timal treatment through review of larger case series and global comparison of patient management.

EPENDYMOMAS: SURGEON CASE VOLUME AND PATIENT OUTCOMES

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AIMS: Ependymomas (tumours arising from ependymal cells) are rare in the adult population and therefore there is limited class 1 evidence on the treatment and management of these patients. We present our experience from a large single center. We address whether management should be undertaken by sub-specialised surgeons with high volume experience. METHOD: Retrospective comparative study. RESULTS: High volume surgeons operated on larger volume (16.14 mm3, 8.31mm3, p=0.10) and more complex tumours (multi-centric cases p=0.10). We find a non-significant improvement in complication rate (p=0.77), extent of gross total resection (70.8% against 65.7%) and a positive change in performance status for high volume surgeons (p=0.84). Length of hospital stay is significantly prolonged when complications occur (14.2 and 48.4 days, p<0.05). CONCLUSION: Surgeons who have higher case load of ependymomas operate on more complex tumours. In addition, our results indicate there is a technical advantage of high volume surgeons compared to low volume surgeons, which translates into improved clinical outcomes for patients. We show that this has a significant impact on length of hospital stay, as well as the associated economical implications. For rare tumours such as ependymomas, super-specialisation and referral to surgeons with higher case volume will likely improve patient outcomes. We call for a multi-centre, prospective studies to combine data in demonstrating statistical significance (power calculation for complication rate, N=150, p=0.05).

ARGININE DEPRIVATION THERAPY INDUCES APOPTOTIC CELL DEATH IN MELANOMA BRAIN METASTASIS

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AIMS: The development of melanoma brain metastasis (MBM) occurs in ~50% of metastatic melanoma cases, and significantly worsens prognosis to a median survival of 12.8 months. Melanoma is often reported as an arginine auxotroph due to transcriptional silencing of argininosuccinate synthase 1 (ASS1). Arginine deiminase (ADI) is a non-mammalian enzyme which depletes blood arginine by converting it to citrulline and ammonia, and in its pegylated form ADI shows clinical efficacy in the treatment of a number of cancers via exploiting tumour arginine auxotrophy, resulting in targeted arginine deprivation of tumour cells. While cutaneous melanoma is the prototype cancer for this therapy, studies to date have excluded central nervous system metastasis. We have demonstrated that patient derived primary MBM models are sensitive to arginine deprivation in vitro, confirmed suitable clinical biomarkers of sensitivity, and established the mechanism of tumour cell specific cytotoxicity. METHOD: Patient derived primary cultures of MBM were established and subject to treatment with arginine deprivation. Gene expression and methylation analysis was examined by RT-qPCR, western blot, Illumina mRNA sequencing and Illumina methylated DNA immunoprecipitation-sequencing (MeDIP-seq) on ADI treated and untreated samples. Cell death, cytotoxicity induction and caspase-3 and-7 recruitment was analysed using an Incucyte S3 live-cell imager, by fluorescently labelling cells with Incucyte Cytolight Red Rapid dye, Cytotox Green dye and Caspase-3/7 Green dye, and imaging cells every 2 hours over the course of 2 weeks. 3D spheroid growth and invasion was measured by culturing cells as tumour spheroids before treating with ADI, and imaging spheroids every 2 hours for 2 weeks using an Incucyte S3 live-cell imager. Nuclear leakage and mitochondrial morphology was observed by fluorescently staining treated and untreated cells with DAPI and MitoTracker Red, and imaging on a Leica DMi8 confocal microscope. RESULTS: Primary MBMs differentially express ASS1 at substantially lower levels than non-cancerous melanocytes, however some models are capable of upregulating ASS1 following confrontation with arginine deprivation. Despite this, long-term sensitivity of primary MBMs to arginine deprivation was observed in both 2D and 3D models. In addition, arginine deprivation was seen to inhibit MBM invasion in a 3D model – an important feature in MBM pathogenesis. Initially, autophagy was induced in arginine deprived MBM, however in all models the induction of cytotoxicity correlated with recruitment of caspase-3 and -7, and intrinsic apoptotic cell death confirmed. Nuclear leakage, and eventually complete nuclear destruction was observed, in addition to mitochondrial fragmentation. CONCLUSION: Arginine deprivation is highly effective in reducing 2D and 3D MBM growth, as well as limiting invasion. While apoptotic cell death was observed in all models, the initial induction of autophagy could pose threat of resistance development in a clinical setting, and so combinational therapies with autophagic inhibitors and/or additional apoptotic inducers should be investigated. It is unclear whether nuclear leakage and mitochondrial degradation are the cause or product of apoptosis. Considering the strong clinical evidence for the use of arginine deprivation in non-CNS metastatic melanoma and the results of this study, arginine deprivation is a highly suitable treatment for pre-surgical MBM to limit invasion and increase resection, and for post-surgical continuation.

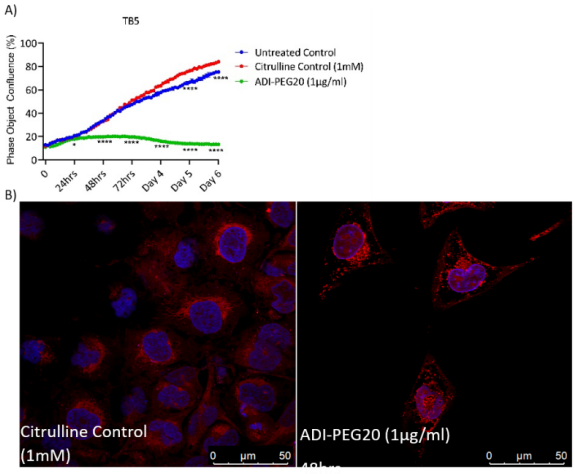

Figure 1: Arginine deprivation inhibits the growth of primary MBM and induces mitochondrial fragmentation. Primary MBM cell cultures (TB5 shown) were treated with media only (untreated control), vehicle control (citrulline control) or 1µg/ml ADI. **A)** Cells were imaged at 10X in bright-field using an Incucyte S3 every 2hrs for 6 days, and % confluence quantified using Incucyte Basic Analyser software*. **B)** After 48hrs treatment, cells were fixed in PFA and stained with MitoTracker Red and DAPI. Images were taken on a Leica DMi8 at 63X.

**Significance is shown to citrulline control. Data displayed as mean ± SEM (some error bars are shorter than data point symbol). n=3. Two-way ANOVA with Tukey's multiple comparisons test was performed to determine significant differences between groups (*P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001, ****P $<$ 0.0001).*

AN AUDIT ON THE DIAGNOSIS OF PRIMARY CNS LYMPHOMA

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AIMS: Primary central nervous system lymphoma (PCNSL) is a rare form of non–Hodgkin lymphoma with exclusive manifestations in the central nervous system, leptomeninges and eyes. It forms around 5% of all primary brain tumours. It is an aggressive tumour which has a poor prognosis if left untreated. It is imperative that diagnosis is made timely so treatment can be started promptly. Therefore, we performed an audit looking into the speed of diagnostic process of PCNSL in our tertiary Neuro–oncology Unit. METHOD: Single-centre retrospective review of PCNSL cases referred to a tertiary Neuro–Oncology Unit over a six month period from June to November 2020. RESULTS: A total of 1309 cases were discussed in the Neuro–oncology MDT meeting over the study period. Fourteen cases (6 male, 8 female; median age [range] 66 [59–83] years) were identified as highly likely PCNSL. Neuroimaging suggested PCNSL as the likely diagnosis in twelve patients. Twelve patients were started on steroids after CT or MRI brain scans. Nine patients had a surgical target and proceeded to have diagnostic brain biopsy. Two patients had different working diagnoses and three patients were deemed unsuitable for brain surgery. One patient required repeat brain biopsy. A tissue diagnosis was made in twelve patients. One patient deteriorated rapidly and one patient had a brain lesion that was deemed too high risk for surgery. The median time between neuroimaging and biopsy was 25 days. The median time taken from first investigation to the pathological confirmation of PCNSL was 36 days (range 6–86 days). CONCLUSION: The

chief reason for delay in diagnosis of PCNSL was that patients were started on steroids before diagnostic investigations were completed. Steroids caused the brain lesions to become smaller or disappear. Accordingly, time was needed to allow withdrawal of steroids before diagnostic investigations could be repeated. Diagnostic delays may have been exacerbated by logistical issues associated with COVID–19. We propose that there needs to be greater awareness of how early introduction of steroids can markedly delay the diagnosis of PCNSL.

A FEASIBILITY STUDY EVALUATING THE USE OF CELL-FREE DNA ANALYSIS IN LABORATORY BRAIN CANCER INVESTIGATIONS

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AIMS: Circulating tumour DNA (ctDNA), shed from solid cancers in to the plasma, represents an exciting analyte for diagnosis and monitoring of disease in cancer patients. However, its use in glioma brain cancer patients represents a challenge, due to reduced permeability of the blood brain barrier. This pilot study sought to investigate the practical aspects and clinical utility of using cell-free DNA (cfDNA) in glioma tests in a NHS diagnostic laboratory. Firstly, we investigated the potential of ctDNA as a proxy for the brain cancer biopsy; where cfDNA analysis was compared to the paired FFPE brain specimen for relevant glioma genetic biomarkers. Secondly, ctDNA constitutes a portion of the overall cfDNA and there is evidence cfDNA metrics per se may also be of value as prognostic tools and surrogates of tumour burden. Additionally, we investigated a potential role for cfDNA metrics in prognostic impact; linking cfDNA concentrations to clinical outcome measures. METHOD: 10ml peripheral blood was collected in specialist preservative tubes and cfDNA isolated using an extraction kit (Qiagen MinElute ccfDNA kit). cfDNA concentration and purity was assessed using chip-based automated electrophoresis. Where relevant (12/39 cases), cfDNA samples were run though laboratory tests of IDH variant detection, 1p19q co-deletion assessment and MGMT promoter methylation analysis. Results were compared with 'standard of care' brain biopsy tests. A potential correlate of cfDNA concentration and clinical outcomes data were assessed in a sub-cohort of glioblastoma patients (n=32). The cohort was divided in to 2 groups – high cfDNA vs. low cfDNA - based on whether a subject's extracted sample cfDNA concentration fell above or below the mean. Comparison of overall survival in months between subjects was checked for normal distribution using the Shapiro-Wilk t-test. The test of equity of survival distributions for the high cfDNA vs. low cfDNA was then analysed as a Kaplan-Meier curve. RESULTS: The protocol delivered cfDNA of high purity, averaging 91%, within the plasma nucleic acid fraction, however the cfDNA concentrations (mean ≈1ng µl-1) fell below the conventional limit of detection of the laboratory tests. In spite of the low concentration, cfDNA samples did generate test PCR amplicon; however results reflected the germline DNA profile rather than the new somatic changes of the tumour. The cfDNA analysis did not pick up the tumour biomarkers seen in the paired tumour biopsy sample. In a second part of the study, cfDNA concentrations for the glioblastoma cohort were assessed in the context of their clinical outcomes data. The data showed a correlate where high cfDNA concentration in the extracted sample was independently associated with inferior outcome in terms of overall survival, with Log Rank significance p=0.014 (Figure 1). CONCLUSION: The cfDNA yields from a 10ml blood sample were consistently too low to meet the limit of detection requirements of the standard laboratory neuropathology genetic tests and glioma tumour profile could not be picked up against the germline background. Thus, in spite of the considerable advantages to glioma plasma molecular testing, using cfDNA as a proxy for a brain biopsy would currently not be possible in our routine diagnostic environment. However, within the limitations of the pilot project testing strategy, the data showed an interesting correlate where high cfDNA concentration was independently associated with inferior outcome in terms of overall survival for glioblastoma patients. Given the simplicity of obtaining this quantifiable metric, there are grounds for further investigations as to its utility; not only with survival outcomes, but also potential correlation with the clinical assessment of tumour burden, blood brain barrier integrity and disease pseudoprogression.

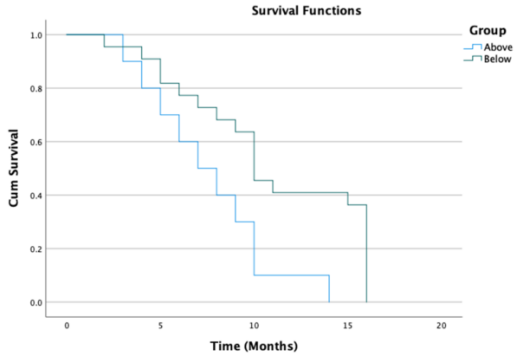

Figure 1
Kaplan Meier Curve for Overall Survival after initial surgery according to cfDNA concentration in the extracted sample

Curves represent:
Cases with high cfDNA, above the mean (blue)
Cases with low cfDNA, below the mean (green)
Log Rank p=0.014

STATIC PERMEABILITY ASSESSMENT METHOD TO DISTINGUISH BRAIN TUMOUR RECURRENCE FROM PSEUDOPROGRESSION

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AIMS: It is common to have adjuvant chemo-radiotherapy after primary brain tumour resection. It is a known side effect that enhancing lesion could be seen in radiation territory after treatment, termed as pseudoprogression. It has been a difficult task to distinguish brain between tumour recurrence from pseudoprogression after radiotherapy. Timing of occurrence of these can overlap. It is important to distinguish the two as management is completely different. Early intervention in recurrence could improve survival time while pseudoprogression could be self-limiting. Surgical resection of pseudoprogression could be counterproductive. The radiological approach has been relying on multimodality investigation and close follow up. It has come to our institution notice that there is a new technique which could distinguish the two conditions efficiently. That's static permeability assessment method, also known as treatment response assessment maps (TRAMs). Our experience with it so far has been beneficial. METHOD: This is a retrospective case series review of primary brain tumour treatment in our neurosurgical institution in 2020. Two high resolution 3D T1-weighted brain MRI images were acquired after a standard dose of gadolinium based contrast agent was injected. The first acquisition began five minutes after injection, and the second began 60 – 105 minutes post contrast injection. The TRAMs technique is based on image subtraction that is post processed after acquisition. The resultant subtracted image set was mapped to grey scale values, where voxels showing contrast clearance were light grey/white, and those showing contrast accumulation were dark grey/black. The zero value (i.e. no clearance or accumulation) was therefore mid-grey. Those with contrast clearance is associated with tumour recurrence. TRAMs images were compared to serial follow up imaging and histopathology results to determine the diagnostic accuracy of the technique. RESULTS: We have identified 21 patients in this period who had concern of either of pseudoprogression or tumour recurrence/progression. There were 6 females and 15 males, mean age 51. There were 14 glioblastoma multiforme (GBM), 5 astrocytoma, 1 oligodendroglioma and 1 post radiotherapy arteriovenous malformation. 17 cases were found to have clear cut recurrence, pseudoprogression or mixture of both in TRAMS. These findings are backed up by histology or repeated follow up scan. 4 cases were considered as equivocal. In retrospect, these cases have challenging interpretation due to poor case selection. TRAMs could distinguish high grade transformation as well as detecting recurrence. In some difficult cases, it is found that both pseudoprogression and recurrence could happen together. CONCLUSION: TRAMs is a useful adjunct to the multimodalities of diagnostic techniques in tricky situation. This has provided an efficient and easy to use tool for radiologists to come up with the answer. We are the first independent centre to report on this technique. This is still early days and fine-tuning of its use is still undergoing. It is clear this has saved precious resources and has given patients more suitable care. We think it would be beneficial for us to share our experience with others and hope to get future collaboration with other centres.