Differentiating solid and infiltrative component of malignant glioma with two-peak visualization of Protoporphyrin IX after 5-ALA administration

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Background
Fluorescence guided-surgery mediated by 5-ALA-induced protoporphyrin IX (PpIX) fluorescence has proven to be a useful surgical adjunct in the neurosurgical treatment of malignant glioma. However, the sensitivity of this method is still not high enough to confidently discriminate between the solid and the infiltrative (low cellularity) component in malignant gliomas. Recent work has demonstrated not one but two different PpIX peaks (620 and 634nm) that are simultaneously present, creating PpIX fluorescence emission. It is presently assumed that PPIX in low cellularity, alkaline and/or infiltrative zone tumor tissue predominantly peaks at 620 nm, whereas in high cellularity, acidic, high-grade tumor tissue PPIX peaks at 634 nm. To date, local emission spectrum measurements are performed with different methods. However, these methods might not encompass important spectral portions of PpIX, since they are mainly based on the assumption that only the 634 nm PpIX state along with its corresponding products are present in tumor tissues. PpIX is lipophilic and in aqueous solution the microenvironment will have a direct influence on his physicochemical states. The emission peak at 634 nm is well known and has been studied intensively in high-grade glioma (HGG). A $Ratio_{620/634}$ has been described by Montcel et al. for discriminating infiltrative from solid component of glioma, being 1 in infiltrative or low-grade glioma (LGG), and 0 in high-grade glioma. We aimed to corroborate this theory using hyperspectral measurements.

Methods
Patients harboring lesions with pre-operative imaging suggestive of malignant glioma received 5-ALA (20 mg/kg b.w.) 4 hours before surgery. Estimated PpIX concentration were measured implementing a hyperspectral camera. cPPIX was assessed at the peak of both states 620 and 634 nm. cPPIX was indirectly measured by evaluating fluorescence phantoms with known cPPIX.

Results
387 tissues from 120 patients where measured and included in this study. $Ratio_{620/634}$ differed significantly between grade IV, III and II gliomas ($p<0.001$) and it tended to be close to 0 in HGG and up to 10 in LGG. Interestingly, tissue from grade II gliomas with and without visible fluorescence differed significantly between measured ratios.

Conclusion
The measured $Ratio_{620/634}$ in brain tumor tissue was helpful in discriminating high- from low-grade tumor tissue. These findings corroborate recently published data. PpIX is lipophilic and

in vivo this protein is in contact with the lipid membrane of mitochondria with a complex microenvironment. It raises the question if pH sensitive methods could improve our understanding of these tumors. Further data is required to correctly evaluate the clinical value of this ratio and analyze if optical imaging systems should be calibrated towards both simultaneously present fluorescence states in PpIX.