving a diagnosis of glioblastoma is a devastating personal stroke of fate. The incidence of glioblastoma is 3–4 cases/100 000 population per year according to the current World Health Organization (WHO) Classification Tumors of the Nervous System, 3.19 cases/100 000 population according to Central Brain Tumor Registry of the United States (CBTRUS) 2011 (http://www.cbtrus.
The social impact of glioblastoma might even be worse when young adults are affected. Each year, nearly 70 000 adolescents and young adults (AYA) aged 15–39 years receive a diagnosis of cancer in the United States. Although the incidence of GBM among young adults is low, approximately 2000 persons <40 years of age were affected in the United States in 2008 (2).

Patients with GBM usually receive a diagnosis after a sudden onset of symptoms resulting from the involvement of eloquent brain areas and/or from increased cranial pressure. They undergo neuro-imaging and a neurosurgical procedure before being told their diagnosis of a malignant brain tumor. The actual standard therapy of glioblastoma includes maximal feasible surgical resection followed by 6 weeks of radiochemotherapy combining involved field radiation therapy of 2 Gy per fraction up to 60 Gy with concomitant oral chemotherapy with temozolomide; this is followed by a 4-week break and 6 months of adjuvant oral chemotherapy with temozolomide.

The peri-operative period and the 6 weeks of radiochemotherapy are busy and highly challenging for patients and families. Fatigue is a very common adverse effect near the end of radiation. Furthermore, scars from the neurosurgical procedure, hair loss after surgery and/or radiation, and the visible adverse effects of dexamethasone treatment change the physical appearance of the patient and reveal presence and site of disease to friends, workmates, employers, acquaintances, and everyone looking. These circumstances hamper the patient’s ability of keeping his or her diagnosis unknown to others, especially to his or her employer and to maintain his or her ordinary life, including professional activities.

In this situation, many patients lose their employment under the pretence of the prolonged sick leave. Not only in the situation of an economical crisis, the opportunities on the job market for patients with brain tumor are very restricted. At least in Austria, social security is not prepared for the needs of young adults with cancer. Because young adults cannot already have worked for decades, they get only very limited payments from social security.

With increasing numbers of patients with malignant gliomas surviving for prolonged periods, treating physicians are repeatedly confronted with patients with social and economic problems. Such issues might have a strong negative impact on a patient’s quality of life, causing depression and the sensation of being a burden to their loved ones.

In a retrospective survey, we wanted to reflect the fate of young adults who receive a diagnosis of glioblastoma and to investigate their potential special juvenile features in clinical, genetic, and socio-demographic characteristics. Because we found an unexpected high prevalence of IDH1-mutated glioblastomas in our cohort of young patients treated at the Medical University of Vienna, we performed confirmatory analysis in an independent series of AYA patients with glioblastomas at another Austrian neurooncologic center (Wagner Jauregg Hospital, Linz, Austria).
Materials and Methods

Study Design

Data on patients with GBM aged <40 years were retrieved from treatment records. Forty years was chosen as the limit for young adulthood in accordance with the definition of AYA (adolescents and young adults with cancer by the National Cancer Institute\textsuperscript{31–45}), corresponding with the period during which an individual usually completes his or her development and establishes a career and private life foundations.

We recorded the following parameters: symptoms leading to diagnosis, duration of symptoms, date of first neurosurgical intervention, extent of surgery, start of postsurgical treatment, dose of radiotherapy administered, chemotherapy, number of cycles given, toxicities, time to progression, date of progression, treatment at progression, and survival duration. Special attention was paid to all information on the living situation and the actual situation of surviving patients.

Tissue blocks from the Institute of Neurology were reviewed by neuropathologists (M.P., A.W.) to confirm the diagnosis of glioblastoma and for the immunohistochemical analysis of IDH1 mutation status.

Patients

All patients with newly diagnosed, histologically proven de novo GBM treated at the Medical University of Vienna from January 1, 2003 through December 31, 2010 who were aged <40 years at the time of diagnosis, defined as the day of surgery, were included in this retrospective survey. Furthermore, no prior diagnosis of a lower-grade glioma, no prior imaging evidence of a brain tumor, and no history of seizures exceeding 3 months before surgery were allowed. The requirements for radio- and chemotherapy at our institution mandate adequate hematologic blood counts, adequate renal and hepatic functions, and the absence of infection or serious concomitant illnesses. All patients gave written informed consent to the treatment. The study was reviewed and approved by the local ethics committee.

The patients treated at the Wagner Jauregg Hospital in Linz also had exclusively primary GBM treated during 1998–2011.

Treatment

The patients underwent maximal feasible neurosurgical tumor resection (biopsy, partial resection, or total resection), followed by standard focal radiotherapy at a dose of 2 Gy per fraction for a total dose of 60 Gy. Because the survey covers a delay prior to the establishment of temozolomide as standard of care, temozolomide was not available for patients treated prior to March 2005. However, as a center participating in the pivotal trial of the European Organisation for Research and Treatment of Cancer (EORTC), we had already adopted concomitant treatment with a nitrosourea-based compound as standard treatment. The nitrosoureas used were either lomustin (CCNU) (80–120 mg/m\textsuperscript{2} given orally every 6 weeks for 1 year) or fotemustine, as published previously, and dacarbazine (fotemustine: 100 mg/m\textsuperscript{2} day 1, for 21 days; dacarbazine: 150 mg/m\textsuperscript{2} day 1, for 21 days).\textsuperscript{46} The concomitant therapy with daily temozolomide, followed by adjuvant temozolomide, according to the EORTC/National Cancer Institute of Canada regimen\textsuperscript{37,38}, became standard of care after March 2005.

Immunohistochemistry for Detection of IDH1 Mutation

IDH1-R132 H protein expression was determined immunohistochemically in formalin-fixed and paraffin-embedded tumor tissue blocks from tumor material. Tissue blocks were cut at a thickness of 3–4 microns. Sections underwent heat-induced antigen retrieval for 60 min and were incubated with the monoclonal IDH1-R132H antibody (clone DIA-H09; Dianova) at a dilution of 1:30 for 60 min. The antibody specifically recognizes IDH 1- R 132 H mutation status. Detection of immunolabeling was performed using the Flex + Mouse system (Dako) with diaminobenzidzin as chromogen. Presence or absence of tumor cell immunolabeling was evaluated by one observer (A.W.). The expression of IDH1-R132H was determined by a 2-tiered semiquantitative scoring system. Cases with cytoplasmic/nuclear expression of the mutant IDH1—R132 H protein were scored as positive. Absence of immunostaining was scored as negative. No case with partly positive and partly negative staining of tumor cells was encountered.

DNA Modification and Methylation-specific PCR (MSP)

DNA was extracted from frozen surgery specimens or paraffin-embedded tissues with the use of a QIAmp DNA Blood Mini Kit and FFPE Tissue Kit (QIAGEN), respectively. Bisulfite conversion was done using an Epitect Bisulfite Kit (QIAGEN) according to the instructions of the manufacturer. MSP was performed with primers specific for either methylated or modified unmethylated MGMT promoter,\textsuperscript{47} as previously described.\textsuperscript{48} CpGenome Universal methylated and Universal unmethylated DNA (Chemicon International) were used as controls. PCR products were separated by 6% polyacrylamide gel electrophoresis, stained with ethidiumbromide, and visualized under UV illumination (ChemiDoc; BioRad). Expression levels were quantified by Quantity One Quantitation software (BioRad) and calculated relatively to the indicated controls. All results were confirmed in at least 2 independent experiments.

Statistical Analysis

Survival was estimated as overall survival (OS) and time to tumor progression (TTP). OS was defined from the
day of surgery that led to histological diagnosis of GBM to death of patient, whereas TTP was defined from the day of surgery to the first day of suspicion of a later confirmed eventual disease progression by MRI. Survival data were calculated according to the Kaplan Meier method. Data regarding patients who survived until the end of the observation period were censored at their last follow-up. For the purpose of testing hypotheses about survival of subgroups, the log rank test and the more complex Cox proportional hazards model were used. Categorical data were analyzed by \(\chi^2\) contingency analysis using Fisher’s exact test. Results on IDH1 mutations were correlated to age, sex, and survival data.

Results

Patients Characteristics

Forty-seven patients entered this retrospective survey. The 28 men and 19 women were aged 18–39 years. The median age at the time of diagnosis was 32 years (Table 1). Thirty-three patients were \(\geq\) 30 years of age at the time of diagnosis. Before starting radiochemotherapy, 44 patients (94%) showed a WHO performance status of 0 or 1; 3 male patients were classified as WHO performance status 2. The most common symptoms at diagnosis were severe headache and emesis (29 patients [61.7%]). Seizures occurred in 15 patients (31.9%), which is slightly more than in older patients with GBM. An organic psycho syndrome was observed in 15 patients (32%), with concentration deficits, personality changes, and mood and character alterations. Fourteen patients (30%) suffered neurological deficits: 7 patients (14.9%) remarked defects of vision, and 7 (14.9%) had problems with speaking.

Immunohistochemistry

Seventeen tumors were positive for IDH1 mutation and 16 were negative for IDH1 mutations. Despite intense efforts, no tumor tissue was available for further molecular genetic analyses in 14 patients (29.8%). The mostly small stereotactic biopsies have either been already used for diagnostic purposes or surgery was performed in other hospitals that did not participate in the present study. Because the percentage of IDH1 was that high, the medical history of the patients was rechecked to search for indicators for secondary glioblastoma, and we found that one patient had a long-lasting history of focal seizures that could have been related to an undetected precursor glioma.

Table 1. Characteristics of 70 adult patients with glioblastoma aged <40 years, treated at the Medical University of Vienna \((n = 47)\) and at the Wagner Jauregg Hospital in Linz, Austria

<table>
<thead>
<tr>
<th></th>
<th>Vienna (%)</th>
<th>Linz (%)</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td>N</td>
<td>47</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>15</td>
<td>34</td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>32</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Range</td>
<td>20–39</td>
<td>20–39</td>
<td>20–39</td>
</tr>
<tr>
<td>WHO performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24 (51)</td>
<td>8 (35)</td>
<td>32 (45.7)</td>
</tr>
<tr>
<td>1</td>
<td>20 (42.5)</td>
<td>13 (56)</td>
<td>33 (47)</td>
</tr>
<tr>
<td>2</td>
<td>3 (6.5)</td>
<td>2 (9)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Extent of resection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>7 (15)</td>
<td>2 (9)</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>16 (34)</td>
<td>13 (56)</td>
<td>29 (41)</td>
</tr>
<tr>
<td>Gross total</td>
<td>24 (51)</td>
<td>8 (35)</td>
<td>32 (46)</td>
</tr>
<tr>
<td>MGMT promoter methylation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>20</td>
<td>11</td>
<td>31 (44)</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>11</td>
<td>10</td>
<td>21 (30)</td>
</tr>
<tr>
<td>Not available</td>
<td>16</td>
<td>2</td>
<td>18 (26)</td>
</tr>
<tr>
<td>IDH1 mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>16 (34%)</td>
<td>14 (60)</td>
<td>30 (43)</td>
</tr>
<tr>
<td>Mutated</td>
<td>17 (36%)</td>
<td>6 (26)</td>
<td>23 (33)</td>
</tr>
<tr>
<td>Not available</td>
<td>14 (29)</td>
<td>3 (13)</td>
<td>17 (24)</td>
</tr>
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</table>

Therapy

Gross total resection was possible in 24 patients (51%); 16 patients underwent partial resection (34%), whereas in 7 (15%), biopsy only was done. In all of the samples, the diagnosis of GBM was histologically confirmed. All patients completed initial radiochemotherapy with a radiation up to 60 Gy. All patients completed concomitant chemotherapy, and all but 6 patients who experienced severe hematological toxicities (5 thrombocytopenias, 4 leucopenias) completed 6 cycles of adjuvant chemotherapy (87%).

Recurrence occurred in 35 patients (74.5%). Second surgeries were done in 43% of patients, much more than in older GBM patients. Four persons underwent surgery 3 times, and 1 patient underwent surgery 4 times.

Altogether 22 (46.8%) of 47 patients received salvage therapy with second-line chemotherapy or targeted therapies. The most frequently administered substances were temozolomide, thalidomide (9 patients each), and imatinib (11 patients). Second-line chemotherapeutics prescribed more rarely were erlotinib, CCNU, irinotecan, temsirolimus, bevacizumab + liposomal doxorubicin, fotemustine, and combinations of these.

Survival

Results of survival and progression-free survival were calculated separately for both sites and showed no
difference. Therapeutic outcomes are shown for the whole cohort of 70 patients.

Median OS was 28 months (95% confidence interval [CI], 24–31.6 months) (Fig. 1). The first patient died after 3 months. Eighty-seven percent of patients survived at 1 year, 61.5% at 2 years, and 16% (11 patients) at 5 years. Three patients are still alive at the time of writing: 133, 165, and 182 months in progression-free survival after initial diagnosis. One patient died in remission of his GBM 66 months after diagnosis due to sepsis, which occurred as complication of a myelodysplastic syndrome 6 years after chemotherapy with fotemustine. Patients aged <30 years at diagnosis had a significantly shorter survival (n = 23; OS, 24 months) than did patients aged ≥30 years (n = 47; OS, 29 months; P < .05) (Fig. 2). Sex and presence or absence of seizures were not significantly related to survival. Extent of resection and the performance status showed a significant impact on survival. Patients with methylated MGMT promoter showed a trend toward prolonged survival (28 vs 25 months). Patients with IDH1 mutation lived for a median of 44 months, whereas patients with IDH1 wild-type lived for a median of 24 months (see Supplementary material, Fig. S1). This difference was not statistically significant.

Of interest, both patients whose tumors showed IDH1 mutation without MGMT promoter methylation survived for >5 years and had a TTP of 45 months, followed by the patients with IDH1 mutation and MGMT promoter methylation (n = 19), who survived a median of 38.8 months (see Fig. 3).

**TTP**

Median PFS reached 12 months (95% CI, 9.5–14 months). For patients treated before 2008, it cannot be ruled out that pseudoprogression was misinterpreted and the therapy regimen was changed earlier than it would be done actually, because 2 of the long-term surviving patients underwent a repeated resection within 8 months after initial surgery for radiation necrosis. Age <30 years or ≥30 years, sex, or extent on resection showed no statistically significant impact on time to progression. Patients with glioblastomas showing IDH1 mutations showed a significantly prolonged time to progression (19 vs 13 months; P < .05) (Supplementary material, Fig. S2) whereas no such difference was observed for the promoter methylation status of MGMT (12 vs 13 months for unmethylated and methylated tumors).

**Linz Cohort**

Because of the unexpected high percentage of patients with IDH1 mutation, we asked the Wagner Jauregg Hospital in Linz to analyze their cases of primary GBM in patients aged <40 years from the past years. They provided us with 23 cases of primary GBM in patients who received a diagnosis during 1999–2011. In 21 of these patients, material for IDH1 immunohistochemistry and MGMT promoter analysis was available. Results are shown in Table 1.
Sociodemographic Outcomes

At the time of diagnosis, 20 of 47 patients were employed. The patients worked in a wide variety of jobs before their illness, including as sawmill assistants, butchers, shop assistants, overhead cooks, lawyers, teachers, midwives, and medical doctors. Three patients were students; 2 completed their studies successfully after diagnosis (pedagogics, law). Eight patients had achieved university degrees before developing glioblastoma. After diagnosis, patients working in large enterprises with several hundreds of employees or patients working in family businesses were able to keep their jobs. The individual job situation of the patients is shown in Fig. 4. Of note, persons working either in administrative jobs or with and on computers showed the highest probability of keeping their employment. The person working full time for the longest period was a clerk at a tax office.

The majority of patients (27 of 47) were not employed shortly after diagnosis, and none of them succeeded at starting or returning to a job after diagnosis of glioblastoma. Moreover, 21 patients (44%) moved back to living in the household of their parents, a decision showing their loss of independence and the leakiness of social security.

Discussion

The data in this retrospective series show that among patients with GBM diagnosed before the age of 40 years the best subgroup of patients survive a median of 2.5 years. The time of progression (ie, the period with minimal symptoms from the brain tumor in young patients) is twice as long, compared with patients who receive a diagnosis of GBM in later adulthood. Nevertheless, most of these patients died within 5 years. Of note, therapeutic outcomes were similar in both centers (Linz and Vienna).

Of interest, GBM arising in young adult patients showed a higher than expected proportion of IDH1 mutations. This was found in a monocentric retrospective survey, but was confirmed in a second unrelated sample from another hospital in a distinct geographic area. Patients with IDH1 mutation showed longer event-free survival (18 months [95% CI, 11–25 months] vs 8.2 months [95% CI, 1.5–15 months]; \( P = \) .055) and longer OS (44 months [95% CI, 36.7–51 months] vs 24.2 months [95% CI, 15.7–32.7 months]; \( P = .2 \)), compared with young patients with GBM without the mutation and unselected patients with GBM, although these differences did not reach statistical significance.
However, Jha et al\textsuperscript{18} evaluated the age-specific molecular profile of 75 patients with glioblastomas and described similar findings as those in our cohort. Although they found IDH1 mutation only in a minority of patients either >40 years of age or <18 years of age, IDH1 mutation was present in 40% of young adult patients with GBM.

Cases with a history of secondary GBM (eg, a previous diagnosis of anaplastic or diffuse glioma) were excluded from this study. The current diagnosis of secondary GBM is purely clinical, based on a history of a less malignant glioma than GBM. The data of this series suggest that this clinical definition does evidently not discriminate between the established classifications based on gene expression profiles and that, in young patients with GBM, an IDH1-mutated tumor might not always be diagnosed before it has evolved to GBM. However, this will become more important when targeted therapies for IDH1-mutated GBM become available.

On the other hand, the socioeconomic implications of glioblastoma for young patients are alarming. Our retrospective survey shows that only a minority of patients were able to maintain an adult, independent life after the diagnosis of glioblastoma. Still, most patients lost their jobs because of their illness, were not able to restart working, and faced substantial financial problems, although in Austria, they do not have to afford any treatment costs.\textsuperscript{18} In most jobs, employers are not willing and cannot take the responsibility for a staff member with GBM in professions, including sawmill assistants, butchers, salespeople, midwives, lawyers, and medical doctors. This issue is, to date, an open challenge. A utopian fantasy of meaningful employment for persons with persisting symptoms of cancer is remunerating them for community-based (no commuting) duties without fixed working times, including gardening in public spaces; assisting children, adolescents, and older persons; and other cultural, community-based, and even scientific activities. Individuals with GBM cannot compete successfully on the job market as long as the therapeutic options do not improve significantly.

Moreover, a high number of patients returned to live with their parents, which is a highly different pattern than in other patients with cancer and might illustrate that caring for patients with GBM at the end of life is a too high of a burden for the partner of a young adult, who usually also has to work and perhaps raise children. Indeed, this survey bears all uncertainties associated with retrospective studies. The exact reasons for the unemployment and the return to the parents’ homes were not recorded prospectively. However, employment status is reflected by the social security within 1 month after any change of the employment status and address changes that are recorded for prescriptions and contact information by the hospital administration.
In conclusion, young patients with glioblastoma deserve special attention and thorough follow-up and counseling to ameliorate their situation within the limited time left by the still fatal glioma.

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References


