Frameless stereotactic biopsy for precision neurosurgery: diagnostic value, safety and accuracy

Tommaso Sciortino[1,2] M.D., Bethania Fernandes[3] M.D., Marco Conti Nibali[1,2] M.D., Lorenzo G. Gay[1,2] M.D., Marco Rossi[1,2] M.D., Lopci Egesta[4] M.D., Anna E. Colombo[3] M.D., Maria G. Elefante[3] M.D., Federico Pessina[1,5] M.D. and Lorenzo Bello[1,6] M.D., Marco Riva[1,7] M.D.

1. Unit of Neurosurgical Oncology, Humanitas Research Hospital, IRCCS, Milano, ITALY.
2. Università degli Studi di Milano, Milan, Italy.
3. Unit of Pathology, Humanitas Clinical and Research Hospital, Rozzano, Milan, ITALY.
4. Unit of Nuclear Medicine, Humanitas Research Hospital, Rozzano (MI), ITALY.
5. Department of Biomedical Sciences, Humanitas University, Rozzano (MI), ITALY.
6. Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy.
7. Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, ITALY.

Corresponding Author: Marco Riva, M.D. Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan (MI), ITALY; Humanitas Research Hospital, Via Manzoni 56, 20089 – Rozzano (MI). E-mail: marco.riva@unimi.it

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Abstract

Background: Stereotactic biopsy is consistently employed to characterize cerebral lesions in patients who are not suitable for microsurgical resection. In the past years technical improvement and neuroimaging advancements contributed to increase the diagnostic yield, the safety and the application of this procedure. Currently, in addition to histological diagnosis, the molecular analysis is considered essential in the diagnostic process to properly select therapeutic and prognostic algorithms in a personalized approach. The present study reports our experience with frameless stereotactic brain biopsy in this molecular era.

Methods: 140 consecutive patients treated from January 2013 to September 2018 were analyzed. Biopsies were performed using the Brainlab Varioguide® frameless stereotactic system. Patients' clinical and demographic data, the time of occupation of the operating room, the surgical time, the morbidity and the diagnostic yield in providing a histological and molecular diagnosis were recorded and evaluated.

Results: The overall diagnostic yield was 93.6% with 9 procedures resulting non-diagnostic. Among 110 patients with glioma, the IDH-1 mutational status was characterized in 108 cases (98.2%), resulting wild-type in all subjects but 3; MGMT methylation was characterized in 96 cases (87.3%), resulting present in 60 patients and 1p/19q codeletion was founded in 6 of the 20 cases of grade II-III gliomas analyzed. All the specimens were apt for molecular analysis when performed. Bleeding requiring surgical drainage occurred in 2.1% of the cases; 8 (5.7%) asymptomatic hemorrhages requiring no treatment were observed. No biopsy-related mortality was recorded. Median length of hospital stay was 5 days (IQR 4-8) with mean surgical time of 60.77 minutes (±23.12) and 137.44 ±24.1 minutes of total occupation time of the operative room.

Conclusions: Stereotactic frameless biopsy is a safe, feasible and fast procedure to obtain a histological and molecular diagnosis.

Key words: Glioma, molecular markers, frameless biopsy, diagnostic yield, IDH1

Introduction

Stereotactic biopsy is a robust technique, representing one of the most important and minimally invasive procedure to characterize lesions of the central nervous system in vivo [8, 31, 35].

Since its inception, this technique progressively developed from frame-based [2, 3] to frame-less (or *less framed*) setup [4–6] with the aid of instrument holders, trajectory guides, lockable or robotic arms [7, 24] to grant accurate targeting and to ease the workflow.

Despite the frame-based technique is still considered the gold-standard for a stereotactic approach to the brain, frame-less devices progressively evolved. Frame-based techniques are considered troublesome by several factors, such as frame structure, patient's discomfort, imaging after frame placement, calculations of the entry point, prolonged surgical time and risk of infection at the frame's fixture points [34]. Therefore, frame-less procedure have been becoming a useful choice for their easiness of use and comparable diagnostic yield [1, 16].

Neuroimaging advancements further benefited the stereotactic approach, contributing to increase both the diagnostic yield and the safety by allowing accurate planning and intraoperative check of sampling at the correct target with several approaches [11, 32, 45]. Both morphological and metabolic imaging, such as conventional and advanced MRI [14, 39] and positron-emission tomography (PET) with dedicated radiotracers [26]can now be co-registered and uploaded in the navigation system and used as image-guidance to target the most informative lesion area and thus grant an optimal diagnosis.

In fact, when microsurgical resection is not indicated, stereotactic biopsy is crucial for obtaining a definite histopathologic diagnosis in order to select the appropriate therapeutic modality for a specific patient and his/her pathology.

In addition to histopathology, recent refinements of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) established the need of stratification through molecular features [19, 27, 37] for a conclusive diagnosis. In particular, the status of isocitrate dehydrogenase (IDH) 1 and 2, the co-deletion of complete chromosome arms 1p and 19q and the methylation status of the O^6-methylguanine-DNA-methyltransferase (MGMT) gene are the most relevant molecular markers for glioma characterization [9, 18, 19]. As therapeutic options become increasingly depending upon molecular features for both clinical and experimental management, an accurate and precise integrated histo-molecular diagnosis is thus mandatory, especially in a time where *precision medicine* looms.

Previous studies explored the feasibility, safety, experimental and clinical accuracy of a frame-less system with a lockable arm with real-time visual feedback of the target area [38] when diagnosis for

glioma was based on a previous WHO classification exclusively based on histological features . We herein reported a study expanding previous experiences of the stereotactic biopsy in the updated context of a molecular era of neuro-oncology. In particular, this study was conceived to assess the performance of the frameless stereotactic biopsy in providing tissue samples appropriate to meet both the histological and molecular demands of the updated diagnostic criteria of the 2016 WHO CNS tumors classification.

Materials and Methods

Subjects

One hundred and forty (140) adult subjects affected by a lesion of unknown etiology affecting the brain not amenable of microsurgical resection, as established by the consultant neurosurgeons either independently or during weekly institutional neuro-oncology group discussions, were prospectively enrolled from January 2013 to August 2018 [**Figure 1**]. All patients signed an informed consent for the procedure. No patients underwent open surgical treatment or radiation therapy before the bioptic procedure. Demographic, clinical and pathological features were collected, along with surgical and pathological data [Tables 1- 3]. Histology was classified according to the 2016 WHO brain tumor classification [27].

Neuroradiological Protocol and Image processing

MR imaging was performed on a 3 Tesla MR scanner (Siemens Verio, Germany), as previously described [40]. Lesion volumes were computed onto volumetric sequences with a semiautomatic segmentation method using iPlan Cranial 3.0 (Brainlab AG, Munich, Germany). 11-C-MET-PET was available for "hot-spot" identification in 49 patients (35%), serving as additional hint for the appropriate selection of the target [26]. The pre-operative MR and PET imaging dataset was co-registered with a CT scan, where 7 radiopaque fiducials were applied. CT and MRI were performed within 24 hours of surgery. The co-registered datasets were uploaded to the neuronavigation system and registration was based on fiducials. A post-operative CT scan was performed to rule out any acute complication.

Biopsy targets and entry points were planned on MRI with contrast enhancement and 11-C-MET-PET hot spots, when available. In order to avoid larger vessel damage, trajectories were controlled for any crossing vessels in contrast enhanced volumetric MR images. Targeted lesion volumes and trajectory length, from dura mater to the target, were measured.

Frameless Stereotactic Biopsy

Patients were operated on general anesthesia. After placement in the 3-point Mayfield head clamp, the procedure was performed under navigation guidance with on-site planning. The surgical plan (entry point, biopsy target, and needle trajectory) was determined using Brainlab navigation software module within the Cranial application. After accuracy of the system was confirmed, a burr hole was placed, and

biopsy samples were obtained with image-guidance using Brainlab Varioguide® frameless stereotactic brain biopsy system; a pre-calibrated needle with 2 reflective markers is inserted through the lockable stereotactic arm with 3 rotational joints, serving as trajectory guide. The navigation system provided a real-time visual feedback of the position of the sampling window; the system is a not-rigid device allowing to change the trajectory in any moment during the procedure, if needed **[Figure 2].**

All tissue samples were verified by a pathologist attending the operating room. In each procedure, the samples extraction was continued until the quantity and the quality of the tissue taken were considered suitable for definitive analysis by the pathologist. Time of occupation of the operating room and the surgical time, from skin incision to suture, were recorded. Both histological and molecular features were collected and stored prospectively. No retrospective pathological re-assessment of previously acquired samples were performed.

Statistical Analysis

The statistical analysis and the collection of data were performed with IBM SPSS Statistics 22.0 for Mac software (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± standard deviation (SD) or median plus the range between the minimum and the maximum value or the interquartile range (IQR). Continuous variables were compared with a Student's t-test and categorical variables were compared with the Fisher exact test. We considered, as statistically significant, a two-tailed P-value < 0.05.

Results

Demographic and lesions characteristics

One hundred and forty patients (73 males, 67 females) underwent the stereotactic frameless procedure. Mean age was 58.6 years (±15.3). Median performance status (KPS) was 90%, ranging from 40-100%. Median length of hospital stay was 5 days (IQR 4-8). Lesion sites are reported in **Table 1** and lesion volumes are reported in **Table 2**.

Frameless stereotactic biopsy

Targets were selected onto MRI. The mean trajectory length was 46.04 mm (±32.54) and ranged from 8.5 to 140 mm, measured from cortical entry to the target. A single needle pass was used for sequential biopsies taken along the trajectory in all cases but 3; 84 (60%) and 56 (40%) cases were approached from the left and right side, respectively. Nine posterior fossa lesions (6.4%), in particular, were approached through a retro-sigmoidal burr-hole.

The surgical procedure lasted 60.5 minutes on average (±23.1), measured from skin incision to complete suture. The time spent in the operating room, measured from patient entry to the exit, was 137.44 ± 24.1 minutes.

Integrated Histo-molecular diagnosis

The overall diagnostic yield was 93.6%: a definitive histological diagnosis was obtained in 131 patients. Grade IV, III and II gliomas were reported in 76 (69.1%), 15 (13.6%) and 6 (5.5%) cases, respectively; glioma not otherwise specified (NOS) was reported in 12 (10.9%) subjects. A B-cell Non-Hodgkin lymphoma was diagnosed in 13 (11.8%) patients; 2 (1.8%) cases of abscess and 2 of germ-cell tumor were reported. One metastasis from melanoma and a colloid cyst were also diagnosed. The final pathology report resulted inconclusive in 9 cases (6.4%). In this group, 5 out of 8 patients had a 11-C-MET-PET available for the biopsy planning. No differences ($p>0.05$) were recorded in the diagnostic yield dividing the sample by gender, median age, lesion site and 11-C-MET-PET availability.

Considering the new classification of the WHO, the power to characterize 3 relevant molecular determinants of gliomas, such as IDH status, 1p and 19q codeletion and MGMT methylation, was also analyzed when applicable. The IDH-1 status was characterized in 108 cases (98.2 % in the glioma group), resulting wild-type in all subjects but 3; MGMT methylation was characterized in 96 cases

(87.3%), resulting present in 60 patients, and absent in 36 cases. 1p/19q codeletion was founded in 6 of the 20 cases of lower grade gliomas (i.e. grade II and III) where the material provided by the biopsy was successfully analyzed in 100% of the cases.

Morbidity

An asymptomatic intracerebral hemorrhage (ICH), detected by routine post-operative CT scan and requiring mere observation, occurred in 8 (5.7%) patients. A symptomatic ICH requiring surgical drainage occurred in 3 cases (2.1%), with no permanent neurological dysfunction at follow-up. No in-hospital or 30-day mortality were recorded. There was not statistically significant correlation between the number and the occurrence of surgical complication and the patient's sex, age (using the median age of 60yr) and the location of the hemispherical tumors (superficial versus deep location).

We did not observe any statistical significant difference between the numbers of complication in patients underwent surgery from January 2013 to April 2016 (6 cases) and patients underwent the procedure from April 2016 to July 2018 (2 cases).

Discussion

The aim of stereotactic biopsy is to provide the diagnosis of a cerebral lesion of unknown etiology in an easy, safe and fast way. In the present study, we reviewed all the biopsies performed in our institute from January 2013 to August 2018. We analyzed the safety and the diagnostic yield according to the new 2016 WHO CNS tumors classification of the frameless stereotactic biopsy using the Varioguide system to provide essential histological and molecular features. Bradac et al, in a recent prospective and randomized study, showed that the frameless biopsy procedure has the same trajectory accuracy, rate of complications and diagnostic yield of the frame-based technique that is still now considered the gold-standard. Although they showed that the frameless biopsy is better accepted and tolerated by the patients [10], a conclusive argument about tolerance to either frame-based or frameless technique is still controversial.

In the past years a lot of studies showed the importance of adding the molecular data to the histological and morphological evaluation for a better prognostic and therapeutic characterization of patients with tumors of the central nervous system. The data supporting these evidences were so strong that the molecular markers are currently essential in the new 2016 classification of the central nervous system tumors [27]. In particular, to characterize a glial tumor, the pathologist employs the status of mutation of IDH1/2, the 1p/19q codeletion and the MGMT promoter methylation [9, 18, 19, 26, 37, 42]. Therefore, we focused our analyses on the histological result of the glial lesions and related molecular investigations, thus expanding previous findings of the performance of the stereotactic biopsy in contemporary neuro-oncology[29].

We obtained a diagnostic yield, defined as "the likelihood that a test or procedure will provide the information needed to establish a diagnosis" [22], of 93.6%. This data is comparable to others reported by different authors [17, 20, 33, 38, 44, 45] . For example, Khatab et al [22] reviewed 16 different works in which 1628 frameless biopsy procedures were analyzed whit an average diagnostic yield of 93.8% (range 87-100%). Similar result are reported, for example, by Lu et al [28] with a diagnostic yield with frameless biopsies of 91.8%, also without any statistical significant difference in comparison to frame-based biopsies (96.9%), and intraoperative MRI guided biopsies (89.9%). In addition, these results independently confirming previous results of the application of stereotactic frameless biopsy in the context of the 2016 WHO CNS tumors classification, thus providing more evidence for supporting the clinical practice[29].

We did not find any demographic or lesion characteristics associated more likely to a diagnostic yield; in particular there was no association with the gender, median age, lesion site. We did not found any

statistically significant association even with the availability of 11-C-MET-PET for "hot spot" targeting and the diagnostic yield. [25, 36] The use of stereotactic PET may increase the diagnostic yield of brain biopsy, like showed in several previous studies, but our data doesn't' show this correlation. The lack of association between the diagnostic yeld and all the demographic, tumoral and availability of PET can be likely explained by the relative low prevalence of non-diagnostic procedure in our semple. Khatab et al showed that patients younger than 30 years were more likely to obtain a non-diagnostic biopsy. Other reported predictive factors for non-diagnostic biopsies were right-sided lesions, long surgical time and the number of biopsy for single patient [17]. All the procedures were performed with a pathologist attending the operating room. Dammers et al, in fact, showed that the intraoperative frozen-section analysis statistically improved the diagnostic yield, the number of biopsies needed and the operating time [16]. Although a control group was not available, the high diagnostic yield in the current series is further determined by the ability to easily adjust the trajectory according to the visual feedback provided by the navigation platform and by the frameless setup of the stereotactic arm, in cases where the pathologist does not find the sample appropriate for final diagnosis.

Among the 110 patients with a histological diagnosis of glioma, the specimen was useful to characterize the status of the mutation of IDH1/2 in 108 patients (98.2%) and in 103 patients it was wild-type. This shows that even in the molecular era, a needle biopsy is perfectly able to provide the correct amount of tissue useful for a molecular analysis. The status of mutation of IDH1 is an important prognostic factor and in the current tumor classification is the first characteristic that is used to correctly classify the glioma lesions [27].

The analysis of the 1p/19q codeletion was performed according to the clinical, radiological and pathological characteristics. In our study, the 1p/19q codeletion was found in 39 patients with a presumptive glioma and the analysis was successfully performed in all the specimen analyzed. The presence of the 1p/19q codeletion is a good prognostic factor [12, 13] and drive the pathologist to the diagnosis of an oligodendroglial tumor with different implication in term of post-surgical adjuvant treatment and prognosis.

The analysis of the status of methylation of the of the MGMT promoter gene was performed in 94 patients with a presence of 60 patient with a methylated promoter and 34 patients without methylation. The information derived from the analysis of the MGMT promoter methylation status from small-sized specimen obtained by stereotactic biopsies are reliable and it can be considered a representation of the whole tumor tested, as showed by Grasbon-Frodl et al [21] in patients undergoing multiple biopsies of the same tumor. Similarly, different tumor regions show an homogeneous distribution and concordant

findings in the detection of the codeletion of the 1p/19q [23, 43] with misleading results only in presence of significant contamination of the sample (for example by blood or other contaminant).

In our experience, the frameless stereotactic biopsy resulted a safe technique: we had only 12 (8.7%) cases of surgical complication in 140 patients and only 3 (2.1%) of them required a surgical intervention. In 1 case, we observed a cerebral abscess at the piking site successfully treated with medical therapy. We also observed 8 small asymptomatic hemorrhage detected only with the CT scan routinely performed after the procedure. These findings are in line with other work where the rate or complication vary from 0% to 20% [15, 22, 28, 44]. Analyzing possible cause of complication, we did not find any correlation with age, KPF, site of tumor, tumor volume or other patients or tumors characteristics (all the $p > 0.05$). This data likely stems from the limited number of adverse events in our simple that cannot allow to reach the statistical significance. In fact, as showed by Malone et al. [30], analyzing big registry with more than 7.500 patients is possible to find some characteristic associated with hemorrhagic adverse events like old age, presence of edema, hydrocephalus and other.

The mean time of stay in the operative room in our sample was 137.44 minutes (+/- 24.1) but analyzing the actually duration of the procedure it falls to 60.77 +/- 23.12 minutes. This mean time duration is similar to the other centers and series [20, 41] and is important because a short duration of the procedure is related to shorter duration of the anesthesia and a shorter exposure to infection.

We also hypothesized that the occurrence of complications could have been related to the number of total procedures carried out in a single center in a given time; however, no statistically significant difference was observed dividing the current series into two halves, ruling out the hypothesis of an effect of the learning curve onto the likelihood of complications.

Conclusion

In the era of the integrated histologic and molecular diagnosis, the treatment of glial tumors, the most common intra-axial primitive lesions of the CNS, is strongly determined to their molecular profile. When a surgical open procedure is not possible, a stereotactic frameless biopsy is an important tool in the hands of the neurosurgeon. Our data shows that the stereotactic frameless biopsy is an efficient procedure to provide a molecular diagnosis that is currently essential for the correct management of the neuro-oncological patients. This yield could become even more relevant in the near future, when multiple therapeutic approaches should become available, such as immunological or cell-based therapies.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Humanitas Research Hospital) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

Informed consent: Informed consent for stereotactic biopsy was obtained from all participants included in the study.

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Table 1

Lesions location

Site	No. (%)
Side of the entry point	
Left	84 (60)
Right	56 (40)
Cerebral Lobe	
Frontal	51 (36.4)
Temporal	23 (16.4)
Parietal	10 (7.1)
Occipital	4 (2.8)
Corpus Callosum	27 (19.2)
Diencephalon	9 (6.4)
Basal Ganglia	7 (5)
Cerebellum	9 (6.4)

Table 2
Clinical characteristics

Characteristics	
Age (%)	
<40	18 (12.8)
40-65	68 (48.6)
>65	54 (38.6)
Mean Age (range)	58.68 (17-86)
Sex (%)	
Male	73 (47.9)
Female	67 (52.1)
11-C-MET-PET available (%)	49 (35%)
Median KPS before surgery (range)	90 (40-100)
Median Length of hospital stay (IQR)	5 (4-8)
Time in OR (mean +/- SD)	137.44 +/- 24.1
Duration of surgery in minutes (mean +/- SD)	60.5 +/- 23.1
Lesions volume in cm^3 (mean +/- SD)	26.09 +/- 26.6 (range 0.56-98.83)

Table 3
Histo-molecoular and bioptic results

Histological diagnostic yield (%)	131/140 (93.6)
No. Of trajectories (%)	
1	137 (97.9)
>1	3 (2.1)
Mean Trajectories lenght in mm +/- SD	46.04 +/- 32.54 (range 8.5-140)
Histological diagnosis	
Glioma I/II/III/IV/NOS grade	1/6/15/76/12 (total 110)
Lymphoma	13
Metastasis	4
Abcess	2
Germ cell tumor	2
Unconclusive	9
Molecular diagnosis in glioma patients	
IDH-1 status obteinded (% of glioma)	108 (98,2)
Mutated	3
Wildtype	105
MGMT promoter methylation status (% of glioma)	96 (87.3)
Methylated	60
Unmethylated	36
1p/19q codelation (n° of glioma investigated)	6 (30)
Surgical Complication (%)	
Asymptomatic haemorrhage	8 (5.7)
Abscess	1 (0.7)
Surgical complication requiring surgery	3 (2.1)

Figures Caption

Figure 1

The flow chart describes the approach for newly diagnosed intra-axial lesions and the selection process of the study cohort.

Figure 2

Axial (**A**), coronal (**B**) and sagittal (**C**) post-contrast T1-weighted and 11-C-MET-PET CT scans (**D-F**) are co-registered before surgery and used for trajectory planning. The trajectory is showed (yellow), with the entry point located in the left middle frontal gyrus and the target, a deep presumptive high-grade glioma, located in the basal ganglia area. The sampling window is showed in purple and allows the surgeon to check the part of the lesion is to be taken and analyzed by the pathologist. The 11C-MET PET scans are fused to the other MRI scans to increase the likelihood of targeting the portion of the lesion with an enhanced methionine metabolism, corresponding to an increased cellular turn-over. The histological evaluation demonstrated a Glioblastoma (Grade IV WHO), IDH-1 mutated, MGMT promotor methylated (56%), proliferative fraction (Ki-67): 20%.