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ORIGINAL ARTICLE

Pathological assessment of epilepsy surgery brain tissue

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Key words

Hippocampal sclerosis • Focal cortical dysplasia • Glioneuronal tumor • Neuropathology • Seizure outcome • Epilepsy surgery

Summary

Surgical resection represents a successful strategy to achieve seizure control in patients with drug resistant epilepsy. In the last years increasing importance has been recognized to pathological substrate for epilepsy classifications and for predicting seizure and neuropsychological outcome after surgery. The current histopathological classifications of epilepsy-associated abnormalities certainly represent an amazing effort to overcome the limits of the previous classifications and constitute a formidable tool in the management of patients after epilepsy surgery. However the correct application of the recent ILAE classification systems begins with a proper epilepsy surgery technique, able to provide "en bloc" and "spatially oriented" surgical specimens and continues with the use of an appropriate pathological workup and reproducible stains. This methodological approach permits to relate the surgical outcome to the specific pathological findings, the site of the lesion, and the surgical strategy. These data are essential to an adequate preoperative patient and family counselling. Furthermore in this paper, besides the workup and the classification systems, we evidence some aspects which may be challenging and sometime misleading in clinical practice. In conclusion, a pathology based approach to epilepsy surgery is essential and might improve the interpretation of the outcomes and the comprehension of the causes of failures.

Introduction

Surgical resection represents a successful strategy to achieve seizure control in patients with drug resistant epilepsy. A broad series of lesions can be observed in these patients, including hippocampal sclerosis (HS), focal cortical dysplasias (FCD), long-term epilepsy-associated tumors (LEATs), vascular malformations (e.g., cavernomas, arteriovenous malformations), glial scars (traumatic brain injury, bleeding, perinatal infarcts, or any other ischemic insult), inflammation (e.g., Rasmussen's or limbic encephalitis) or an association of these pathologies [1].

In the last years increasing importance has been recognized to pathological substrate for epilepsy classifications [2] and for predicting seizure and neuropsychological outcome after surgery [3-6]. Furthermore a pathology based approach to epilepsy surgery might improve the comprehension of the causes of failures and possibly advance imaging-pathology correlations [7-9].

The relevance on epilepsy surgery of pathological substrate entail some effects. First of all the epilepsy surgeon should be aware of the relevance of the histopathological assessment and provide an adequate specimens for a proper pathological diagnosis.

The current histopathological classifications of HS [10] and FCD [11] certainly represent an amazing effort to overcome the recognized limits of the previous classifications and constitute a formidable tool, useful in the interpretation of seizure outcome after epilepsy surgery. These recent epilepsy classification schemes for subtype-specific clinicopathologic diagnosis are supported by evidence from peer-reviewed research studies, with demonstrated good interobserver and intraobserver reproducibility for histopathological categories [12], but pathological diagnosis must follow and strictly observe an adequate pathological protocol [13].

In order to obtain this goal it is important that an efficient

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and reliable pathology-based assessment of brain tissues is accomplished in close collaboration with the different team members, clinical and research colleagues (neurologists, epileptologists, neuropediatrics, neuroradiologists, neurosurgeons), involved in epilepsy surgery. This recommendation requires, for example, the presence of the pathologist in the operating room to document anatomic landmarks of the surgical specimen [13].

Methods and histopathological findings

All cases should be histologically diagnosed according to the WHO classification of tumors of the central nervous system [14] and the more recent classifications for HS [10], FCD [11], and granular cell pathology (GCP) [15].
The correct application of the recent ILAE classification systems begins with a proper epilepsy surgery technique, able to provide "en bloc" and "spatially oriented" surgical specimens. In fact anatomically intact tissue samples are fundamental for histopathologic examination.
Suspected lesions or other regions of interest should also be marked, such as the site of an epileptic focus determined by presurgical or intraoperative electrophysiologic recordings [13].
A small portion of surgical specimens could be unfixed and snap frozen in liquid nitrogen and long-term stored at –80 °C, to allow molecular-biological analysis [16], or collected for tissue culture procedure [17]. Obviously it is necessary to collect small samples of tissue for research to ensure that there is no difficulty to the histologic diagnosis.
The remaining tissue should always be fixed in 10% buffered formalin and embedded in paraffin.
We adopt a standardized cutting scheme using anatomic landmarks. Neurosurgeons should label the anteriorposterior or dorsal-ventral axis of each sample with staples or ink (Fig. 1, anterior temporal lobectomy). If a not adequate specimen is provided, it will probably hinder a proper pathological diagnosis (Fig. 2, cortical resection of an MRI-negative epileptogenic brain region).

Fig. 1. En bloc surgical specimen of anterior temporal lobectomy correctly oriented for pathological examination.

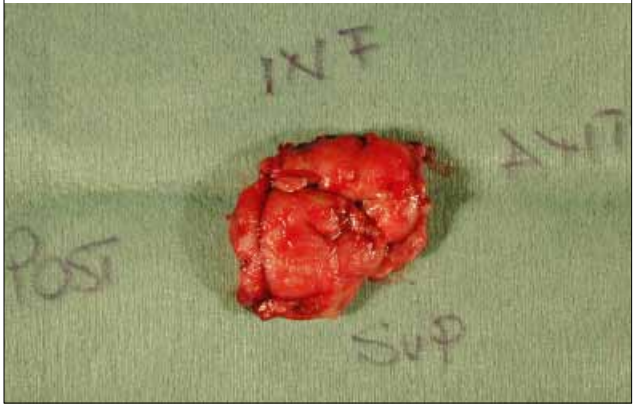
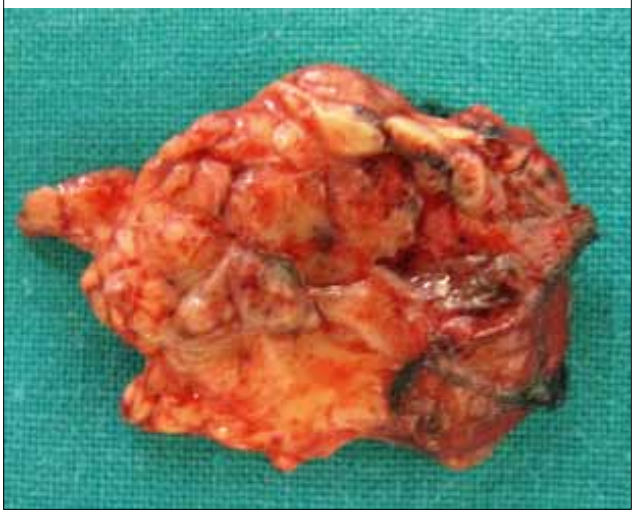
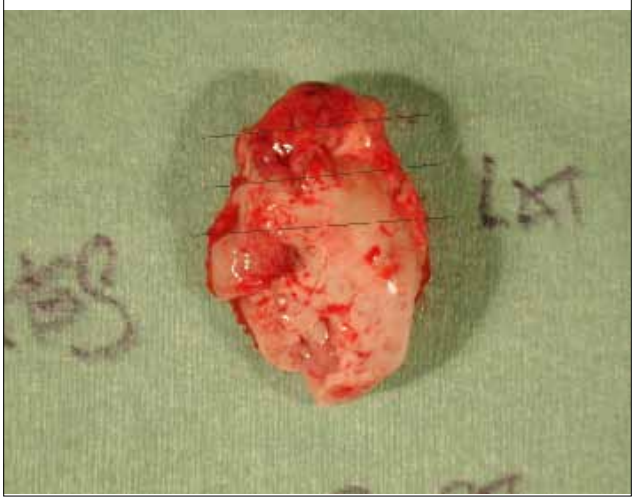


Fig. 2. A not adequate cortical specimen of an MRI-negative epileptogenic brain region.



We usually label margins with different colored inks, in order to determine whether resection borders are lesion-free, a potentially useful parameter, especially applicable to FCD and LEATs.
Systematic cutting of the hippocampal specimens is conducted into 5-mm interval parallel slabs, preferably at coronal planes along anterior-posterior axis (Fig. 3, hippocampectomy); samples from the mid-hippocampal body are particularly useful for evaluation.
Polar temporal specimens are dissected perpendicular to the pial surface in 3- to 5-mm sections, while tangential cutting must be avoided.
Hematoxylin and eosin (H&E) staining should be performed on every slice, whereas additional stains may be conducted after preselection. Paraffin sections of 4 µm are most appropriate for histochemical and immunohistochemical stains. To date it is well established

Fig. 3. En bloc surgical specimen of hippocampectomy correctly oriented for pathological examination.



that pathologic workup of human brain tissue obtained during epilepsy surgery requires a minimum set of appropriate and reproducible stains and antibody immunoreactivities, that can be utilized internationally by neuropathologists or general anatomic pathologists in most hospitals [13].

1. For the classification of HS [10] and GCP [15] NeuN represents the most valuable immunostaining [18] for depicting anatomical structures and assessing the neuronal cell loss in surgical TLE specimens (Fig. 4). At the same time stains with Luxol-fast-blue, Klüver-Barrera (Fig. 5) Nissl (Fig. 6) and GFAP may be useful and very representative.

2. For the classification of FCD [11] sections with anatomically well preserved cortical orientation including adequate white matter areas should be selected. After the preselection with H&E (Figg. 7, 8) the most useful stainings and antibodies are NeuN (Fig. 9), Luxol Fast Blue, Klüver-Barrera and Nissl. For the assessment of neuronal dysmorphism NeuN, MAP2, phosphorylated and nonphosphorylated neurofilament protein (SMI-32) (Fig. 10) may be adopted, while balloon cells can be highlighted using vimentin (Fig. 11) and CD34.

The current ILAE classification system for FCD [11] introduced, in comparison to the previously internationally adopted classification [19], a third FCD category (FCD Type III) in addition to FCD Type I (for abnormalities in cortical architecture) and FCD Type II (characterized by large and dysmorphic neurons, with or without the presence of balloon cells). FCD Type III, the new category, identifies cases where cortical lamination abnormalities are associated/adjacent with/to other lesions: HS (FCD Type IIIa), LEATs (FCD Type IIIb), vascular malformations (FCD Type IIIc), or any other lesion acquired during early prenatal or postnatal life (FCD Type IIId).

In cases suspicious for FCD Type IIIb, the areas identified as dysplastic should be carefully evaluated with

Fig. 6. ILAE HS type 1: in this case the neuronal cell loss has spared the pyramidal neurons in the CA2 subfield, while Dentate Gyrus is characterized by significant cell loss, with a reduced or disappeared granular cell layer and cell-free gaps in the horizontal direction (GCP Type 1) (Nissl, 40X magnification).

Fig. 4. ILAE HS type 1: the neuronal cell loss in this hippocampal specimen is promptly evidenced by NeuN (NeuN, 40X magnification).

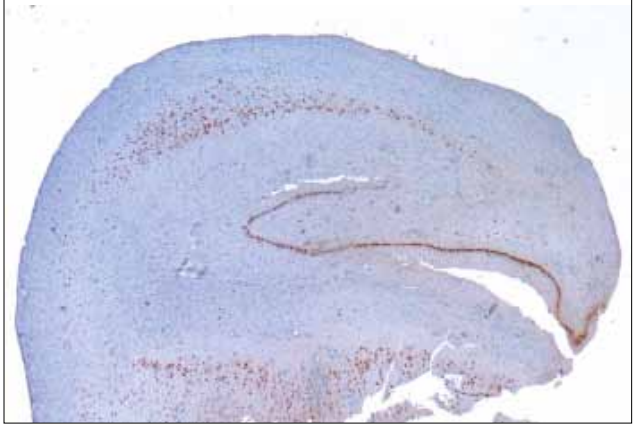


Fig. 7. FCD IIa: In many cases dysmorphic neurons may be highlighted in H&E sections (H&E, 200X magnification).

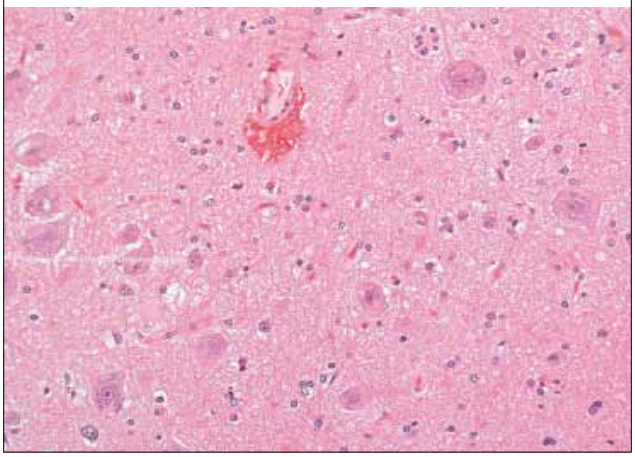


Fig. 5. ILAE HS type 1: a brisk and diffuse neuronal cell loss can be observed in all subfields of Ammon Horn, while Dentate Gyrus is preserved (no GCP) (Klüver-Barrera, 25X magnification).

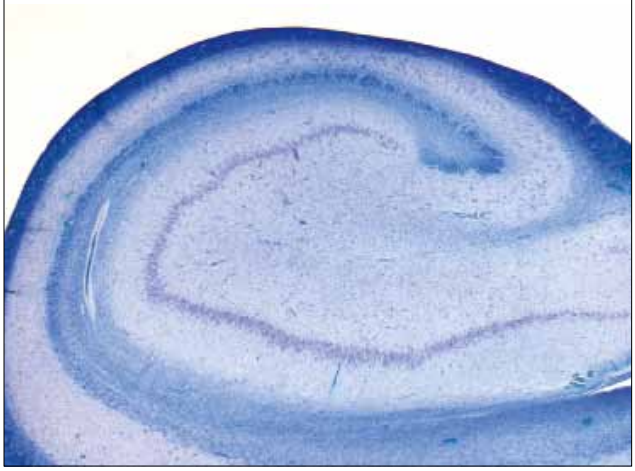


Fig. 8. FCD IIa: This high power field picture evidences dysmorphic neurons characterized by cytological abnormalities, with cell diameters and cell nucleus diameter significantly enlarged in comparison to adjacent neurons. Furthermore Nissl substance is aggregated and displaced towards the cell membrane (H&E, 400X magnification).

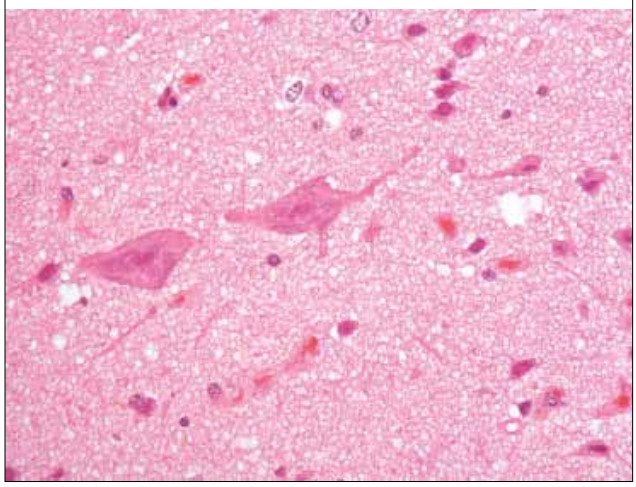


Fig. 9. Dyslamination with dysmorphic neurons in FCDIIa (NeuN, 40X magnification).

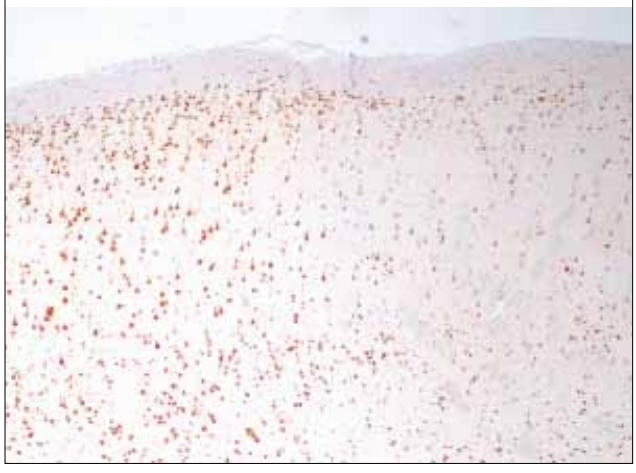


Fig. 10. FCD IIa: Phosphorylated and nonphosphorylated neurofilament isoforms accumulate in the cytoplasm of dysmorphic neurons (SMI-32, 200X magnification).

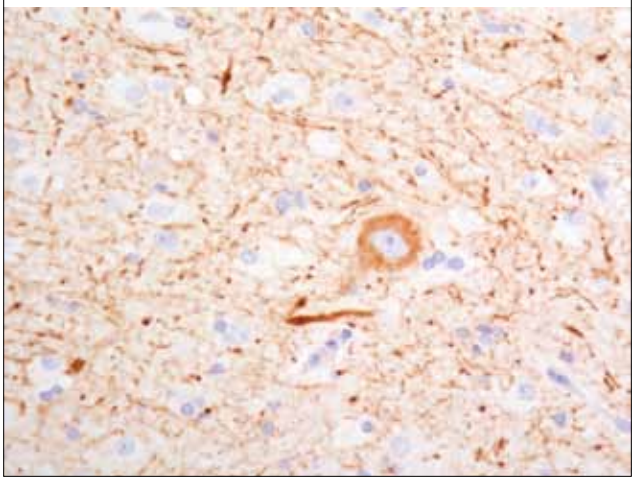
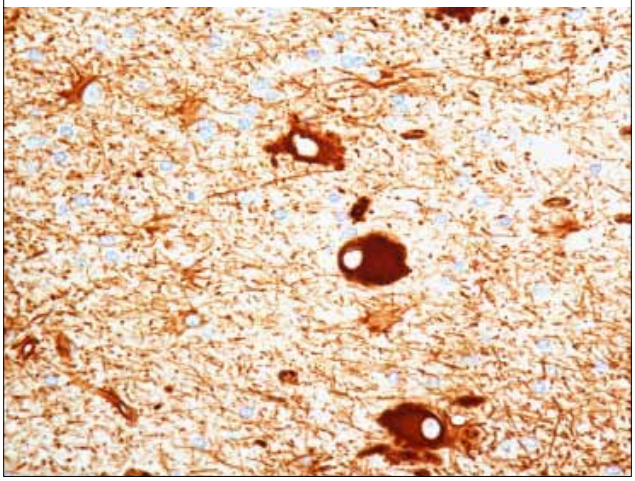


Fig. 11. FCD IIb: Balloon cells commonly accumulate Vimentin in their huge cytoplasm (Vimentin, 200X magnification).



CD34, MAP2, p53, Ki67 and IDH1 antisera, in order to rule out the possibility of tumor infiltration misdiagnosed as dysplastic tissue.

Therefore, according to the current ILAE classification, the principal pathology should always be determined in order to distinguish between isolated and associated FCD variants.

It is well established that a correct histopathological assessment of epilepsy surgery brain tissue is useful for predicting epilepsy and neuropsychological outcome after surgery [3-6]. In our series [6] patients with LEAT, HS, or HS associated with FCD showed the best postsurgical seizure outcome (Engel Class I in more than 80% of cases), whereas only 63% of patients with isolated FCD achieved the same outcome. Interestingly, the analysis of seizure outcome in patients with different subtypes of FCD and of HS showed different prognoses, with worse outcomes associated to atypical HS, absence of GCP and isolated FCD Type I.

Discussion

A pathology based approach to epilepsy surgery is essential and might improve the interpretation of the results and the comprehension of the causes of failures.

A standardized neuropathologic examination of brain tissue obtained from epilepsy surgery allows the correct classification of the clinicopathologic substrate of the epilepsy disorder and contributes to predict the patient's risk for unfavorable postsurgical seizure control.

The procedures above illustrated ensure the best histologic assessment and support research activities. They are based on systematic sampling of 5-mm interval slabs along an anatomically defined plane of section. Further-

more the pathology report should specify the more recent classifications in the definition of subtypes of the epileptogenic lesion, their localization, and extent in the samples submitted [13]. Nevertheless the histopathological, clinical and neuroradiological features of these lesions remain sometimes complex or even controversial.
Molecular tests are increasingly becoming more important, thus tissue storage and archiving is indispensable, also considering that retrospective investigations and additional examinations of stored tissue samples for patients who underwent epilepsy surgery in the past could become the rule.
For the tumors typically observed in patients submitted to epilepsy surgery has been proposed the acronym "LEATs" (Long-term epilepsy associated tumors) [20]. By definition, the expression "long term" means that drug-resistant seizures last for two years or more. The availability of more sophisticated and non-invasive diagnostic tools, such as High-Field Magnetic Resonance imaging associated with advances in histological knowledge of these neoplasms has led, in recent years, the scientific community to a better recognition and to an earlier treatment of these lesions [21 22].
The spectrum of LEATs involves an increasingly wide variety of lesions, e.g. glioneuronal tumors (gangliooglioma, dysembrioplastic neuroepithelial tumor, papillary glioneuronal tumor) and glial tumors (pilocitic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, oligodendroglioma, angiocentric glioma). Furthermore composite LEATs (i.e. ganglioglioma + pleomorphic xanthoastrocytoma, ganglioglioma + dysembrioplastic neuroepithelial tumor) have been highlighted [23]. The biological behavior of these tumors is not completely understood, but it is well known that tumors like pleomorphic xanthoastrocytoma or diffuse gliomas tend to recur and may become high grade gliomas. Recently knowledge about molecular features of LEATs is rapidly growing [24 25], and considering that the last years have seen breakthroughs in the definition of the molecular alterations that characterize diffuse gliomas [26], such background should be carefully considered.
It is well known that LEATs show BRAF mutations in a wide percentage of cases [27 28] and that mutant BRAFV600E protein in gangliogliomas is predominantly expressed by neuronal tumor cells [29]. At the same time IDH1 mutation and 1p/19q codeletion analyses may be helpful in the differential diagnosis with diffuse gliomas. An essential task will be the identification of those tumors with a significant propensity for recurrence or even malignant progression, and the characterization of molecular signatures associated with risk of transformation and progression could be very helpful.
In comparison to the previously used HS classification [30], the most recent one [10] simplified the categories to only three groups, namely the HS ILAE type 1, the most frequently encountered, and the HS ILAE type 2 and type 3, also indicated as "atypical" HS.
In 2009, Blümcke et al. [15] elaborated a classification system for GCP, recognizing 3 different histological patterns: no GCP (normal granular cell layer); GCP Type 1, characterized by significant cell loss, with a reduced vertical thickness of granular cell layer and/or cell-free gaps in the horizontal direction; GCP Type 2, characterized by broadening of the dentate gyrus granular cell layer and presence of ectopic granule cells in the molecular layer or bilamination of the granular cell layer.
It has been already reported the identification of miRNAs differentially expressed in human epilepsy with or without GCP [31]. The status of the dentate gyrus should be no longer regarded as an accessory morphological finding, but rather as an additional parameter in predicting seizure [32] and neuropsychological outcome [33], considering its potential to generate neurospheres from the subgranular zone [17 34].
More than forty years after the introduction of the term "Focal Cortical Dysplasia (FCD)" by David Taylor [35], the current classification of FCD [11] certainly represents an amazing effort to overcome the recognized limits of the previous classifications and constitutes a formidable tool in the definition of seizure outcome after epilepsy surgery. Nevertheless, in our opinion, the group of FCD type III (including cortical lamination abnormalities adjacent to a LEAT or HS) may be challenging and sometimes misleading in clinical practice. Regarding FCD type IIIb, considering that LEATs may need not only epileptogenic but also oncological follow-up, the summarizing histological diagnosis of FCD type IIIb appear misleading, shifting the focus on the not evolutive part (FCD) of this composite disease [36]. Unlike the other FCD types III (a , c , d) in which the principal lesion has not "evolutive" potential (HS, vascular malformations, ischemic or trauma injury, encephalitis), in FCD type IIIb the principal lesion is a low grade tumor, which requires a different clinical and imaging follow-up, involving, for a proper management, neurologists, neurosurgeons and neurooncologists. Therefore, this synthetic denomination may induce the epilepsy surgery team to neglect the most appropriate oncological follow up.
Furthermore, according to ILAE Classification [11], some histopathological findings may represent "atypical" features, that may be still difficult to classify, such as for instance, the association between FCD type II and other structural abnormalities, namely HS or LEATs [6 37].
The combination of these pathological findings has been reported as a rare association and it should not be classified as FCD Type III variant, but as dual (FCD type II–HS) and double (FCD type II–LEAT) pathology. However, the terms "Dual" and "Double" are often considered interchangeable and can be misleading and confusing.
Regarding the essential field of epilepsy surgery failures five major causes are commonly identified in the literature as a cause of failures: (1) insufficient resection the of mesial temporal structures, (2) insufficient or non resection of temporal neocortex, (3) dual pathology, (4) relapse on the contralateral temporal lobe, (5) extratemporal and temporal plus epilepsy [38 39].
Our finding, according with the recent literature, suggest that different pathological subtypes are associated with different postsurgical seizure outcomes [5 6]. This implies

that surgical failure, i.e. seizure recurrence, may occur either because of incomplete resection of the epileptogenic zone or because of an underlying pathological condition associated with a worse outcome.

However it is important to consider that, also adopting a standardized operational procedure, as above illustrated, histopathologic examination could not discover altered cortical brain structure in all cases.

This does not mean that histologically normal tissue is also functionally normal, as some alterations cannot be detected at the resolution level of light microscopy. The essential meaning of the presurgical neurophysiological study allow to precisely identify the epileptogenic zone (i.e. the brain area of seizures onset generation and fast propagation) that may involve both lesional areas or functionally altered networks [40]. In 2012 a study reported infection with human papilloma virus HPV16, transmitting the high-risk oncogene E6 in patients with FCD IIB [41], but afterwards different studies could not provide evidence for any HPV strain [42 43].

In the last years many molecular alterations that increase tissue susceptibility to seizures or reduce seizure threshold escape have been discovered [13]. The more advanced biological techniques permitted to identify some of these alterations (e.g. acquired channelopathies and altered glial networks [44 45]), therefore significant evidences are already available and are changing the interpretation of these diseases. In particular it has been observed that brain somatic activating mutations in MTOR cause FCD and accordingly mTOR may represent a treatment target for intractable epilepsy in FCD [46]. Hence somatic mutations rather than viral infection classify FCD type II as mTORopathy [47].

In conclusion, a pathology based approach to epilepsy surgery is essential and might improve the interpretation of the outcomes and the comprehension of the causes of failures. The described methodological approach aims to relate the surgical outcome to the specific pathological findings, the site of the lesion, and the surgical strategy. These data are essential to an adequate preoperative patient and family counselling. In this paper, besides the workup and the classification systems, we have evidenced some aspects which may be challenging and sometime misleading in clinical practice. Nowadays a current comprehensive epilepsy surgery program should include non-invasive and invasive anatomo-electroclinical, advanced imaging study and a well-established neuropathological assessment protocol.

In this setting our suggestions might contribute to focus some specific issues needing a further deeper definition, for a better management of the epileptic patients.

References

1 Blümcke I, Spreafico R. *Cause matters: a neuropathological challenge to human epilepsies*. Brain Patho. 2012;22:347-9.

2 Berg AT, Berkovic SF, Brodie MJ, et al. *Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009.* Epilepsia 2010;51:676-85.

3 Blumcke I, Russo GL, Najm I, et al. *Pathology-based approach to epilepsy surgery*. Acta Neuropathol 2014;128:1-3.

4 Coras R, Pauli E, Li J, et al. *Differential influence of hippocampal subfields to memory formation: insights from patients with temporal lobe epilepsy.* Brain 2014;137:1945-57.

5 Deleo F, Garbelli R, Milesi G, et al. *Short- and long-term surgical outcomes of temporal lobe epilepsy associated with hippocampal sclerosis: Relationships with neuropathology.* Epilepsia 2016;57:306-15.

6 Giulioni M, Marucci G, Martinoni M, et al. *Seizure outcome in surgically treated drug-resistant mesial temporal lobe epilepsy based on the recent histopathological classifications* J Neurosurg 2013;119:37-47.

7 Schoene-Bake JC, Keller SS, Niehusmann P, et al. *In vivo mapping of hippocampal subfields in mesial temporal lobe epilepsy: Relation to histopathology.* Hum Brain Mapp 2014;35:4718-28.

8 Colon-Perez LM, King M, Parekh M, et al. *High-field magnetic resonance imaging of the human temporal lobe.* NeuroImage Clin 2015;9:58-68.

9 Garbelli R, Zucca I, Milesi G, et al. *Combined 7-T MRI and histopathologic study of normal and dysplastic samples from patients with TLE.* Neurology 2011;76:1177-85.

10 Blümcke I, Thom M, Aronica E, et al. *International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a task force report from ILAE Commission on Diagnostic Methods*. Epilepsia 2013;54:1315-29.

11 Blümcke I, Thom M, Aronica E, et al. *The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission.* Epilepsia 2011;52:158-74.

12 Coras R, De Boer OJ, Armstrong D, et al. *Good interobserver and intraobserver agreement in the evaluation of the new ILAE classification of focal cortical dysplasias.* Epilepsia 2012;53:1341-8.

13 Blümcke I, Aronica E, Miyata H, et al. *International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: a consensus Task Force report from the ILAE Commission on Diagnostic Methods.* Epilepsia 2016;57:348-58.

14 Louis DN, Ohgaki H, Wiestler OD, et al. *WHO classification of tumours of the central nervous system.* Lyon: IARC 2016.

15 Blümcke I, Kistner I, Clusmann H, et al. *Towards a clinico-pathol-4gical classification of granule cell dispersion in human mesial temporal lobe epilepsies.* Acta Neuropathol 2009;117:535-44.

16 Kovacs GG, Rozemuller AJ, van Swieten JC, et al. *Neuropathology of the hippocampus in FTLD-Tau with pick bodies: a study of the BrainNet Europe Consortium.* Neuropathol Appl Neurobiol 2013;39:166-78.

17 Paradisi M, Fernández M, Del Vecchio G, et al. *Ex vivo study of dentate gyrus neurogenesis in human pharmacoresistant temporal lobe epilepsy.* Neuropathol Appl Neurobiol 2010;36:535-50.

18 Wolf HK, Buslei R, Schmidt Kastner R, et al. *NeuN: a useful neuronal marker for diagnostic histopathology.* J Histochem Cytochem 1996;44:1167-71.

19 Palmini A, Najm I, Avanzini G, et al. *Terminology and classification of the cortical dysplasias*. Neurology 2004;62(6 Suppl 3):S2–S8.

20 Luyken C, Blümcke I, Fimmers R, et al. *The spectrum of long-term epilepsyassociated tumors: long-term seizure and tumor out come and neurosurgical aspects*. Epilepsia 2003;44:822-30.

21 Thom M, Blümcke I, Aronica E. *Long-term epilepsy-associated tumors.* Brain Pathol 2012;22:350-79.

22 Giulioni M, Marucci G, Martinoni M, et al. *Epilepsy associated tumors: review article.* World J Clin Cases 2014;2:623-41.

23 Prayson RA, Napekoski KM. *Composite ganglioglioma/dysembryoplastic neuroepithelial tumor: a clinicopathologic study of 8 cases.* Hum Pathol 2012;43:1113-8.

[24] Marucci G, de Biase D, Visani M, et al. *Mutant BRAF in low grade epilepsy-associated tumors and focal cortical dysplasia.* Ann Clin Transl Neurol 2014;1:130-4.

[25] Japp A, Gielen GH, Becker AJ. *Recent aspects of classification and epidemiology of epilepsy-associated tumors.* Epilepsia 2013;54:5-11.

[26] Brat DJ, Verhaak RG, Aldape KD, et al. *Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas.* N Engl J Med 2015;372:2481-98.

[27] Chappe C, Padovani L, Scavarda D, et al. *Dysembryoplastic neuroepithelial tumors share with pleomorphic xanthoastrocytomas and gangliogliomas BRAF(V600E) mutation and expression.* Brain Pathol 2013;23:574-83.

[28] Schindler G, Capper D, Meyer J, et al. *Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma.* Acta Neuropathol 2011;121:397-405.

[29] Koelsche C, Wöhrer A, Jeibmann A, et al. *Mutant BRAF V600E protein in ganglioglioma is predominantly expressed by neuronal tumor cells.* Acta Neuropathol 2013;125:891-900.

[30] Blumcke I, Pauli E, Clusmann H, et al. *A new clinico-pathological classification system for mesial temporal sclerosis.* Acta Neuropathol 2007;113:235-44.

[31] Zucchini S, Marucci G, Paradiso B, et al. *Identification of miRNAs differentially expressed in human epilepsy with or without granule cell pathology.* PLoS One. 2014;9:e105521.

[32] Marucci G, Rubboli G, Giulioni M. *Role of dentate gyrus alterations in mesial temporal sclerosis.* Clin Neuropathol 2010;29:32-5.

[33] Coras R, Siebzehnrubl FA, Pauli E, et al. *Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans.* Brain 2010;133:3359-72.

[34] Marucci G, Giulioni M, Rubboli G, et al. *Neurogenesis in temporal lobe epilepsy: Relationship between histological findings and changes in dentate gyrus proliferative properties.* Clin Neurol Neurosurg 2013;115:187-91.

[35] Taylor DC, Falconer MA, Bruton CJ, et al. *Focal dysplasia of the cerebral cortex in epilepsy*. J Neurol Neurosurg Psychiatry 1971;34:369-87.

[36] Giulioni M, Martinoni M, Marucci G. *Observations on epilepsy associated with low-grade tumors and focal cortical dysplasias.* Childs Nerv Syst 2015;31:507-9.

[37] Cossu M, Fuschillo D, Bramerio M, et al. *Epilepsy surgery of focal cortical dysplasia-associated tumors.* Epilepsia 2013;54:115-22.

[38] Jehi LE, Silveira DC, Bingaman W, et al. *Temporal lobe epilepsy surgery failures: predictors of seizure recurrence, yield of reevaluation, and outcome following reoperation.* J Neurosurg 2010;113:1186-94.

[39] Barba C, Rheims S, Minotti L, et al. *Temporal plus epilepsy is a major determinant of temporal lobe surgery failures.* Brain 2016;139(Pt 2):444-51.

[40] Lüders HO, Najm I, Nair D, et al. *The epileptogenic zone: general principles*. Epileptic Disord 2006;8:S1-S9.

[41] Chen J, Tsai V, Parker WE, et al. *Detection of human papillomavirus in human focal cortical dysplasia type IIB.* Ann Neurol 2012;72:881-92.

[42] Thom M, Liu J, Reeves C, et al. *A cautionary note in the interpretation of human papillomavirus E6 immunohistochemistry in focal cortical dysplasia.* Ann Neurol 2015;77:352-3.

[43] Coras R, Korn K, Bien CG, et al. *No evidence for human papilloma virus infection in focal cortical dysplasia IIb.* Ann Neurol 2015;77:312-9.

[44] Bernard C, Anderson A, Becker A, et al. *Acquired dendritic channelopathy in temporal lobe epilepsy.* Science 2004;305:532-5.

[45] Steinhauser C, Boison D. *Epilepsy: crucial role for astrocytes.* Glia 2012;60:1191.

[46] Lim JS, Kim WI, Kang HC, et al. *Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy.* Nat Med. 2015;21:395-400.

[47] Blümcke I, Sarnat HB. *Somatic mutations rather than viral infection classify focal cortical dysplasia type II as mTORopathy.* Curr Opin Neurol 2016;29:0.