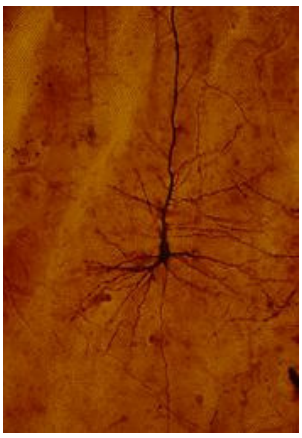
Simplified Methods

in Brain Tumor Biopsies and Diagnostics



Tor Brommeland

Department of Neurosurgery
Institute of Clinical Medicine
University of Tromsø
Tromsø, Norway

2007

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Tromsø, April 2007

2 LIST OF PAPERS

I. **A new procedure for frameless computer navigated stereotaxy**. Brommeland T, Hennig R. Acta Neurochir (Wien) 2000;142:443-448

II. **Mechanical accuracy of a new stereotactic guide**. Brommeland T, Hennig R. Acta Neurochir (Wien) 2000;142:449-454

III. **Does imprint cytology of brain tumours improve intraoperative diagnoses?** Brommeland T, Lindal S, Straume B, Dahl IL, Hennig R. Acta Neurol Scand 2003;108:153-156

IV. **Serum levels of glial fibrillary acidic protein correlate to tumor volume of high-grade gliomas.** Brommeland T, Rosengren L, Fridlund S, Hennig R, Isaksen V. Accepted for publishing, Acta Neurol Scand

3 ABBREVIATIONS

AA:	Anaplastic astrocytoma
AFP:	Alfa feto-protein
CBV:	Cerebral blood volume
CNS:	Central nervous system
CSF:	Cerebrospinal fluid
CT:	Computer tomography
EGFR:	Epidermal growth factor receptor
GBM :	Glioblastoma multiforme
GFAP:	Glial fibrillary acidic protein
HCG:	Human chorionic gonadotropin
LGG:	Low-grade glioma
MBP:	Myelin basic protein
MRI:	Magnetic resonance imaging
ODG:	Oligodendroglioma
PCNSL:	Primary CNS lymphoma
PDGF:	Platelet derived growth factor
PNET:	Primitive neuroectodermal tumor
UNN:	University Hospital of North Norway
WHO:	World Health Organization

4 INTRODUCTION

4.1 Historical perspectives

Even though reports of cranial surgery dates back to the B.C. era, modern intracranial tumor surgery commenced in the 1800's. William Macewen removed the first intracranial meningioma in 1879 and in 1884 Rickman J. Godlee performed the first resection of a glioma in a 25-year-old patient.[1,2] Victor Horsley, Harvey Cushing and Walter Dandy later introduced the curved skin flap, various surgical approaches to anatomical structures of the brain and the pneumoencephalograhy.[1,3] In Norway, Vilhelm Magnus performed the first operation on a deep-seated tumor in the left cerebral hemisphere in 1903.[4] Magnus, being the only neurosurgeon in the country for 25 years carried out more than 200 intracranial procedures with a mortality rate of 8.1%.

Further development in the neurosurgical field included the stereotactic frame. The Horsley-Clarke frame was introduced in the early 1900's for the purpose of animal research. In humans, stereotactic procedures were carried out after ventriculography was integrated with a frame system in 1947 by Spiegel and Wycis.[5] Though originally applied for lesion surgery on mental disorders and pain relief, stereotactic frame-based biopsies of brain tumors were later established.[6] The advent of computer tomography (CT) and magnetic resonance imaging (MRI) opened the possibility for frameless stereotaxy using computers with pointing devices for pre – and per-operative planning and navigation. Frameless computer based neuronavigation is now widely used in brain tumor surgery for biopsies, surgical approaches and resection of neoplasms.[7-10]

The precise diagnosis of a brain lesion has always relied on microscopic examination of tissue. The historical path leading to modern histological techniques has been long and cumbersome: Problems with tissue fixation were overcome when formalin replaced alcohol in the mid 1800s. The research undertaken by the commercial dye industry eventually lead to the use of haematoxylin and eosin among pathologists. As to intraoperative diagnoses, frozen sectioning became greatly improved with the advent of the cryostat in 1938 enabling thin slices of tissue to be prepared.[11] Dudgeon

and Patrick published a paper in 1927 describing the method of imprint cytology for fast diagnosis of tumors.[12] Today, these techniques are widely used for rapid diagnosis of tumors.

4.2 Classification and epidemiology of primary intracranial tumors

Primary intracranial tumors arise from the brain, meninges, cranial nerves, the pituitary and blood vessels. Based solely on histology and immunohistochemical criterias the World Health Organization (WHO) classification system is the most widely used.[13] This system differentiates tumors based on the cells of origin: Neuroepithelial, perhiperal nerves, meninges, haemopoietic system, germ cells and cells of the sellar region. A malignancy grading scheme of I, II, III and IV is commonly applied to distinguish benign or low-grade tumors from atypical or anaplastic lesions.

Astrocytes, oligodendrocytes and ependymal cells are the most common origin of primary brain tumors.[14] The sub-classification of astrocytomas further divides these neoplasms into diffuse astrocytomas, anaplastic astrocytomas (AA) and glioblastoma multiforme (GBM).[13] Diffuse astrocytomas correspond to WHO grade II and include fibrillary, protoplasmatic and gemistocytic astrocytomas. These tumors are often grouped together and referred to as low-grade gliomas (LGG). GBM may arise from anaplastic transformation of LGG or AA (secondary GBM) or directly, “de novo” (primary GBM). The pilocytic astrocytoma, pleomorphic xanthoastrocytoma and subependymal giant cell astrocytoma make up a distinct clinical and pathological entity within this group of tumors.

Oligodendrogliomas (ODG) represent a diagnostic challenge to the neuropathologist as differentiation between this neoplasm and astrocytomas may be difficult. These tumors share histological features and mixed morphology is often encountered with varying degrees of oligodendrocytes and astrocytes throughout the sampled tissue.[15] To date, the WHO criterias are based only on the histologic verification of tumor tissue even though genetic profiling detecting allelic loss of 1p and/or 19q may add important information as this is considered a genetic characteristic of ODG.[15,16]

Meningiomas grow from arachnoid cap cells of the paccionian granulations and constitute 24% of all intracranial tumors in the adult population.[17] The vast majority of these tumors are benign (WHO grade I), with atypical (grade II) and malignant (grade III) variants occurring in approximately 6% and 1,5% of the cases, respectively.[18]

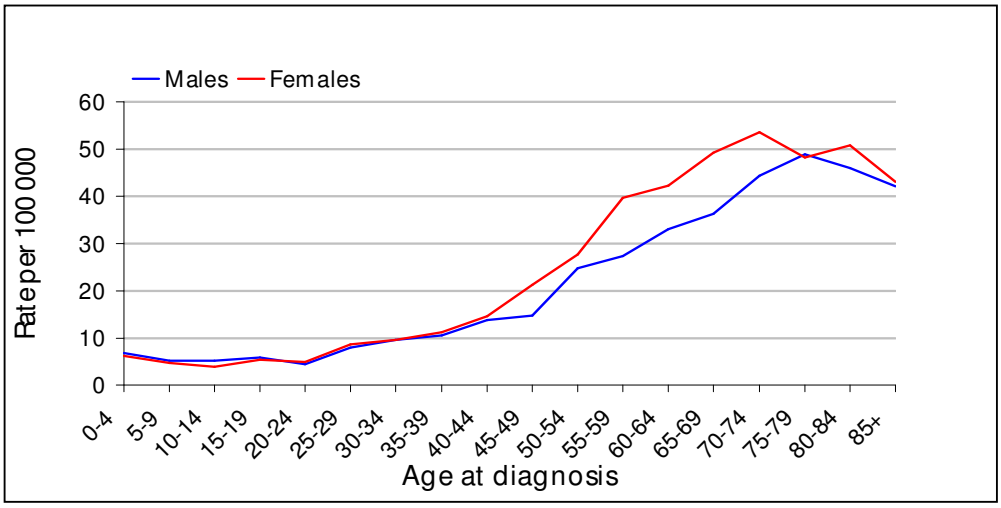

Figure 1. Age-specific incidence rates of tumors within CNS, meninges, cranial nerves and peripheral nervous system, 2000-2004, Norway. Malignant and benign. *Cancer Registry of Norway, 2004.*

The overall incidence of primary intracranial tumors is approximately 12 per 100.000 person-years (Figure 1).[17,19,20] Of these, roughly 50% are tumors of glial origin of which GBM is the most common in the adult population constituting 23-28% of all primary intracranial tumors.[17,21] Age-specific incidence of brain tumors have been demonstrated with an increasing trend in children and patients older than 50 years during the period of 1970-1999.[20,22,23] The etiology of this is not fully understood but improved imaging techniques (CT and MRI) seem to explain a major part of the increase in the elderly population.[24,25]

Brain neoplasms are the most common solid tumors in the pediatric population and account for approximately 20% of all cancers in children.[26,27] Astrocytomas constitute 42% of these followed by PNET (26%), and ependymomas (11%).[26]

A higher incidence of primary CNS lymphoma (PCNSL) has also been reported over the last 20 years.[28,29] This neoplasm now makes up 6% of all primary brain tumors in some materials, which is a 3-time increase compared to earlier figures.[13,28,29] The higher number of immunocompromised patients seen in the 1980's and 1990's due to the AIDS epidemic contribute to this development.[28,29]

Males have a significantly higher rate of neuroepithelial tumors and lymphomas than females. Meningioma represents the only primary intracranial tumor of which females dominate.[17] Apart from ionizing radiation, no other known risk factors are associated with intracranial neoplasms. Contrary to some beliefs, the use of cell phones do not seem to be of concern.[30,31]

Prognosis varies considerably depending on type of tumor. GBMs are among the most aggressive with a mean survival of approximately 12 months despite surgical resection, radiotherapy and chemotherapy.[32] Even though most patients with high-grade gliomas have a dismal prognosis, long-time survivors are seen in all histologic groups and illustrate the heterogeneity of these tumors.[33] In contrast, ten-year survival rates of patients with meningiomas are higher than 80%.[34]

4.3 Tumorogenesis

Alterations in the genetic expression of normal cells are essential and often the initial events leading to a neoplastic lesion. In GBM, some of the principal mechanisms have been identified and may serve as a model of which primary brain tumors develop.[35-37] A few of these will be described here as they have clinical relevance in terms of diagnosing the neoplasms and represent future treatment targets.

4.3.1 Oncogenes and tumor suppressor genes

The tumor suppressor gene p53 plays an important role in cell-cycle arrest. Mutations or loss of this gene seem to promote uncontrolled cell division that, together with overexpression of platelet-derived growth factor (PDGF) ligands and receptors, participate in the development of low-grade gliomas.[38] Inactivation of the p53 gene seem to be a major factor in anaplastic transformation from low-grade to high-grade

gliomas.[35] Similarly, mutations in the PTEN gene (*P*hospatase and *te*nsin homology) have been demonstrated in approximately 40% of high-grade gliomas while the epidermal growth factor receptor (EGFR) gene is the most frequently overexpressed oncogene in astrocytic tumors overall.[13] Genetic alteration of chromosome 10 is present in as many as 75-95% of all GBM.[13,35]

Though primary and secondary GBM have identical histopathology their genetic expression differ: Secondary GBM are characterized by p53 mutations together with overexpression of PDGF as opposed to primary GBM where amplification of EGFR is increased and p53 mutatations are rare.[35-39] This type of genetic profiling has identified subgroups of GBM and ODG having prognostic and clinical impact.[40-42] The discovery of chemosensitive patients with ODG harboring allelic loss of 1p and/or 19q has resulted in routine genetic profiling when this histological diagnosis is encountered.[40,43] Similar findings in AA and GBM illustrate the heterogeneity of these tumors and may partially explain why some patients become long-term survivors despite their aggressive disease.[33]

Knowledge of the genetic alterations that take part in high-grade gliomas has encouraged new therapeutic approaches. Using adenovirus as vectors, "healthy" p53 genes have been injected into GBM tumors by means of a stereotactic procedure.[44] O6-methylguanine DNA methyltransferase (MGMT) status is another example of how genetic mapping may individualize patient treatment.[45] These techniques illustrate future treatment of patient with brain tumors: The integration of modern neurosurgical and oncological expertise based on the histological and genetic expressions of the tumors.

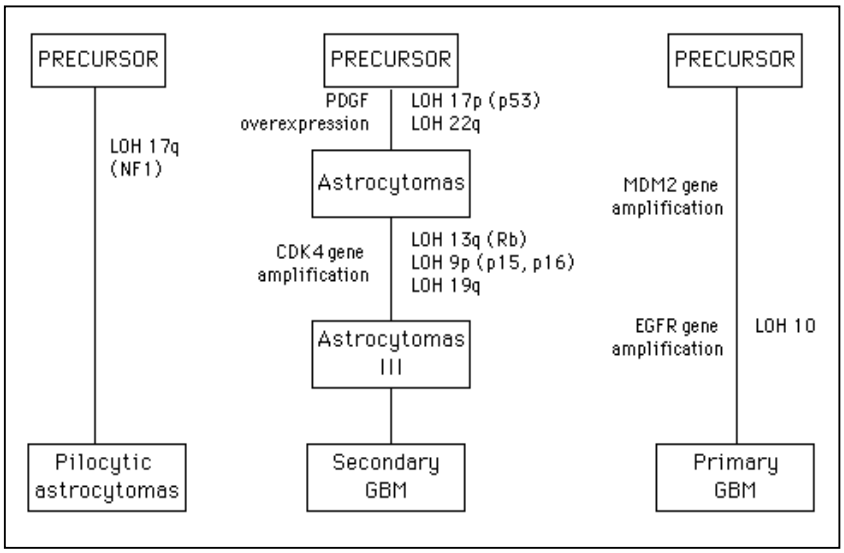

Figure 2. Suggested molecular pathways in astrocytoma formation. LOH; Loss of heterozygosity; NF1; Neurofibromatosis 1. *From Ng and Lam, Pathology, 1998*

4.3.2 Angiogenesis and invasion

The high degree of vascularisation in GBM is obvious both on MRI studies and histologic investigations. Malignant tumors are fast growing and areas with hypoxia may arise. This process is a major trigger for upregulation of receptors and ligands that together stimulate formation of new blood vessels. Such receptors are found in increased numbers on the surface of endothelial cells while tumor cells produce and secrete ligands that stimulate these receptors.[46,47] Vascular endothelial factor (VEGF) produced by tumor cells in a paracrine regulation is significant in this process: Binding of this ligand to the corresponding receptor stimulates angiogeneses and induces increased vascular permeability.[46] The latter process contributes in the development of vasogenic edema surrounding these neoplasms. Studies have shown the use of steroids to downregulate VEGF production and reduce vascular permeability.[48,49] Further tumor growth is mainly along the white matter tracts but can also be seen in cerebrospinal fluid (CSF) and along blood vessels. Tumor cells secrete proteolytic enzymes that destroy cell-to-cell bindings in normal tissue thus facilitating the spread of malignant cells.[50,51]

4.4 Imaging techniques

MRI has become the primary imaging modality for brain tumors and is now widely used in the pre –and postoperative evaluation of these patients.

Conventional MRI with or without contrast medium is the investigation of choice when suspecting a brain lesion.[35] However, this imaging modality has limitations: The findings are often not specific as gliomas, metastasis, lymphomas, abscesses and infarction all may present ring-like contrast enhancement and surrounding edema.[52] In addition, studies have demonstrated that the degree of contrast enhancement poorly correlates to the histologic grade of gliomas.[53-55] In a clinical setting this MRI technique alone has a limited diagnostic yield.[52]

Perfusion MRI measures blood flow to a tumor and the surrounding brain tissue. The most commonly used parameter is regional cerebral blood volume (rCBV) which evaluates the amount of blood passing through a specified region of the brain. The technique enables to a certain extent glioma grading, differentiation of metastasis and high-grade gliomas, selection of an appropriate target for stereotactic biopsy and defininition of tumor margins.[54,56]

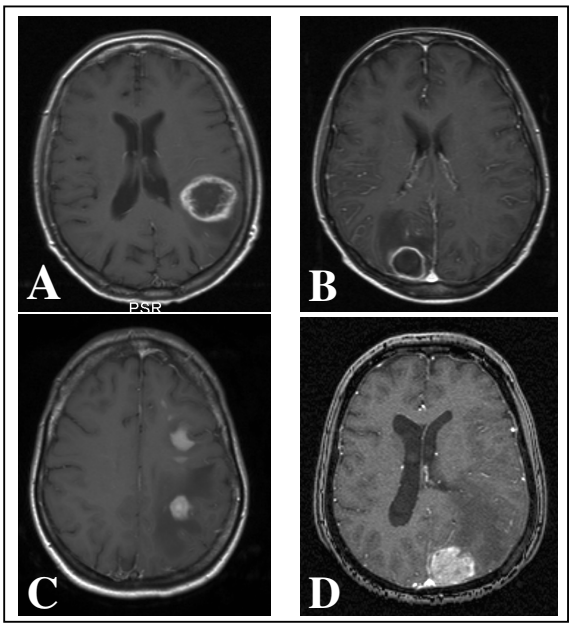

Figure 3. Conventional T1-weighted MRI scans of *A*. Glioblastoma multiforme; *B*. Abscess; *C*. Primary CNS lymphoma; *D*. Metastasis from breast cancer.

Tissue concentrations of the metabolites choline, creatine and N-acetyl are estimated with MR spectroscopy. These concentrations are graphically displayed and ratios calculated relative to creatine (Figure 4). This MR modality opens the possibility of distinguishing between high-grade gliomas, solitary metastasis and even PNET.[57-60]

Radiation induced necrosis and recurrent GBM may appear similarly on MRI scans and represent a challenge even for perfusion MRI and MR spectroscopy [56,61]. Positron emission tomography (PET) can resolve part of this problem though the sensitivity is arguable.[62,63] This technique measures metabolic activity in tumors and may even be used to trace the activity of genes introduced in GBM.[64]

Most of these imaging studies are retrospective and designed to investigate threshold levels of specified parameters in order to attain an acceptable sensitivity and specificity. Even though various imaging modalities may be complementary and contribute to diagnosing a brain lesion, the clinical value is still partially undetermined for some of the techniques: A prospective clinical study on 100 patients with newly diagnosed brain tumors demonstrated that MR spectroscopy contributed in only 6 of these cases regarding the pre-operative diagnosis.[65]

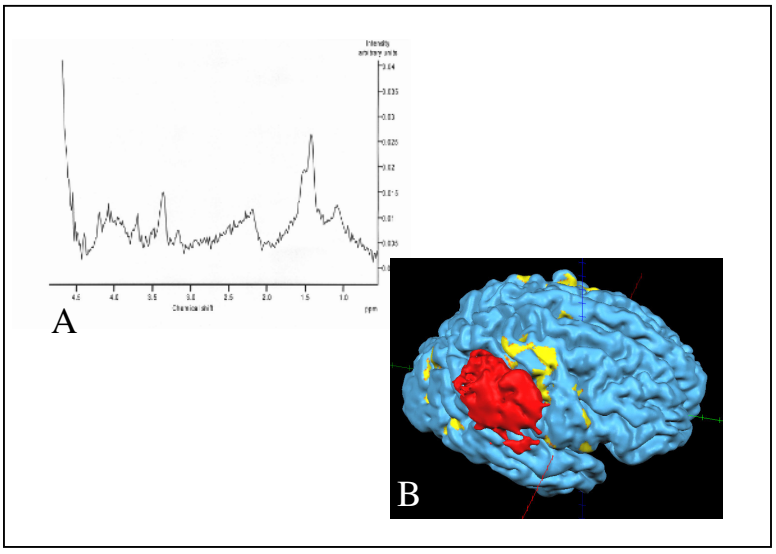

Figure 4. *A*. MR spectroscopy of a primary CNS lymphoma showing abnormal spike-pattern, *B*. Functional MRI (fMRI) of a low-grade glioma (red) during finger movements (yellow).

4.5 Surgery on brain tumors

Basically, two types of surgery may be performed: Craniotomy or a stereotactic biopsy through a burr hole. In cases of glial tumors, controversy still exists whether resection of these tumors contributes significantly to overall survival. A number of studies with different end results have been performed over the past decades.[66-72] These investigations have been criticized because of their retrospective design and analytical flaws.[72,73] To date, no large, prospective, randomized trial has been carried out to investigate the role of surgical treatment in glioma patients. Similarly, patients with a single brain metastasis may benefit from cytoreductive surgery in combination with whole brain radiotherapy in terms of prolonged functionally independent survival though overall survival has not been shown to improve.[74] Meningiomas represent the other end of the scale as these tumors often may undergo gross total resection with high rates of long-term survival.[34]

Even though MRI and PET techniques are capable of distinguishing between several types of brain tumors, surgical intervention in order to obtain relevant tissue for histologic investigations remain the primary diagnostic modality. In cases of brain abscesses, puncture and culturing of sampled material provides a microbiological diagnosis. Stereotactic biopsies using frame-based systems are well documented in terms of precision, high diagnostic yield and low rate of complications. [75-79]

Stereotactic computers are now widely applied in neurosurgery after the introduction in the early 1990's. These systems are considered a helpful tool in pre-operative planning as well as assistance in placing a craniotomy, intra-operative navigation and defining resection borders of tumors.[7,8,80,81] At the Department of Neurosurgery, University Hospital of North Norway (UNN), a stereotactic computer has been used instead of a frame for various intracranial procedures since the mid-1990's.[9] In order to take full advantage of the navigational abilities, a skull-mounted guide system was developed at the department (Figure 5). This system is coupled with a stereotactic computer and permits puncture of intracranial mass lesions as well as introduction of ventricular catheters. The guide system was retrospectively evaluated and the results compared to stereotactic frames after application in 36 patients for a total of 39 procedures *(Paper I).* The mechanical accuracy of the system was later studied on a

phantom model using three different entry points to targets localized within a 3-dimensional co-ordinate system *(Paper II)*.

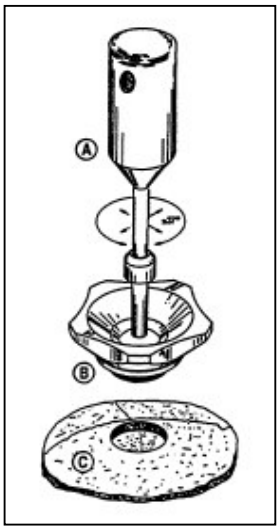

Figure 5. Schematic drawing of the stereotactic guide system. *A*. Computer connected probe; *B*. Stereotactic guide; *C.* Skull with burr hole.

4.6 Laboratory investigations

Morphologic investigations of tumor tissue remain the gold standard for diagnosing brain tumors. A final diagnosis is commonly based on post-operative histologic evaluation of paraffin embedded material. However, intraoperative diagnosis of a brain lesion is a valuable tool in several cases:[82] 1) Verification of adequately sampled tissue in stereotactic biopsies of brain tumors; 2) Differentiation between normal and neoplastic tissue for definition of resection borders; 3) Deciding whether surgical treatment should be continued in cases were the pre-operative diagnosis is undetermined, i.e. abscess, PCNSL or GBM.

Traditionally, frozen sections are most frequently used in the intraoperative setting even though imprints and smear preparations have well documented diagnostic accuracy [83-85]. Imprint cytology was introduced as an intraoperative supplement to frozen sections at the Department of Pathology, UNN in 1999. A retrospective study was carried out to investigate whether the combined use of frozen sections and imprint cytology improved the intraoperative diagnostic accuracy (*Paper III*).

"Biomarker" is a term used to describe the measurement of a substance associated with a condition or disease process.[86] Biomarkers of malignant processes are few in general and almost non-existent in the setting of primary brain tumors. Important exceptions are alfa feto-protein (AFP) and human chorionic gonadotropin (HCG) in the pre-operative evaluation of suspected germinal cell tumors or neoplastic processes of the pineal gland.[87] Various markers of CNS pathology have been studied both in cerebrospinal fluid (CSF) and serum but the clinical implications remain uncertain. In patients with cerebral tumors, a biomarker may find its use in the post-operative phase

in order to monitor treatment or detect relapse of the tumor. As to date, only serial neuroradiological imaging may detect tumor re-growth.

Recent publications have demonstrated several potential markers in serum for glial tumors but some of these lack specificity for the CNS [86,88-90]. Glial fibrillary protein (GFAP) is an intermediate filament protein of the astrocytic cytoskeleton and considered to be specific to the CNS. Increased levels of this protein in CSF and serum have been demonstrated in various neurological conditions.[91-95] However, the expression of GFAP in serum have not to date been investigated in patients with glial tumors. In *Paper IV*, pre-operative serum concentrations of GFAP were measured in patients with high grade gliomas (WHO grade III and IV) and the levels correlated to clinical, radiological and histological variables.

5 AIMS OF THE THESIS

1. To evaluate the clinical accuracy and safety of a new stereotactic guide system when applied for biopsies of intracranial mass lesions.

2. To determine the mechanical accuracy of this stereotactic system.

3. To estimate the stereotactic computer system error.

4. To assess the intraoperative diagnostic accuracy of frozen sections and imprint cytology of brain neoplasms.

5. To investigate whether choice of surgical procedure (craniotomy versus stereotactic biopsy) affected intraoperative diagnostic accuracy.

6. To investigate serum levels of glial fibrillary acidic protein (GFAP) in patients with high-grade gliomas and its correlation to clinical, histological and radiological parameters.

6 MATERIAL AND METHODS

The 337 patients constituting this thesis were patients referred to the Neurosurgical department, UNN between 1995 and 2005. The patient selection is presented in Figure 6.

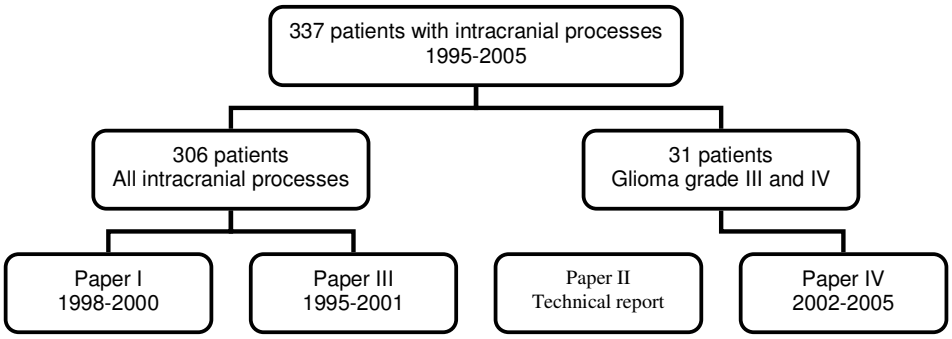

Figure 6. Patient population and selection, 1995-2005.

6.1 Paper I

Over a period of two years, 36 patients with intracranial mass lesions were diagnosed by stereotactic biopsies. Three patients were operated twice for a total of 39 procedures. Mean age and range were 52 years and 15-82 years, respectively. The biopsies were carried out using a newly developed guide system connected to a stereotactic computer through a passive, articulated arm with a sensor probe (Figure 5). Volumetric 2 mm T1-weighted, contrast enhanced MR images were used for all procedures.

In a retrospective study the following parameters were registered and analyzed: Type of anesthesia, operating time, size and location of target, biopsy depth, histological and microbiological findings, estimated computer error after registration, and complications.The results were evaluated and compared to published data on similar frame-based procedures.

6.2 Paper II

In order to determine the mechanical accuracy of the frame-less system a phantom model was developed. The model consisted of a coconut shell with a spherical target located inside a Cartesian three-dimensional co-ordinate system. The coordinates of the target center was x=0, y=0 and z=0. Three burr holes were created as entry points for the stereotactic guide and approaches from these defined: Left, right and midline. For registration and navigation, both MR and CT images were used. The accuracy of the registration procedure was calculated by the computer and defined as the root mean square error (RMS error). The biopsy procedure described in *Paper I* was simulated by establishing a trajectory to the target and inserting the biopsy needle to a computer-calculated depth. The position of the needle tip was defined by the corresponding x, y and z values in the co-ordinate system. The mechanical accuracy of the procedure was found by calculating the distance between target center and needle position for each trial. This so-called *Euclidian distance* in space can be found by $D=[(X_{target}-x_{needle})^2 + (Y_{target}-Y_{needle})^2 + (Z_{target}-z_{needle})^2]^{1/2}$.

Using two MRI sequences and one CT scan, a total of 182 and 60 measurements were performed, respectively. In addition, the error of the stereotactic computer was estimated by placing the probe tip on the target center and registering the visual position on the computer. The distance between target center and probe tip on the computer was measured and defined as the computer system accuracy.

A normal distribution of data was found and students t-tests applied for statistical analysis. The spread of observations was estimated using the coefficient of variance (COV) defined as COV=SD/ME where SD=standard deviation and ME=mean error. COV is a mathematical figure of which a value of <1 in this setting signifies a clustering of observations.

6.3 Paper III

Intraoperative diagnoses of brain tumors are important as they may affect ongoing surgery. In 1999, imprint cytology was introduced as a routine technique and supplement to frozen sections at the Department of Pathology, UNN. A retrospective

study was carried out in order to 1) investigate whether the supplemental technique resulted in improved intraoperative diagnostic accuracy and 2) whether choice of surgical procedure (craniotomy versus stereotactic biopsy) affected this accuracy. Between 1999 and 2001, 153 patients were diagnosed by both imprint cytology and frozen sections (patient group B). The consecutively last 153 patients diagnosed prior to 1999 were used as control group (patient group A). Intraoperative diagnoses were compared to the final results of paraffin embedded material in both groups.
Chi-square and kappa statistics were used for statistical analysis. The kappa value is a statistical measure of agreement between two independent variables with a range of –1 to 1. In clinical settings, the value of 1 signifies perfect agreement while values < 0.4 indicate only moderate agreement better than chance.[96]

6.4 Paper IV

Pre-operative serum samples of 31 patients with newly diagnosed GBM and AA were analyzed for GFAP and S-100B. The serum levels were correlated to clinical data (patient age, sex, steroid use), radiological findings (tumor size and location) and histological analysis (Ki-67 labeling index) by transformed linear regression analysis. Tumor size was estimated by planimetry: The area of the contrast enhancing regions on each digital MRI slice was calculated with a computer software tool and the corresponding volume found by multiplying this area with the slice thickness. These image volumes were summed to find the gross total tumor volume. Ki-67 staining was performed and the number of positive cells counted at 400x magnification in ten visual fields. The area with highest mitotic activity was selected for the analysis and the Ki-67 labeling index defined as the fraction of positive cells.

7 RESULTS

7.1 Paper I

The registration procedure performed pre-operatively was carried out using anatomical landmarks only with an average procedure-related time of 10 minutes. The mean computer error (RMS) after registration was 2.0 mm (range 0.7-4.7 mm). Mean time of surgery was 60 minutes (range 40-120 min) including patient positioning, registration procedure and a histologically confirmed biopsy. Local or general anesthesia was used in 25 and 14 cases, respectively. Mean tumor diameter was 4.6 cm (range 1.5-10.0 cm) with a mean biopsy depth of 4.0 cm (range 0.5-7.5 cm). A histological or microbiological diagnosis was established in all cases except two resulting in a diagnostic yield of 95%. Tumors of glial origin were the most common finding comprising 25/34 neoplasms (74%). In the two inconclusive cases tissue analysis showed necrosis, gliosis or cystic components indicating that the lesions had been reached but further investigations did not establish a final diagnosis.

There were two cases of complications (5.1%): In one patient presenting with an intracerebral abscess puncture triggered further growth of the lesion and a second operation had to bee performed for drainage and installation of antibiotics. In the second case the patient experienced a grand-mal seizure when the dura was electrocoagulated. Both patients recovered to their habitual state.

7.2 Paper II

The overall computer system accuracy was 1.0 mm with both MR and CT images. For the MRI based trials, measurements demonstrated a clustered but skewed positioning of the needle tip when using the right sided approach while CT based navigation revealed no significant difference in accuracy between the approaches. The mechanical accuracy of the system was 3.8 mm using MRI and 2.9 mm with CT (t-test, $p<0.001$). Coefficient of variation (COV) was <0.5 for both the MRI and CT studies signifying a clustering of the observations. The results for the mechanical accuracy measurements are summarized in Table 1.

Table 1. Summary of results for mechanical accuracy measurements

	Mean values (mm)					
Image	**n**	**RMS**	**Error ± 2SEM**	**TD**	**95% CI**	**COV**
MR	182	1.24	3.8 ± 0.3	58 (54-64)	3.5-4.1	0.43
CT	60	0.92	2.9 ± 0.2	61 (58-66)	2.7-3.1	0.31

RMS Root mean square; *ME* Mean error; *SEM* Standard error of the mean; *TD* Trajectory distance; *CI* Confidence interval; *COV* Coefficient of variation.

7.3 Paper III

A total of 117 craniotomies and 36 stereotactic biopsies were performed in patient group A (diagnosed prior to 1999). In group B, 100 craniotomies and 53 stereotactic biopsies were carried out. Tumors of glial origin were the most common findings constituting 44% in group A and 49% in group B. Overall intraoperative diagnostic accuracy improved from 87% to 91% after introduction of imprint cytology (χ^2-test, $p>0.05$). Though a tendency towards greater accuracy was seen when the tissue samples were based on resected material rather than a stereotactic biopsy the difference was not significant. Similarly, kappa-values were high in both groups signifying a high degree of agreement between the intraoperative and final diagnosis. Results are summarized in Table 2. The number of delayed intraoperative diagnosis declined significantly from 30 (20%) in group A to 8 (5.2%) in group B. (χ^2-test, $P<0.001$).

Table 2. Results of intraoperative diagnoses according to patient group and surgical procedure.

	Group A			Group B		
Procedure	**n**	**Correct/Incorrect**	**%**	**n**	**Correct/Incorrect**	**%**
Craniotomy	117	103/14	88	100	94/6	94
Stereotactic biopsy	36	30/6	83	53	45/8	89
Total	153	133/20	87	153	139/14	91

7.4 Paper IV

Mean serum levels of GFAP and S-100B were 239 ng/L and 58.3 ng/L, respectively. Mean tumor volume was 29.9 cm^3. Steroid administration was given prior to surgery and blood sampling in 22 patients for a mean period of 4.8 days. Mean Ki-67 LI was 0.17 with a range of 0-0.42. Multivariat linear regression analysis revealed a significant association only between serum GFAP and tumor volume ($p<0.001$) with a Spearman correlation coefficient of $r=0.67$. Assuming a linear correlation between GFAP and tumor size a cut-off point was found at 20 cm^3. Mean GFAP concentrations for patients with tumor volumes greater than this was 398 ng/L (n=17) compared to 47.9 ng/L (n=14) for smaller lesions ($p=0.0002$, Mann-Whitney U test).

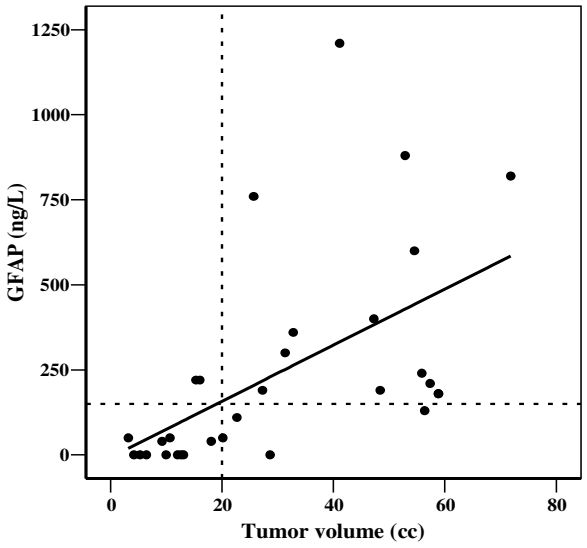

Figure 7. Scatter plot of serum GFAP levels and tumor size. Reference level of 150 ng/L marked with horizontal line. Cross-section of "best-fit" line and reference level marked with vertical line.

8 DISCUSSION

8.1 Surgical perspectives

Space-occupying brain lesions represent a diagnostic challenge to the clinician due to the multitude of differential diagnoses: Neoplasm, infection, vascular or inflammatory/immunological disorders and unrecognized trauma.[97] As demonstrated in section 4.4, imaging technologies such as MRI and PET have improved the accuracy of non-invasive diagnoses. Without these newer techniques, pre-operative diagnoses have been shown incorrect in more than 30% in some series.[98-100] This is

especially true in cases of glial tumors since lack of contrast enhancement on MRI cannot rule out a high-grade lesion. [55,100] Even though some authors question the need for tissue diagnostics of brain lesions,[101] a major part of the literature support the necessity of brain biopsies for correct diagnoses.[97,98,102-104] Traditionally, stereotactic brain biopsies have been carried out using frame-based systems. Indications vary between neurosurgical institutions, but some of the most common are listed in Table 3.

Table 3. Commonly suggested indications for stereotactic biopsy of brain lesion.[102,105]

Indications for stereotactic biopsy	**Examples**
Lesion not amenable for open surgery	Tumor in or near eloquent areas
Patient medical status preclude open surgery	Elderly, heart or lung disease
Multiplicity	Metastatic disease, HIV
Tumor diagnosis unlikely or unresolved	Abscess, PCNSL

PCNSL; Primary CNS lymphoma

The diagnostic yield (percent of cases where a final diagnoses is established based on tissue analysis) of these procedures is usually between 92-99%.[79,103,104,106] The size of biopsies affect this figure in some studies, while others do not report such a trend.[79,102,104] The number of biopsies are important: Brainard et al.[107] showed that sampling up to four biopsies from the same target location increases the diagnostic yield from 67% to 89% in a series of 185 patients using frozen section evaluation.

The mortality rate is less than 1% in the larger series of stereotactic biopsies, death most often being caused by hemorrhage or diffuse edema.[104,108,109] In a review of the literature encompassing 7471 biopsies, Hall et al.[109] found the overall morbidity rate to be 3.5%. Post-operative morbidity may be transient or permanent with paresis, visual field deficits and epileptic seizures being among the most frequently reported complications.[110] Clinically silent hemorrhages after a biopsy may be seen in more than 50% of the patients but seldom affect patient management.[111] Risk analysis have identified lesion location, lesion histology, chronic steroid use (>3 months) and antiplatelet agent intake < 48 hours prior to surgery as independent predictors of

clinically relevant morbidity.[105,110] Hyperglycemia in diabetic patients increased the risk of neurological deficits three-fold with glucose levels above 20 mg/ml having a positive predictive value of 100% for complications in a study by McGirt et al.[110] However, in a similar investigation Sawin et al.[105] did not find diabetes as a significant predictor of morbidity. In this study the number of biopsy attempts was also noted as a factor influencing the risk of neurological complications. The number reported in this study is surprisingly high (mean of 22 biopsies per patient in the morbidity group) and differs significantly from others.[103,104,110] Contrary to many belifs, patient age, type of anesthesia, hypertension or surgeon experience do not increase the risk of post-operative morbidity.[105,110] The significant predictors of complications are summarized in Table 4.

Table 4. Independent risk factors of post-operative morbidity in two patients series

Risk factor	**Odds Ratio (OR)**
Antiplatelet agents (<48 hrs prior to surgery	35.0 [a]
Steroids (>3 months)	13.9 [a]
Deep location (basal ganglia and thalamus)	4.7;3.29-4.06 [a,b]
High-grade glioma	4.0 [a]
Diabetes mellitus	3.73 [b]

[a]Sawin et al. [105], [b]McGirt et al. [110]

The introduction of stereotactic computers in the early 1990's made frameless stereotaxy possible. These commercially available systems have proven accurate and reliable through extensive laboratory testing and clinical experience.[7,8,112-114] Parallel to this, frameless stereotactic biopsies of intrinsic brain lesions have developed and in some instances made the use of frame-based systems obsolete: A variety of devices for stereotactic guidance have been presented in the literature.[10,115-118] All of these systems utilize the navigational abilities of a stereotactic computer with rigid, guidance of a biopsy needle by locking a set trajectory from entry point to the target of interest. Some of the techniques employ a flexible arm in order to line up the trajectory while others depend on a skull-mounted system. In the latter case the burr hole is often larger than that made for arm-based systems which often use a twist drill. However, the advantage of a large burr-hole is a greater range of motion through the

same opening with the possibility of biopsies from multiple trajectories. This can easily be done by altering the computer determined trajectory without any further planning as demonstrated in Paper I. Such is not the case in the frame-based setting where new targets have to be selected, calculated and mechanically plotted before actually performing the biopsy.

In frameless stereotactic procedures the temporal separation of imaging and surgery enables scanning of the patient at almost any given time before the operation. However, this is mostly true when anatomical landmarks are used for the registration procedure rather than skin fiducials. Fiducials seem to result in improved registration accuracy compared to anatomical landmarks but limits the time span between imaging and surgery.[7,119] As presented in Paper I, anatomical landmarks only were used and found to be sufficient and convenient especially in non-cooperative patients. The registration errors (RMS errors) reported by Golfinos et al.[7] using fiducials are greater than those obtained with anatomical landmarks in our study. The stereotactic computer was the same in both investigations (*The Viewing Wand*®, ISG Technologies). It is important to bear in mind that the registration error not necessarily reflects the actual localization accuracy as this depends on the number and geometric spacing of the registration points. Since the head of a patient is not spherical and registration points not perfectly geometrical, volumes of greater or less accuracy than the registration error may be found.[120,121]. At our institution, a total of 10 surface locations are most often used (ears, lateral orbital edges and nose). In addition, a surface-fit is performed at the end of the registration as this gives an even distribution of a high number of registration points and a low RMS error (this was not done in the patients constituting Paper I). However, a technical study on a phantom model reported that the addition of such surface points ("surface fit") in fact increased the degree of error.[119] This has not been our experience in the clinical setting and the surface fit is today a routine part of our registration procedure.

Complication rates, tumor histology and localization, target depth, diagnostic yield and operating time were registered in Paper I and found to compare favorably with similar studies.[116,118,122,123] The total time spent on the procedure relies heavily on whether general or local anesthesia is applied: In the study by Smith et al.[124] both operating and in-hospitalization time was significantly less for patients biopsied with

a stereotactic frame than with a frameless system due to the high number of procedures with local anesthesia with the former technique. However, both the pre – and per-operative times for the frameless part of this study are considerably longer than in our study and what is generally reported in the literature where frameless biopsies show clear advantages.[10,116,118,122,123,125]

Disadvantages apply, of course, to frameless stereotactic procedures. Some of the first stereotactic systems applied an articulated arm that could be sensible to displacement during surgery.[9] The newer systems using wireless LED registration have eliminated this but are vulnerable to disturbances in the line-of-sight as equipment or personnel may interfere with the signals. The degree of accuracy is a continuous matter of concern and debate. Limited results of biopsies in the posterior fossa have been reported with frameless systems. This is probably caused by increased skin mobility creating greater registration errors.[116]

Frame-based systems have proven very reliable both for biopsies and functional surgery though disparate to many beliefs accuracy with these systems is not sub-millimetric. In the landmark study of four different frame-based systems, Maciunas et al.[126] showed that the mechanical accuracy was 2.28 mm and demonstrated a linear correlation between application accuracy and CT slice thickness. This report also emphasizes the distinction between mechanical accuracy, which is the optimal performance of the system, and application accuracy. The latter signifies the "real life" setting where a multitude of factors influence accuracy.

This application accuracy of frameless stereotactic guidance systems have been investigated usually by simulating a biopsy procedure on a phantom model.[115,120,127] These studies demonstrate mean application accuracies between 1.0-4.8 mm using MR images on the stereotactic computer. In the series by Quiñones-Hinojosa et al.[127] reporting an error of 1.0 mm, a probe rather than a biopsy needle was used to optimize system accuracy. Henderson et al. [115] found a mean accuracy of 1.25 mm which was significantly better than the two frame-based systems used for comparison. The reported accuracy was however the combined results of MRI and CT series and the authors do not report the accuracy of the separate measurements. In Paper II, MR and CT images resulted in mean errors of 3.8 mm and 2.9 mm, respectively which

positively compares to other reports.[115,120,127] In our study, accuracy measurements using the right approach generated significantly poorer results when using MRI sequences on the computer. CT based measurements showed no such tendency and distortion of the magnetic field was found to be the most likely explanation for this.[128] This may be questioned as Quiñones-Hinojosa et al.[127] in a very similar study did not demonstrate any difference in accuracy between their different approaches with MR images.

Paper I and II demonstrate that frameless stereotactic biopsies can be carried out safely, effectively and accurately. Frameless navigation techniques represent an alternative to stereotactic frames and have now also been applied in functional neurosurgery with good results.[129]

8.2 Intraoperative diagnosis

Intraoperative diagnoses are usually based on frozen section histology or cytological investigations, the latter being composed by smear and imprint cytology. The diagnostic accuracy of these methods is most often displayed as the number or percentage of cases where the intraoperative diagnosis is consistent with that of paraffin embedded material. The definition of agreement between these two diagnosis vary in the literature but the distinction between primary and secondary tumors as well as neoplasms and inflammation is essential.[83,130,131] The heterogeneity of gliomas makes classification of high and low grade tumors challenging.[132] For clinical purposes distinction between grade II and grade III-IV is probably most relevant in the per-operative setting. In Paper III, complete agreement between the two diagnoses was demanded with the exception of distinguishing AA from GBM. The intraoperative diagnosis was reported as the combined analysis of cytological and frozen sections in patient group B since the neuropathologist examined both preparations and independent observations could not be claimed. The intraoperative diagnostic accuracy improved after the introduction of cytological investigations though not to a significant level. However, the number of cases where the intraoperative diagnoses were noted as “uncertain” declined. This may reflect an increased confidence or certainty of the neuropathologist as the diagnoses reported to the operating room were based on two supplementary tissue investigations. Similar

results were obtained by Martinez et al.[84] increasing their diagnostic accuracy from 88% to 95% by using cytology in addition to frozen sections. The overall diagnostic accuracy of 91% in our study is in agreement with similar reports regardless of preparation methods.[83,84,131,133,134] In both our patient groups, diagnoses were based on specimens from open resection or stereotactic biopsy. The small sample size is a well described problem with stereotactic biopsies.[132] However, we did not find a significant difference in intraoperative diagnostic accuracy between biopsies from open and stereotactic procedures even though a trend towards poorer results was seen in the latter group. This issue has also been addressed by others: Woodwoorth et al.[135] and Feiden et al.[136] compared the final diagnosis based on stereotactic brain biopsies with that of resected material in patients with glial tumors. The final diagnosis based on a stereotactic biopsy accurately represented the greater lesion in 76-89% of cases. In the remaining cases, the WHO grade was underestimated, a problem also documented by others.[137] Jackson et al.[132] reported a 38% discrepancy in diagnosis between stereotactic biopsies and later resected gliomas. The erroneous diagnosis could have affected treatment in 26% of the patients and prognosis in 38%. Although these results are disturbing this study represents a small subset of gliomas from a specialized center focusing on gliomas in or near eloquent areas of the brain. The reported trend of lower malignancy grade in stereotactic specimens compared to open resections may raise the question of whether the appropriate samples had been collected from the contrast-enhancing parts of these tumors.

The combined use of frozen sectioning and cytology are the most frequently applied intraoperative techniques among neuropathologists.[83] Even though Reyes et al.[131] found frozen sections to be superior to cytology in terms of intraoperative diagnostic accuracy the sole use of cytology techniques have reported acccuracy rates between 76-90%.[83,84,131,133,138] The two distinct methods for tissue preparations both have advantages: Cytology provides the opportunity to study single cells and details of the nucleus while frozen sections generate better impression of the tissue composition and vascular proliferation.[84,130,139] Examples of this are shown in Figure 8.

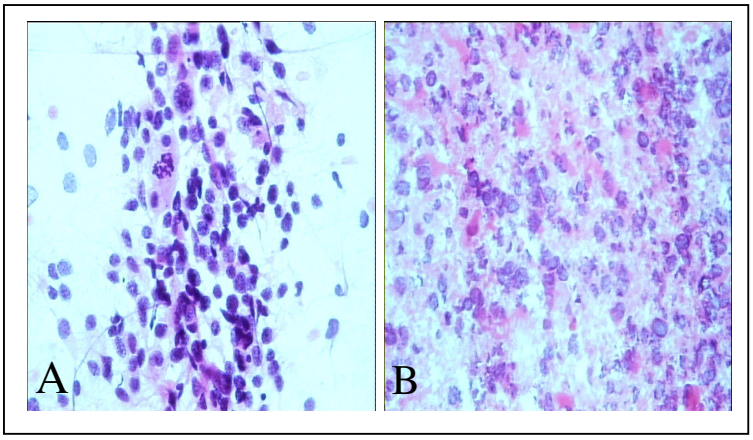

Figure 8. Imprint cytology (*A*) and frozen section (*B*) of a GBM.

When considering the tissue handling, cytologic investigations come in handy when the samples are small or potentially hazardous, i.e. in patients with HIV, tuberculosis or Creutzfeldt-Jakobs.[83,140] Hard or rubbery specimens leave few cells on the object glass and frozen sections may in these cases be the only valid technique.[131] Most centers seem to recommend the combined use of cytology and frozen sections for improved intraoperative diagnostic accuracy, which is in line with our experience and what was reported in Paper III.[83,131]

8.3 Serum investigations

Pathological processes within the CNS may cause increased levels of various biochemical markers either in cerebrospinal fluid (CSF) or serum.[141-145] Lack of diagnostic specificity make routine investigations for such biomarkers of limited value in the clinical setting. This is especially true for patients with brain tumors; No known biomarker has specificity for malignant disease within the CNS and several markers have proved to be of no value.[146,147] However, in some brain neoplasms such as pineal or germ cell tumors, PCNSL or pediatric tumors, biomarker evaluation may be helpful as part of a pre-operative diagnostic work-up.[87,148-150]

Recent publications have identified proteins in serum with promising applications for glioma patients even though some of these markers lack specificity for the CNS.[86,88,90,151] Paper IV investigates the pre-operative serum levels of S-100B and

glial fibrillary protein (GFAP) in patients with AA and GBM. S-100B was chosen for comparison as this protein is among the most thoroughly studied biomarker in CSF and serum.To our knowledge, this is the first report of serum GFAP measurements in glioma patients. GFAP was elevated in 16 of 31 patients and showed a significant correlation with tumor volume. For S-100B, no such trends were seen and only two patients had pathological levels of this protein. A linear correlation between tumor volume and GFAP levels was indicated with a Spearman coefficient of 0.67 (Figure 7). This is in line with reported results on patients with ischemic lesions and serum measurements of GFAP. [95,152]. A tumor volume of 20 cm^3 represented a cut-off point at which patients with larger tumors had significantly higher GFAP levels than the smaller lesions. Assuming tumor sphericity this volume correlated to a lesion with a diameter of 3.4 cm.

Though the S-100B levels reported in our study are markedly lower than those reported by Vos et al.[153] and Kanner et al.[154], these studies included glioma patients already treated with surgery, radiotherapy and/or chemotherapy. This may affect the measured levels as destruction of brain parenchyma or the blood brain barrier cause leakage of proteins to serum.[155]

The wide range of serum GFAP concentrations cannot be fully explained by tumor volume as some patients with large lesions had low serum levels of the protein. This may reflect the heterogeneity of these neoplasms as demonstrated in both survival times and varying histopathological expressions.[156,157] Whether the increased serum GFAP levels signify disruption of the blood brain barrier or astrocytic destruction remain largely unexplained, as demonstrated in S-100B studies.[154,158]

To date, no ideal biomarker of glial tumors have been identified even though some investigations are encouraging.[86,88,90,151] These proteins as well as GFAP are not commercially available for measurement as of today and analysis are confined to a research setting. The clinical application is undetermined but most authors advocate the possibility of monitoring brain tumor patients with serum tests as a supplement to, or instead of, serial neuro-imaging. In a theoretical setting, serum tests could be used for early detection of recurrence in order to commence treatment, for instance with temozolomide.[159] GFAP measurement in serum has no diagnostic value as this protein

is a non-specific marker of CNS injury. Further studies are needed in order to investigate whether GFAP analysis is clinically applicable in glioma patients.

9 SUMMARY AND REFLECTIONS

The incidence of brain tumors is increasing in an aging population, possibly due to improved imaging techniques.[24] Tissue sampling of brain lesions for histologic and immunocytochemical investigations remain the gold standard in order to establish a reliable diagnosis. In addition, genetic analyses of brain tumors have inspired new treatment options and contributed to our understanding of the underlying patophysiology.[44,160] In the future, we will probably rely more on genetic analyses of tumor tissue than today. Hence, the need for surgical techniques offering safe, accurate and simple ways of sampling tissue for various investigations will not decline in the coming years. This is important to bear in mind in a time when MRI has become almost our sole technique in the diagnostic work-up of a brain tumor.

Stereotactic frames have for years provided both the accuracy and safety required in this process. Various investigations disagree as to whether the frameless, computer neuronavigation represent a true clinical improvement.[124,125] However, experience with stereotactic computers is increasing and so is the documentation of safety, accuracy and versatility of these systems. [7,113-116] We have found the temporal spacing of imaging and surgery with anatomical landmarks especially valuable as this allows easier planning and reduces the need for co-ordination between operating room and the MRI lab. In the majority of intracranial mass lesions, we feel that stereotactic biopsies with a frameless system provide the required accuracy and safety.

A fast and reliable intraoperative tissue diagnosis supplies the neurosurgeon with important information: Intraoperative diagnostics verifies that the target lesion has been reached in a stereotactic biopsy. In the case of tumor resection further surgery may be halted or continued based on the neuropathologist's report. The introduction of imprint cytology did not significantly improve the intraoperative diagnostic accuracy in our institution. However, these methods seem to be complementary with distinct advantages and the combined use of these has become routine at our hospital.

Investigations of serum proteins in patients with brain tumors have so far contributed little. We have shown that patients with large high-grade gliomas seem to release GFAP to serum with a linear correlation between tumor size and serum levels of the protein. The results indicate that this protein serve as a potential biomarker in patients with high-grade gliomas. GFAP and other biomarkers may represent a simple method of evaluating on-going treatment or detect recurrence but further studies should be performed to investigate this in detail.

The final diagnosis of a brain lesion is already the combined results of neuro-imaging and tissue investigations. The team effort of a neuro -radiologist, -surgeon and -pathologist will remain essential also in the future as diagnostic methods and treatment options develop.

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