Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique.

Dr Krishan Pratap Singh

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www.sctimst.ac.in

TITLE OF THESIS

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THESIS SUBMITTED BY

Dr Krishan Pratap Singh

TO

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF

DM in Neuroradiology and Intervention Neuroradiology

2020

DECLARATION BY THE STUDENT

CERTIFICATE

I, Dr Krishan Pratap Singh, hereby certify that I had personally carried out the work depicted in the thesis titled,

"*Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique".*

No part of this thesis has been submitted for the award of any other degree or

diploma prior to this date.

Signature

Dr Krishna Pratap Singh,
Senior Resident

Dept of IS and IR

Date 10 Aug 2022

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

CERTIFICATE BY THE RESEARCH GUIDE

Name of the Guide: Dr C Kesavadas, Professor (Senior Grade) Radiology

Division/Department: Dept of ISIR

This is to certify that Dr Krishan Pratap Singh, Senior Resident , department/division of Intervention Radiology and Imaging Sciences of this institute has fulfilled the requirements prescribed for the MD/DM/MCh degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum.

The thesis entitled, "*Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique.*" was carried out under my direct supervision. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

*Clearance was obtained from the Institutional Ethics Committee for carrying out the study.

Signature

Dr C Kesavadas
Prof (Sr Grade) Radiology
Dept of IS & IR, SCTIMST
Thiruvananthapuram, India

Date 10/08/2022

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

CERTIFICATE BY THE RESEARCH CO-GUIDE

Name of the Guide: Dr Bejoy Thomas, Prof and HOD

Division/Department: Dept of IS & IR

This is to certify that Dr Krishan Pratap Singh, department/division of Intervention radiology and Imaging Sciences of this institute has fulfilled the requirements prescribed for the DM degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum.

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*Clearance was obtained from the Institutional Ethics Committee for carrying out the study.

Signature

Dr Bejoy Thomas
Prof and HOD Radiology
Dept of IS & IR, SCTIMST
Thiruvananthapuram, India

Date
10/8/2022

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
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SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

CERTIFICATE BY THE RESEARCH CO-GUIDE

Name of the Guide: Dr Krishna Kumar K, Professor

Division/Department: Dept of Neurosurgery

This is to certify that Dr Krishan Pratap Singh, department/division of Intervention radiology and Imaging Sciences of this institute has fulfilled the requirements prescribed for the DM degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum.

The Thesis work under the thesis entitled, "*Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique.*" was carried out under my supervision as co guide. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

*Clearance was obtained from the Institutional Ethics Committee for carrying out the study.

Signature Krishnakumar, K

Dr Krishna Kumar K
Professor Neurosurgery
Dept of Neurosurgery, SCTIMST
Thiruvananthapuram, India

Date 10/Aug/2022

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
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Thiruvananthapuram - 695 011, Kerala, India
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Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

CERTIFICATE BY THE RESEARCH CO-GUIDE

Name of the Guide: Dr Deepti AN, Addl Prof

Division/Department: Dept of Pathology

This is to certify that Dr Krishan Pratap Singh, department/division of Intervention radiology and Imaging Sciences of this institute has fulfilled the requirements prescribed for the DM degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum.

The Thesis work under the thesis entitled, "*Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique.*" was carried out under my supervision as co guide. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

*Clearance was obtained from the Institutional Ethics Committee for carrying out the study.

Signature
Dr Deepti AN
Addl Prof, Pathology
Dept of Pathology, SCTIMST
Thiruvananthapuram, India

Date 10/8/22

APPROVAL OF THE THESIS

The thesis entitled

*Role of multi delay arterial spin labeling (ASL) as a novel imaging biomarker for magnetic resonance imaging grading of glial neoplasms as compared T1 dynamic contrast enhancement (T1 DCE) and T2*dynamic susceptibility contrast (T2*DSC) perfusion technique.*

Submitted by

Dr Krishan Pratap Singh

for the degree of

DM Neuroradiology and Intervention Neuroradiology

of

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM

is evaluated and approved by

Name and signature of the guide

Name and signature of thesis examiner

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Dr. Krishan Pratap Singh
Senior Resident,
Dept of IS & IR,
SCTISMT,
Thiruvananthapuram, India

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LIST OF ABBREVIATIONS

S No	Abbreviation	Full Form
1.	ASL	Arterial spin labeling
2.	T1 DCE	T1 dynamic contrast enhancement
3.	T2*DSC	T2* dynamic susceptibility contrast
4.	CBV	Cerebral blood volume
5	rCBV	Relative cerebral blood volume
6.	aCBV	Arterialized cerebral blood volume
7.	naCBV	Normalised aCBV
8.	CBF	Cerebral blood flow
9.	nCBV	Normalized cerebral blood volume
10.	rCBF	Relative Cerebral blood flow
11.	MTT	Mean transit time
12.	TTP	Time to peak
13.	ATT	Arterial transit time
14.	IDH	Isocitrate dehydrogenase
15.	LGG	Low grade glioma
16.	HGG	High grade glioma

LIST OF ABBREVIATIONS

17.	ROC	Receiver operating curve
18.	Ve	Volume of extracellular space
19.	SAR	Specific absorption rate
20.	AIF	Arterial input function
21	PLD	Post labeling delay

INRODUCTION:

Arterial spin labeled (ASL) perfusion MRI is one of its kind in perfusion imaging because of the fact it uses magnetically labeled blood as contrast and is completely non invasive.

The principle of ASL was introduced in early 1990 by Alan P. Kortesky, Donald S Williams, John A Detre and John S Leigh in clinical imaging but the true potential of ASL with improved signal to noise ratio (SNR) useful for clinical needs is materalised after advent of modern high field strength scanners with advanced instrumentation, software and coil systems[1],[2],[3],[4].

ASL is based on the basic principle of magnetically tagging inflowing arterial blood water proton before its entry into tissue of interest. So is is basically a tracer technique using endogenous tracer[5],[6]. The labeling of water protons in blood is done by applying radiofrequency (RF) pulses to invert their net magnetisation. Once the tagging is done the images are acquired after an interval called transit time, which is the time taken by flowing blood to reach area of interest. Images post labeling are acquired by rapid imaging techniques like echo-planar imaging, gradient and spin echo hybrid technique or 3 dimensional fast spin echo using a stack of spiral approach[7],[8]. In ASL imaging always a pair of images are acquired one of which is a tag/labeled image and a control image. The signal difference between these imaging set is the amount of magnetisation inverted and delivered to the tissue of interest. Assuming all the tagged blood has arrived the imaging voxel at the time of image acquisition then the signal difference is directly proportional to the cerebral blood flow(CBF) of the brain tissue.

ASL has a host of applications in clinical practices ranging from dementia, acute stroke, cerebrovascular reserve testing, epilepsy, neurodegenerative disorders, CNS neoplasms and many other.

ASL perfusion has been useful in studying characteristics of brain neoplasms based on perfusion parameters. Various studies have compared ASL parameter of CBF with already established perfusion techniques like T1dynamic contrast enhancement and T2* dynamic susceptibility contrast (DSC) perfusion methods and has found good correction.

Recently with the advent of multiple delay ASL it has become possible to calculate arterial transit time (ATT) with good SNR. Using ATT and CBF we can actually calculate arterial cerebral blood volume (aCBV) non invasively without giving exogenous Gadolinium contrast using mathematical calculations. In a study by Wang D et al[9], aCBV has been studied in acute ischemic stroke to decide on the ischemic core and penumbra estimation depending on the cut off values. But this parameter has never been studied in brain glioma to differentiate various grades non invasively. We have undertaken this study with a view to study usefulness of aCBV in grading glial brain neoplasms. We hypothesise that aCBV derived from ASL perfusion is useful novel imaging biomarker in cases where surgery is delayed and regular follow up is needed and for planning biopsy for accurate representative tissue sample yield. Any change in the pattern of perfusion may alert the treating physician about possible change in mitotic behaviour of tumor and need for early intervention. Moreover, for post operative cases on follow up this may be also useful for detecting recurrence.

This novel study is a step in this direction where we will be evaluating the diagnostic utility of aCBV will be tested against two well established perfusion techniques i,e. T1 DCE and T2*DSC parameters for grading brain glioma.

AIMS and OBJECTIVES:

Hypothesis: ASL perfusion derived, arterial cerebral blood volume (aCBV) is as good as T1 DCE and T2*DSC perfusion parameters in grading glial neoplasms.

Objectives:

1. Compare diagnostic utility of ASL perfusion derived aCBV with K-trans of T1 DCS and rCBV of T2*DSC in grading glial neoplasm
2. Find out a cut off for differentiating high grade vs low grade glial neoplasms in all three MRI perfusion techniques and how they correlate with histopathology.

REVIEW OF LITERATURE

In the past before the advent of perfusion techniques, conventional MRI and post contrast enhancement of the tumor used to be the defining criteria in grading brain neoplasms. But now as our understanding of tumor has grown we see high grade brain neoplasms without post contrast enhancement and vice versa. The perfusion imaging has now become an indispensable tool in clinical evaluation of brain neoplasms. Perfusion can be calculated by various methodologies each with certain advantages and limitations, namely T2WI dynamic susceptibility contrast (T2* DSC) MRI, T1 dynamic contrast enhancement (T1 DCE), CT perfusion, single photon emission tomography (SPECT), PET CT and many others.

A relatively new development in the field of perfusion imaging is advent of ASL perfusion MRI. One of the advantages of ASL perfusion is its unique feature of non use of Gadolinium contrast media, and is one of the only options in patients with compromised renal function. Amongst its multitude of applications in brain imaging it is also being relied upon to characterise brain neoplasms. With the growing evidence on ASL efficacy in perfusion imaging, more and more scientific published data is certifying its utility in characterising brain neoplasms including grading of brain glioma.

ASL has an advantage over other perfusion techniques in that it generates image by "magnetically labeled" water molecules in blood which work as endogenous tracers as they reach organ of interest. The greatest advantage of ASL is its non invasive nature and it does not require exogenous Gadolinium contrast eliminating the risk of contrast induced toxic effects in patients with compromised renal function[10]. In addition, the technique can be repeated if needed especially in pediatric patients and

in uncooperative patients. Also the temporal changes in brain neoplasms perfusion pattern can be appreciated because of repeatability of this technique[11].

ASL also has few drawbacks associated with it, the main drawback being signal to noise ratio (SNR), which is inherently low in ASL perfusion because inflowing labeled water molecules comprise only 1% of the static tissue signal[12]. Flow quantification in ASL is a complex process because of dependence of signal on a multitude of physiological parameters like transit time, inversion pulse profile and labeled blood that bypasses perfusing the tissues[13]. Moreover in brain glioma when there is hemorrhage or calcifications, the artifacts due to susceptibility significantly erode the signal to noise ratio (SNR) thereby compromising the perfusion parameters.

ASL technique and acquisition:

ASL is a technique in which arterial blood is magnetically labelled or tagged using an adiabatic inversion pulse to invert the spins of water protons in the blood at a plane caudal to the brain most likely in the region of upper neck. Once tagged the inflowing magnetically inverted water protons acquire negative magnetisation which mixes with the positive magnetisation of static tissue water protons. This results in a small signal intensity decrease of approximately 1-2 % in the brain tissue. Depending on the specific post labeling delay (PLD) which in most of the cases lies between 1.5-2.5 seconds, tagged images of the brain are acquired. Similarly a control image of the brain is also acquired before tagging. Subtracting this control image from tag image will indirectly give us an estimate measure of this small signal intensity change which is proportional to CBF which can be quantified in units of milliliters of blood per 100 gram of brain tissue.

Similarly multiple acquisitions of the tagged and control paired images is done to enrich the signal quality due to poor SNR associated with ASL.

The difference of these set of images (labeled image minus control image) ASL aims to assess the tissue perfusion which differs from tissue blood flow. Tissue perfusion refers to exchange of water and nutrients within the tissue and happens along the entire length of capillaries. ASL in true sense follows labeled blood water molecule from the arterial compartment all the way till the tissue capillary bed by using them as free diffusible tracer molecules.

Most ASL techniques aim to avoid signal coming either from the flow in vessel or from static tissue thereby employing additional gradient and RF pulses. For the same purpose numerous pulse sequences have been developed to maximise optimisation of SNR by reducing all confounding and potentially artefactual signals. To further enhance SNR multiple cycles of control and labeled images are acquired in 4-6 minute acquisition time.

Despite so much technical refinement, many physical and physiological parameters also affect the quality of ASL image like, labeling efficiency, arterial blood T1 and relaxation time and blood transport through vessels and tissue capillaries (blood flow velocity) and magnetisation transfer effects.

One of the most important determinants is arterial labeling efficiency which is the key aspect to a good quality ASL acquisition with good SNR. Efficiencies designated with symbol alpha can range from 80-98% with currently available pulse sequences which needs to be taken into account when quantifying CBF[14],[15],[16],[17],[18].

Blood transit time and T1 relaxation are in order of seconds which means about two thirds of the label will decay by the time reaches capillary bed. So the optimal PLD is a compromise between the arterial blood T1 and transit time. Arterial T1 and feeding

arteries transit times both need to be taken into account to get a proper absolute quantification by physiological modelling[19]. Ideally the arterial blood relaxation time (T1 Blood) should be measured on individual basis. In the capillary bed the labeled arterial blood water will exchange with the surrounding tissue. The ratio of tissue water with intra-vascular labeled water in the brain is in the order of 20:1, so only a very small portion of measured signal is affected by the magnetic labeling. When this labeled water enters tissue more than 90-95% of this exchanges with the tissue water instantly. This is also the reason it is very difficult to measure any labeled venous blood flowing out of the tissue due to very short T1 relaxation time compared with the equilibrium time (1-2 seconds versus 10-15 mins for a normal CBF of 50 ml/min/100 gm respectively)[20]. Once a bolus of labeled arterial blood is delivered to the target tissue the amount of label deposited in each imaging voxel and hence the ASL signal is directly proportional to the local CBF.

Arterial blood water in ASL can be labeled by various methods: continuous ASL (CASL), pseudocontinuous ASL (PCASL) and pulsed ALS (PALS).

In CASL there is simultaneous application of both a constant RF pulse and gradient Gz and inflowing blood is labeled continuously for 2-4 seconds and after a PLD image is acquired. CASL is affected by the magnetisation transfer effects due to long RF pulse. A;so due to continuous application of low amplitude RF labeling this sequence is difficult to implement on clinical scanners.

PCASL, uses a series of short RF pulses and gradient pulses and results in inversion with same effect as CASL. It has reduced specific absorption rate (SAR) and does not need additional hardware for implementation. One disadvantage is its sensitivity to resonance offset at the labeling plane which can cause it to shift.

In PASL a large slab is inverted along feeding arteries during a very brief labeling phase. The size of labeled bolus depends on the slab size and not the labeling duration. With this method SAR is reduced but at the same time SNR is also significantly compromised to the extent of 70% reduction in SNR[21],[22],[23].

Another method of acquiring ASL is velocity selective ASL (VSASL) where those spins are labeled which are moving above a cut off velocity.

Quantification from ASL data: One approach is to use a range of PLDs between label and image acquisition and fitting this data in kinetic model for the inflow of label, typically extracting blood flow and transit delay (arterial transit time) from the data[24]. Once we get this data set we can calculate the third parameter of arterial cerebral blood volume (aCBV) which is akin to the relative CBV parameter of T2DSC perfusion MRI. ASL imaging interpretation depends on the model used for interpretation. One compartment model of Kety describes exchange of water between capillary and tissue spaces and assumes a single well mixed compartment[25]. Model by Park and Tofts considers blood and extravascular compartments as two separate models[26]. A new approach for compensation of transit delays combines the Look Locker readout with deconvolution methods derived from DSC to estimate blood flow in a model free manner[27].

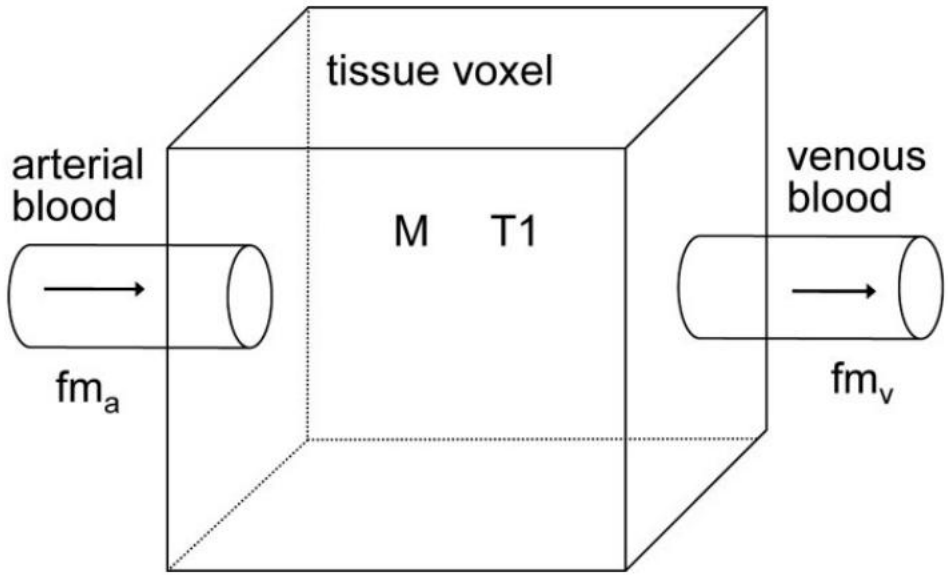

Fig 1.1: Single compartment model depicting entry of labeled blood entering tissue voxel with magnetisation ma and perfusion f and leaving with magnetisation mv. The tissue voxel has magnetisation M and longitudinal relaxation time T1. (Sourced from Park LM Tofts PS: Magn Reson Med 2002;48:27-41)

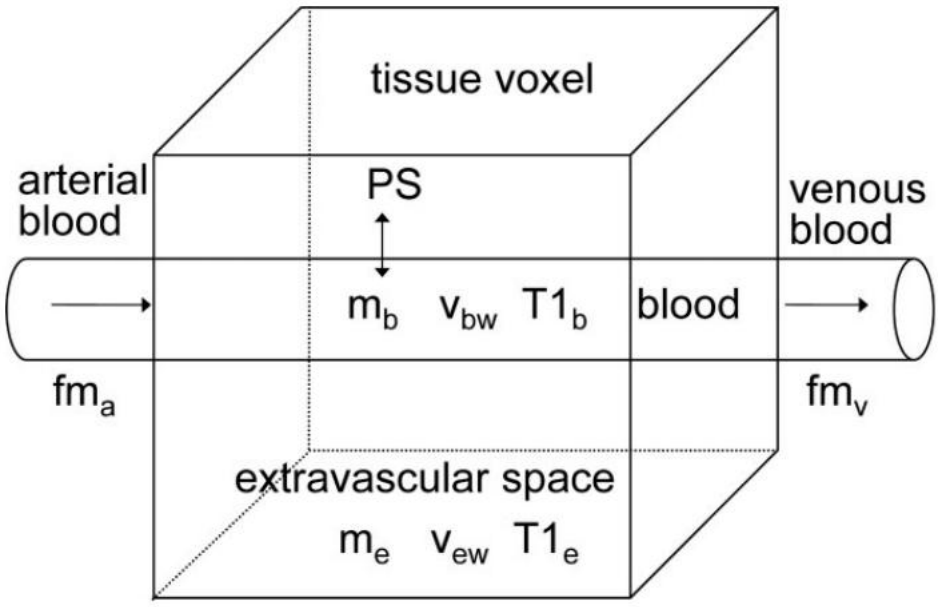

Fig 1.2: Two compartment model with restricted permeability. Labeled water exchanges between a blood compartment and an extravascular compartment via the

capillary wall with PS The blood compartment has magnetisation mb, longitudinal relaxation time T1b, and water volume *v*bw. The extravascular compartment has magnetisation me, longitudinal magnetisation T1e and water volume *v*ew (Sourced from Park LM Tofts PS: Magn Reson Med 2002;48:27-41

Many studies on ASL perfusion have employed single post labeling delay(PLD)for estimation of CBF. As a consequence of this, those cases with increased arterial transit time due to slow blood flow may result in decay of labeled intra-vascular signals thereby underestimating perfusion in region of interest in brain. So it would be prudent to take care of this confounder by acquiring serial SL images at multiple PLDs so both ATT and CBF can be estimated simultaneously. This multi delay ASL gives improved accuracy of CBF quantification and also calculation of additional parameter of arterial CBV. The main limiting factor for multi delay ASL is prolonged scan time but this issue has been addressed recently by combining single shot 3D GRASE (gradient and spin echo) background suppression and pseudo continuous ASL (pCASL) which resulted in dramatic improvements in the temporal stability of ASL image series[28],[29].

Similarly other perfusion techniques like T2*DSC and T1DCE are used to characterise and grade glial neoplasms. Perfusion derived parameters like rCBV (T2* DSC derived) and K-trans (T1 DCE derived) represents tumoral blood volume based on extent of Neo-angiogenesis and permeability/leakiness across these newely formed tumoral blood vessels respectively and correlates to areas of most histological dedifferentiation. Sometimes the areas with most enhancement and the areas showing abnormal perfusion parameters are not necessarily same and therefore for targeted biopsy the perfusion images can provide a better road map[30].

T2* DSC perfusion:

In perfusion MRI the most robust technique is T2*DSC perfusion due to availability of large amount of published data, ease of applicability and relatively simpler post processing technique. The T2*DSC studies on grading glial brain neoplasms have shown strong correlation between tumor grade and T2*DSC derived perfusion parameter of rCBV. DSC perfusion is based on first pass effect of contrast as it passes through tumor bed. There are inherent limitations of T2 DSC which includes relative qualitative nature, sensitivity to magnetic field inhomogeneties, high contrast inflow infusion and contrast leakage effects. First pass effect measures only the permeability in steady state which is different from permeability measured in T1 DCE perfusion where bidirectional exchange between interchanging compartments (plasma and extracellular space) is taken into consideration.

rCBV (relative cerebral blood volume) derived from T2 DSC represents the volume of blood in a given amount of brain tissue (ml of blood/100 g of brain tissue)and is calculated by assessing the area under the concentration time curve following contrast administration. CBV is assumed to be proportional to the area under the contrast agent concentration time curve[31], but the technique does not yield reliable results in all cases in absolute terms. As an alternate to T2*DSC parameter of CBV, ASL perfusion parameters have also shown considerable potential in glial neoplasm characterisation [32].

Due to recirculation of contrast in the blood and capillary permeability the cerebral blood volume obtained is only relative and not absolute. Therefore, another useful parameter of normalised CBV (nCBV) is used which is a ratio of tumor CBV to contralateral normal white matter CBV. nCBV gives a better estimation of tumor characterization as it is free of confounding factors because it uses contralateral white

matter as internal control. So lot of confounding factors like rate of blood flow, concentration of contrast in blood and strength of scanner are obviated.

It is important to calculate pre-contrast longitudinal relaxation time ($T1_0$) to negate effect of one of the confounding factors for obtaining accurate DSC derived parametric values. One of the methods which is used to calculate exact baseline T1 values prior to contrast administration is variable flip angle method[33],[34]. Similarly another technique of variable saturation time delay can be employed but is used less commonly.

For reliable measurement of pharmakokinetic parameters the arterial input function (AIF) is an important consideration. The direct measurement of AIF is generally preferred over standard or averaged AIFs, however no consensus exists over manual or automatic vessel detection. Optimal voxel selection using automatic AIF detection is based on multiple factors i,e. early bolus arrival, a steep wash in slope, large maximum peak concentration and large area under the curve. It has been that AIF obtained from T2 DSC has demonstrated higher grade of reliability as compared to AIF T1 DCE derived parameters in differentiating high grade glioma from low grade glioma. The possible explanation of this cause is large degree of signal change in T2 DSC MRI as compared to T1 DCE MRI and also introduction of unwanted T2* effects due to increased concentration of Gadolinium based contrast during T1 DSC perfusion imaging[35].

There is also disagreement on the whether standard hematocrit value should be used or hematocrit be calculated separately for each patient undergoing T2*DCE MRI. Another common assumption is that there is fast water exchange between compartments with the influence of restricted water exchange highlighted in studies, but this concept has not received much attention during planning a study.

Lesions with high rCBV corresponds to increased microvascular density, higher tumor grade and worse prognosis. It also helps in directing biopsy towards a site which may yield representative sample[36],[37]. Similarly there are studies which have highlighted utility of rCBV from T2*DSC perfusion in differentiating infectious lesions from high grade gliomas[38]. A study by Floriano has suggested a cut off of 1.3 in differentiating infectious lesions from neoplastic lesions with sensitivity of 97.8% and specificity of 92.6%. Despite multiple studies there is overlap of imaging finding in differentiating non neoplastic vs neoplastic findings.

At the same time there is no agreeable threshold to differentiate low grade from high grade glioma. The cut offs suggested by studies vary considerably from each other. Study by Ji Hoon Shin et al suggested a rCBV cut off of 2.93 in differentiating high grade from low grade glioma. Similarly studies by Nail Bulakbasi et al, Meng Law et al and Riyadh et al has suggested rCBV cut off of 2.6, 1.75 and 1.75 respectively to differentaite high grade from low grade glioma[39],[40],[41].

Study by Law et al has suggested that gliomas with rCBV more than 1.75 progresses faster and are associated with poor prognosis.

One reason for this varied range of rCBV across various studies could be non standardization of parameters across various scanners and varied scanning protocols. The post processing corrections can cause lot of heterogeneity in the results thereby bringing lot of variations in results.

One possible solution to this could be adopting a standarised imaging protocol tightly controlling the scan parameters, contrast flow rate, perfusion model selection. There should also be some sort of uniformity in post processing protocol to get comparable results. But since the scanners are manufactured by different vendors with patented technology so achieving uniformity in scanning parameters is not possible. One

possible way out to obtain homogeneity is having an internal control in scanned image, which is by using normalised CBV value in T2*DSC. Also using multiple parameters may enhance the diagnostic accuracy as compared to using single parameter.

T1 DCE perfusion:

This perfusion technique is based on dynamic contrast enhancement of tumor bed as contrast passes in tumor bed. Using gradient recalled echo images a signal intensity curve is acquired during contrast passage within tumor bed. This signal intensity curve represents parameters like perfusion, permeability and extravascular volume measures. One of the basic properties of tumor cells is release of growth factors necessary for neoangiogenesis and growth of tumor cells also known as 'angiogenic switch'[42]. As the tumor bed is composed of newly formed abnormally leaky vessels with immature large endothelial junctions a significant portion of contrast leaks out into extra cellular extravascular space (EES)[43].

In T1 DCE contrast perfusion we use K-Trans also known as volume transfer constant in unit time and it represents transfer of contrast from the vessel into extracellular extravascular space (EES) reflecting the intratumoral microvascular permeability. Increased expression of vascular endothelial growth factor (VEGF) by high grades of tumor promotes endothelial proliferation, proportion of immature vessels and consequently the intra-tumoral microvascular permeability[44] . The K-trans depends on both flow of blood (F) and permeability surface area (PS) . So increased K-trans indirectly reflects neo-angiogenesis with increased permeability suggestive of higher grade of tumor. The early research in T1 DCE perfusion postulated a linear relationship between contrast uptake and signal enhancement but later on details

models it is realised that the signal depends on multiple factors[45],[46]. Scanner field strength, pre contrast longitudinal relaxation time of tissue, patient hematocrit, type of contrast media, AIF assessment and type of pharmakokinetic model applied are few important factors on which perfusion parameters depend upon. Reliance of longitudinal and transverse relaxivities on different contrast agents can influence perfusion parameters calculation in T1 DCE, so type of contrast agent used need to be specified for calculation while processing perfusion parameters. The technique most frequently used for T1 DCE perfusion is a spoiler gradient echo sequence and its ultra fast variants. Recently introduced methods commonly employed includes dual temporal resolution scanning methods and combined and consecutive T2*DSC and T1 DCE measurement[47],[48],[49],[50].

Another perfusion parameter derived from T1 DCE is Ve which represents volume of extra vascular extra cellular space (EES). It represents volume fraction of contrast medium leaking into EES and can be calculated mathematically as a ratio of contrast agent quantity that leaked into EES to the contrast agent that returned to plasma space[51]. Despite the current understanding the true physiological meaning of Ve remains debatable with various terminologies trying to decode this parameter like leakage space, EES or interstitial volume. Considering the variable results showing poor correlation between Ve and cellularity it has been suggested that Ve provides independent information about tumor microenvironment. It has no unit.

Vp (plasma volume): represents plasma volume and is derived from T1 DCE using modified Tofts and Kermode's model. Ti is assumed that is is closely related to tumor neoangiogenesis. It has no unit. Basic features of signal intensity curve like area under the curve (AUC) can be extracted easily without need of pharmakokinetic model but other parameter calculations needs a suitable model for accurate quantification.

Since pharmakokinetic model selection is a vital step in perfusion imaging, a word about pharmakokinetic models is pertinent.

Pharmakokinetic modeling was initially introduced in early 1990s by Brix et al and Tofts for DCE perfusion MRI analysis. The conventional Tofts model assumes negligible blood volume (contrast agent in capillaries can be neglected) and a two way transport. So this model can be applied only in poorly vascularised tissues[52]. The modified Tofts model assumes non-negligible blood compartment and a two way transport. This model is valid only in well vascularised tissue. Patlak model assumes non negligible plasma compartment and one way transport of contrast agent. For both the modified Tofts and Patlak model K-trans equals to PS may be assumed for any solution fitting the data well with a non-negligible Vp. So the Patlak or modified Tofts models would be appropriate in situation with well vascularised tissues (high plasma flow). A one way model like Patlak may be more appropriate in situation with slower blood brain barrier (BBB) leakage and short T1 DCE perfusion acquisition duration. In a similar way by applying a two compartment model with situations having sub temporal resolution is undesired.

Use of Akaike information criteria to assess the most suitable model in a particular situation is considered valuable[53],[54].

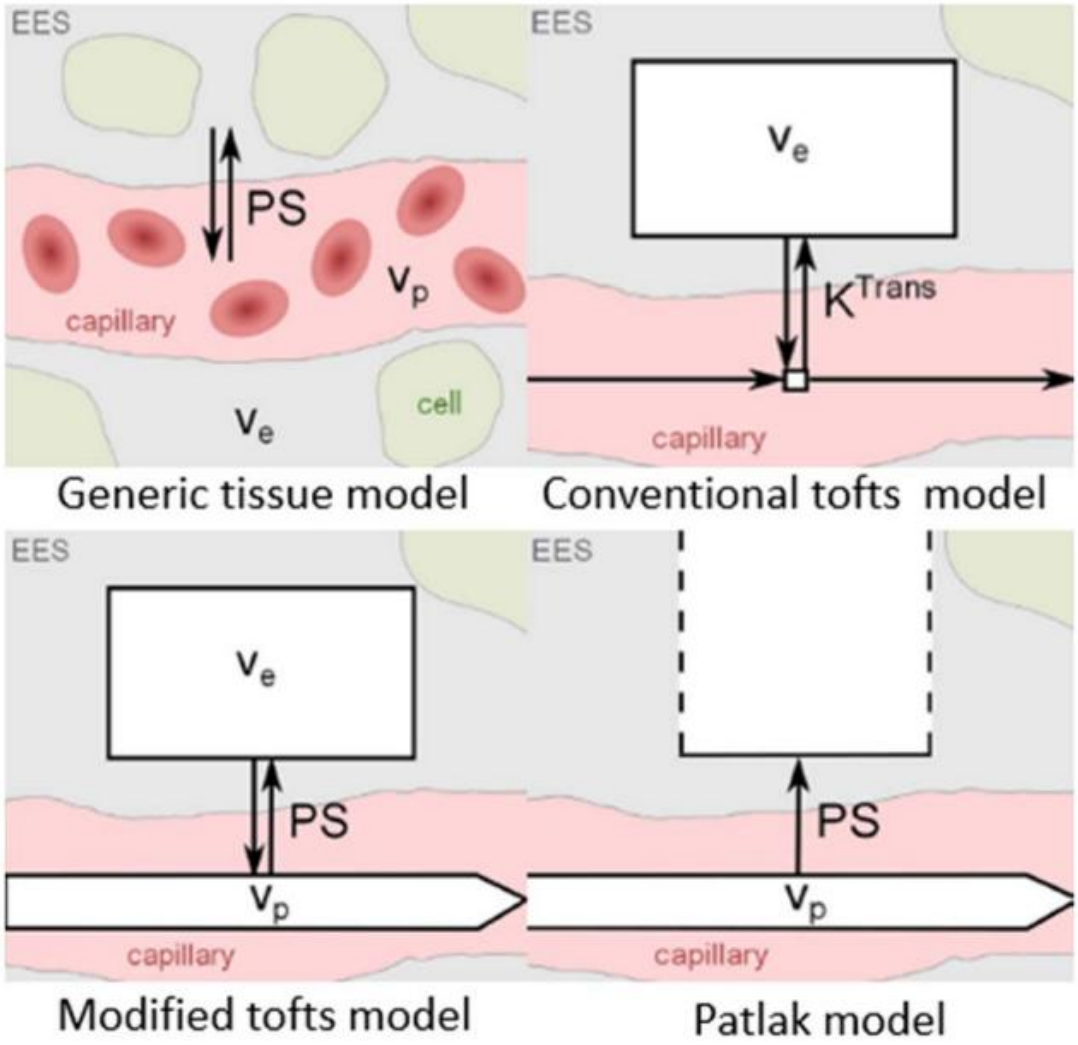

Fig 1.3 Perfusion models: EES: Extracellular extravascular space, Ve: volume fraction, K-trans:transfer constant, Vp: plasma flow, PS: permeability surface area product

Functional integrity of blood brain barrier (BBB) can be evaluated by T1 DCE perfusion in brain pathologies where BBB is disrupted likewise in high grade brain tumors. The extravasation of contrast via leaky newly formed tumor vasculature following BBB disruption leads to accumulation of gadolinium in EES thereby causing T1 shortening. This extravasation attains an equilibrium state with EES and intra-vascular compartment depending on the neo-vascular permeability and rate of

flow of blood. The T1 DCE exploits these characteristics, measures signal enhancement as a function of time and thus evaluates regions of BBB disruption.

One common finding in various studies is that as the tumor grade increases there is consistent increase in T1 DCE parameters like K-trans, Ve and Vp but there is need to consider various confounding factors like pharmakokinetic model, contrast agent used and $T1_0$.

Study bu Takashi et al suggested cut off of 0.0848 for K-trans and 0.18 for Ve for diagnosis of LGG with good combination of sensitivity and specificity[55]. Study by Corrado Santarosa et al documented sensitivity and specificty of 100% for both Vp (cut off 2.25) and K trans cut off of 0.019[56]. Li et al suggested cutoff values of Ktrans of 0.054 and Ve of 0.296 provided the best combination of sensitivity and specificity.

One of the studies by P.Alcaide Leon et al compared voxel by voxel data in GBM cases and have fond very weak correlation between K trans, Vp and CBV thereby suggesting different physiological information provided by these parametric maps.

Perfusion imaging provides very vital information about vascular proliferation within the tumor which is very critical information for assessing the nature of brain neoplasms. Angioneogenesis is the core of neoplastic activity which allows tumor to have access to unhindered nutritional supply and is vital for cellular proliferation[57]. Most commonly studied perfusion parameter in MRI is T2 DSC perfusion derived relative CBF, which has been used to grade glial neoplasms. Various studies have compared diagnostic utility of ASL perfusion derived CBF as compared to T2 DSC derived relative CBF in grading and follow up of brain neoplasms and they have found good correlation[58],[59],[60],[61].

Studies by Lehman et al[62] and Jarnum et al[63] has concluded that ASL perfusion detected brain neoplasms as accurately as T2*DSC perfusion. More recently studies by Hirai et al has also certified that ASL parameters matched closely with DSC quantification in glioma classification[64]. Classification of glial neoplasms is important for timely and effective intervention. A recent study by Yamashita K[65] found good co-relation between ASL perfusion and DWI and FDG PET in differentiating brain gliomas from primary CNS lymphomas. Similarly study by Van Westen et al[66] has proved efficacy of ASL in differentiating various brain neoplasms.

Study by Warmuth C et al[67] has shown good correlation between ASL and T2*DSC in grading brain neoplasms and has also commented on cut offs between low and high grade neoplasms.

Study by Cebeci H et al, has also suggestive cut off values for differentiating low grade vs high grade glioma. A cut off of 1.8 and 1.36 for T2*DSC derived rCBV and ASL derived rCBF respectively for differentiating high grade vs low grade glioma.

Though the literature has ample studies of comparing T2 DSC with ASL but scientific studies comparing ASL perfusion with T1 DCE perfusion are not found in our search.

Study by Noguchi et al has compared ASL percentage signal intensity and histopathological microvascular area in brain tumor patients and has found a positive correlation between these. He has also found positive correlation with rCBV of T2* DSC perfusion imaging[68].

It is also pertinent to know the basis classification scheme of gliomas based on WHO classification of CNS tumors 2016 update.

WHO classification of CNS glioma 2016

Before the WHO CNS tumors 2016, the classification of brain tumors was solely on the basis of factors that could be assessed at microscopy. But since the last update it

was seen that the treatment response of CNS tumors depends primarily on the molecular composition and genetic mutation they are harboring. An aggressive looking tumor with no lethal genetic profile may behave much better than the one harboring lethal genes. Because of this, the updated 2016 WHO CNS tumor classification gave lot of stress to tumor genetics and molecular composition. But still there are few entities which remained uncovered and which were taken care of in WHO 2021 classification of CNS tumors.

Under the new classification **layered diagnosis** approach many entities are being reclassified a per the new scheme with molecular and genetic data supplementing rather than replacing the histological classification. An example of layered approach os being provided:

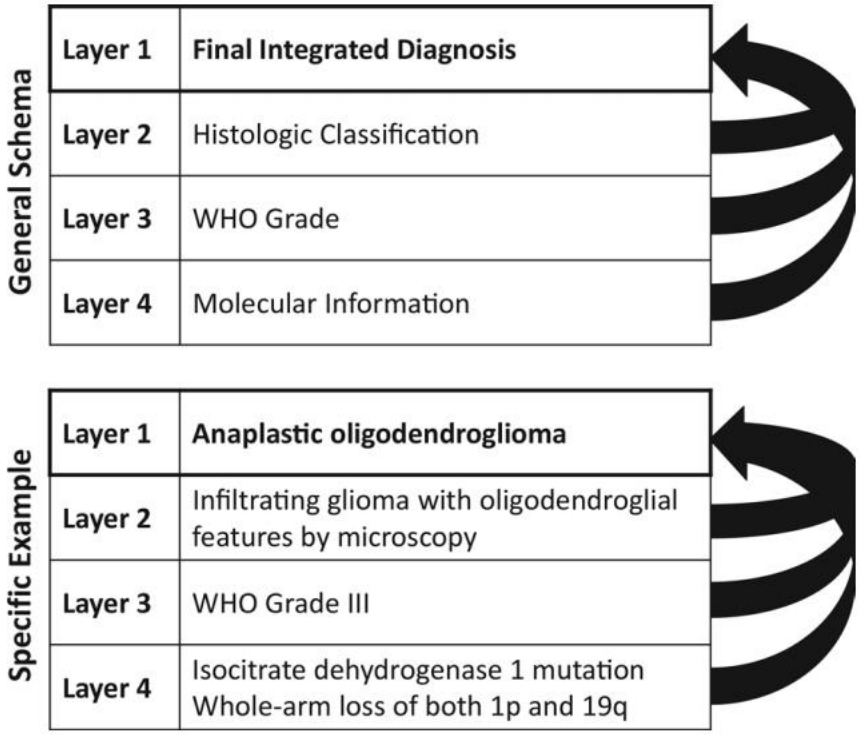

Fig 1.4: WHO brain tumor classification(Sourced from: 2016 Updates to the WHO Brain Tumor Classification System: What the Radiologist Needs to Know: by R Derek et al.)

Of all the changes to the WHO system for 2016 the classification for infiltrating gliomas is definitely the most significant. More recently it is recognised that the concept of IDH mutation has a direct link with prognosis. The tumors having IDH mutation show better prognosis than IDH wild type tumors[69]. Likewise presence of a characteristic balanced translocation of the p arm of chromosome 1 with the q arm of chromosome 19 known as 1p/19q codeletion is associated with improved prognosis and good response to chemotherapy. For the WHO 2016 revised classification IDH mutation has become important to define infiltrating gliomas in adults with 1p/19q codeletion further characterising the type. When a glioma carries both IDH mutation and 1p/19q codeletion it is apt to call it Oligodendroglioma. In the presence of only IDH mutation without 1p/19q codeletion it is called as diffuse astrocytoma.

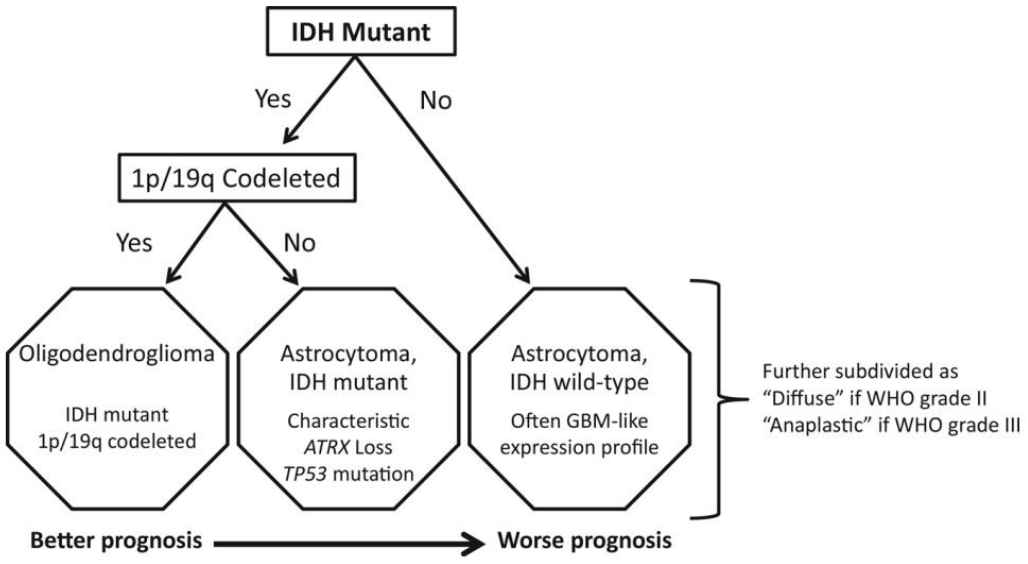

Fig 1.5: IDH mutant glioma subtypes (Sourced from: 2016 Updates to the WHO Brain Tumor Classification System: What the Radiologist Needs to Know: by R Derek et al.)

Glioblastoma (GBM) is another term for WHO grade IV astocytoma. With the advent of WHO 2016 revision GBM is subdivided into two sub-types on the basis of presence of absence of IDH mutation.

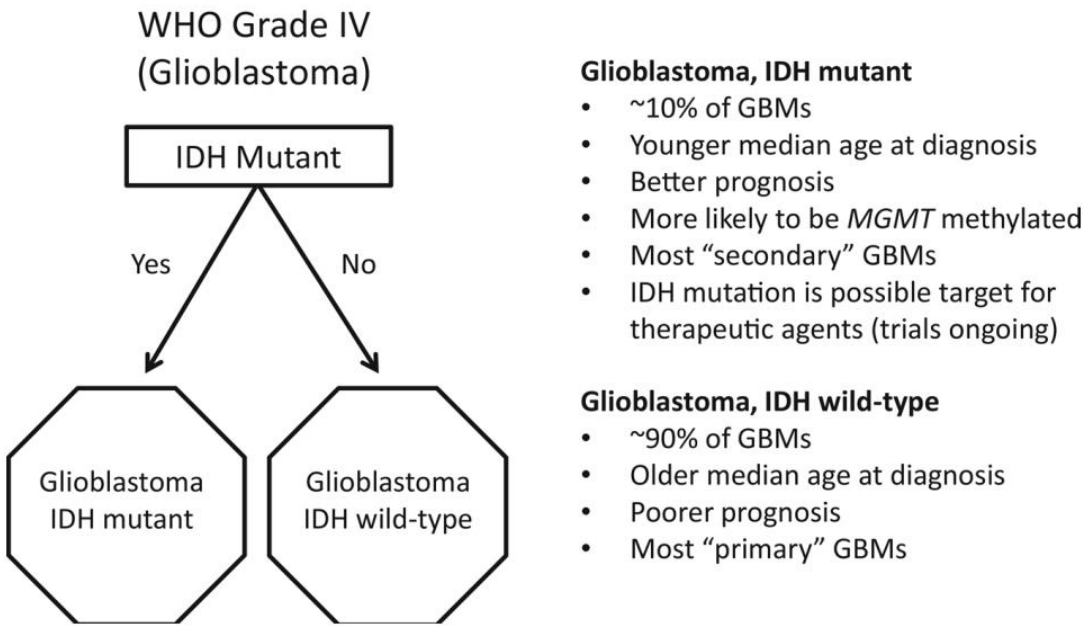

Fig 1.6: WHO grade IV glioma subtypes (Sourced from: 2016 Updates to the WHO Brain Tumor Classification System: What the Radiologist Needs to Know: by R Derek et al.)

In the present study we have focused on grading glial neoplasms of brain into high (histological grade 3 and 4) and low grades(histological grade 2) based on their perfusion characteristics and its correlation with histopathological sub type.

In the recently updated WHO classification of CNS tumors (WHO CNS5) released in 2021 CNS tumor classification there is slight change in classification of gliomas. The adult gliomas are classified as diffuse adult type glioms which includes three subtypes:

1. Diffuse astrocytoma IDH mutant: Grades 2,3,4), Oligodendroglioma IDH mutant,

1p19q co-deleted, Glioblastoma IDH wild type. Grading is not only histological but includes molecular and genetic characteristics.

Despite the advent of ASL technology in clinical use for four decades now its routine clinical use is limited to few centers. Prominent authors like Detre et al find it quite surprising that despite many advantages this technique has not been incorporated into routine clinical practice across many centers[70] . Golay and Guenther[71] think that there is lack of enthusiasm among clinical community for ASL and they contribute it to number of potential limitations which is associated with ASL like, low SNR, compatibility issues with old scanners, prevalence of other techniques which are considered more robust like T1 DCE and T2*DSC perfusion and probably lack of awareness about potential benefits of ASL. Moreover some more causes include difficulties with post processing, presence of artifacts, variability in techniques across various scanners and lack of standard guidelines for interpretation[72]. To address these issues there has been efforts by scientific community to establish ASL network which will rectify the ASL information gap by providing a centralised communication platform. Recently with efforts of scientific community a white paper on clinical implementation of ASL has been published which aims to reduce the prevalent confusion about implementation and provides cleat cut guidelines for implementation of ASL sequence[73].

In addition permeability of contrast while passing through blood vessels is a source of confounder for T2* DSC perfusion, but for ASL it is not a confounder as tagged water is a diffusible tracer. Another advantage is that ASL can be completely operator independent.

Though there are studies comparing the diagnostic utility of T2 DSC perfusion parameters with T1 DCE parameters and also studies comparing ASL perfusion vs T2

DSC perfusion but till date no study has simultaneously compared the diagnostic utility of ASL derived arterialised cerebral blood volume (aCBV), T2DSC derived rCBV and T1 DCE derived K-trans. This study is planned to determine the diagnostic utility of ASL perfusion derived aCBV in grading of glial neoplasms of brain as compared to other two perfusion techniques i,e. T2*DSC and T1 DCE. It will also help in better understanding of tumor micro structure based on the perfusion parameters used in these perfusion techniques and its utility in grading glial neoplasms. Moreover, this study will also lay ground and provide data for further research in brain neoplasms based on which further studies to determine role of ASL perfusion in tumor recurrence and post radiotherapy and chemotherapy changes can be planned.

MATERIALS AND METHODS

Study design: This study is a prospective cross sectional observational study comparing diagnostic utility of one MRI perfusion technique with the others in grading glial neoplasms of brain and gold standard being the histopathological grading.

Study duration: This study is done for a period of 18 months from Oct 2020 to Apr 2022.

Sample size: A total of 56 consecutive adult patients without any gender bias were included after applying the inclusion and exclusion criteria.

The patients are referred for brain imaging by neurosciences out patient department for symptoms suspicious of pathology in brain. After the MRI scan the patient having positive intracranial neoplastic pathology is followed up till surgery and histopathological data is collected.

All the patient with confirmed histopathological diagnosis (after curative surgery or diagnostic biopsy) are included in the study. Those patients who did not have confirmed histopathological diagnosis or who are on imaging follow up and not willing for surgery are not included in the study. The patient with histological grade of 2 are classified as low grade glioma and histological grades 3 and 4 are classified as high grades. We have based our glioma grading on WHO CNS 2016 classification since the study was already started and way past half mark before the publication of WHO CNS 2021 classification.

MRI protocol:

After applying the inclusion and exclusion criteria the patients with no contraindications for MRI examination were explained in detail about the MRI procedure and written informed consent obtained. After this patients underwent MRI

on a GE Discovery MR750w 3.0 Tesla system with 70 cm bore size using 24 channel phased array head coil. The brain tumor imaging protocol consists of T1WI, T2WI, 3D FLAIR, SWAN, DWI with ADC, T1 multi planar post contrast, MRS, T1 DCE T2 DSC and multi delay ASL perfusion(7 delays).

The entire imaging takes place in 40-45 minutes. The tumor imaging protocol is modified to take care of leakage effect in T2 DSC perfusion. ASL perfusion is done before T1 DCE and T2 DSC perfusion, because contrast administration renders the ASL perfusion ineffective with signal drop in the scan. First of all, T1 DCE perfusion is done followed by T2 DSC perfusion. This takes care of leakage effect in T2 DSC because the contrast given in T1 DCE acts as pre-loading dose. Total amount of gadolinium contrast administered is calculated on the basis of body weight at the rate of 0.1mmol/kg after carefully excluding all contra- indications for gadolinium administration. Out of this total contrast volume, 40% of total contrast volume will be used for T1 DCE perfusion and remaining 60% contrast volume for T2 DSC perfusion. To maintain uniformity in scanning we have used same Gadolinium contrast in all patients.

ASL is done with 7 delays(post labeling delays of 1.0, 1.22, 1.48, 1.78, 2.15, 2.63, 3.32 seconds) FOV: 22.0, slice thickness 4 mm, bandwidth 62.4, NEX of 1.

After obtaining plain MRI images and multi delay ASL we performed T1 DCE perfusion. The flow rate is 1.5 ml/sec followed by a saline chase of 20 ml. The image acquision takes approx 5-6 minutes to take 50 phases of scans (5 phases pre contrast and 45 phases post contrast). TR of 4.5 ms, flip angle 25 degrees, NEX 0.75, slice thickness 5 mm, pixel size 2 x 2 and SAR of 0.74 for head region.

This is followed by T2 DSC perfusion with a flow rate of 3 ml/sec followed by saline chase of 20 ml of saline. TR of 2000 ms, TE of 30 ms, flip angle 60 degrees, NEX 1.0, slice thickness 5 mm, pixel size 2.5 x 2.5 and SAR of 0.10 for head region.

Image acquisition takes 2-3 minutes to take 42 phases of scans (5 phases pre-contrast and 37 phases post contrast).

After T1 and T2 perfusion, 3D post contrast T1WI images are acquired followed by MR spectroscopy.

Performing T1 DCE before T2 DSC takes care of leakage correction artefact in T2 DSC.

After image acquisition, post processing of T1 DCE and T2 DSC perfusion data is done in Gen IQ portal provided by GE healthcare along with the scanner. Various T1 DCE and T2 DSC parameters are calculated and values saved for future references.

Multi delay ASL raw data is analysed with the inbuilt software provided by GE after correcting for motion, pairwise subtraction for label and control images followed by averaging to generate the mean difference image, for each PLD respectively.

A weighted delay (WD) was calculated and converted into ATT based on the theoretical relationship between WD and ATT[74]

$$WD = \left[\sum_{i=1}^{4} w(i)\Delta M(i)\right] \Big/ \left[\sum_{i=1}^{4} \Delta M(i)\right]$$

Where w(i) is the PLD. The estimation of ATT based on WD through a monotonic function provided a robust solution for pCASL data with 7 PLDs.

CBF at each delay, f(i), was calculated by

$$f(i) = \frac{\lambda \Delta M(i) R_{1a}}{2\alpha M_0 [\exp((\min(\delta - w(i), 0) - \delta) R_{1a}) - \exp(-(\tau + w(i)) R_{1a})]}$$

Where R1a is the longitudinal relaxation rate of blood, M0 is the equilibrium magnetisation of brain tissue, α is the tagging efficiency, τ is the duration of labeling pulse and λ is the blood/tissue partition coefficient. The final CBF was the mean sum of estimated CBF at each PLD.

The equation used for final CBF calculation is:

$$F = \frac{6000 \cdot \lambda \cdot \left(1 - e^{-TR_{PD}/T1_T}\right) \cdot \left(SI_{cont} - SI_{inv}\right) \cdot e^{PLD/T1_b}}{2 \cdot \alpha \cdot \sigma \cdot T1_b \cdot SI_{PD} \cdot K_{SF} \cdot NEX_{PW} \cdot \left(1 - e^{-LT/T1_b}\right)}$$

Factor of 6000 is used to convert units into ml/100 g of brain tissue. λ is the blood/tissue partition coefficient, TRPD, repetition time of the saturation recovery proton density calibration sequence; T1T and T1b are relaxation times of tissue blood (assumed to be 1.2 sec and 1.5 sec at 3T respectively). SIcont, SIinv and SIPD being the signal intensities of corrosponding control, label/inverted and proton density weighted piexels; PLD being the post labeling delay time between the end of the pCASL inversion component and image acquisition. LT, labeling time, α is the tagging efficiency, KSF a scaling factor for the perfusion weighted sequence and NEXPW being number of excitations for ASL sequence.

With so many parameters being evaluated for calculation a small variance or error in any variable can lead to alteration in result in significant manner.

ASL raw data, post processing gives rCBF and arterial transit time maps. The data is collected and further processed in MatLab 2018b and using a dedicated written code for arterial CBV calculation using rCBF and ATT data.

Arterial CBV (aCBV) map was generated by the product of ATT and mean CBF of multi-delay ASL USING MatLab 2018b.

aCBV = ATT x CBF

T2*DSC images were corrected for motion artifact followed by deconvolution of the image series with automatic arterial input function to generate multi parametric perfusion maps. After the perfusion maps are generated, co-registration of perfusion images from T1 DCE, T2*DSC and multi delay ASL is done with a structural image and average of 3 ROIs is calculated from the representative tumor region. Mirror ROI's having same area are also drawn in the contralateral white matter for internal control. Care is taken not to include the necrotic areas or areas having hemorrhage into lesion ROIs. Care is also taken not to keep the ROIs beyond tumor margins in normal brain parenchyma. Any blood vessel lying in the vicinity is also obviated.

After the scan patient is observed for some time in the department for any possible side effects of contrast administration.

Statistical analysis: Stata 17 software is used for ROC curves generation by calculating and plotting the true positive rate against the false-positive rate for a single classifier at a variety of thresholds. With the same ROC curve, we also calculated cut-off values for differentiating low-grade from high-grade gliomas. Mean values are used for defining various cut-offs across variables. To study the correlation amongst variable parameters we have used Pearson's correlation coefficient and $p<0.05$ is considered statistically significant in the study.

Institutional ethics committee approval is taken prior to starting the study.

RESULTS:

A total of 56 patients of brain glioma were included in the study comprising of 39 (70%) males and 17 (30%) females. The age of patients in the study ranges from 20 to 69 years with median age of 45.5 years (Inter quartile range of 40.8- 58.2 years). Out of 56 glioma cases 30 patients are of low grade gliomas (grade 2) and 26 of high grade gliomas (grade 3 and 4). The median age of patient with low grade gliomas is 44 years (Inter quartile range of 40- 50.8 years) and of high grade gliomas is 56.5 years (Inter quartile range of 45- 63.2 years).

Most common presentation in patients with glioma is seizure (64%), followed by headache in 59% cases, followed by motor weakness and sensory symptoms in about a quarter of patients. Most common location of the glioma is frontal lobe followed by temporo-insular region, however most common location of high grade gliomas is temporo-insular region.

The arterialised CBV (aCBV) signal intensity values calculated from multi delay ASL in low grade gliomas (LGG) is 79.7 (+/- 32.9) as compared to 131.9 (+/-67) in high grade gliomas (HGG), while the mean of normalised aCBV (na CBV) (ratio of lesion signal intensity to control signal intensity) value in LGG is 0.91(+/-0.3) as compared to 1.8 (+/-0.7) in HGG. The absolute cutoff value of signal intensity in aCBV for differentiating LGG from HGG is 110 with sensitivity of 57.7 % and specificity of 86.7 % and AUC of 0.738. The cutoff value of normalised aCBV(naCBV) for differentiating LGG from HGG is 1.12 with sensitivity of 88.5 % and specificity of 83.3 % and AUC of 0.901.

Characteristic	Low, N = 30	High, N = 26
Lesion aCBV	LGG	HGG
Mean (SD)	79.7 (32.9)	131.9 (67.0)
Median (IQR)	77.0 (56.8, 99.7)	115.3 (84.5, 189.0)

Table 1.1. Arterialised CBV (aCBV)

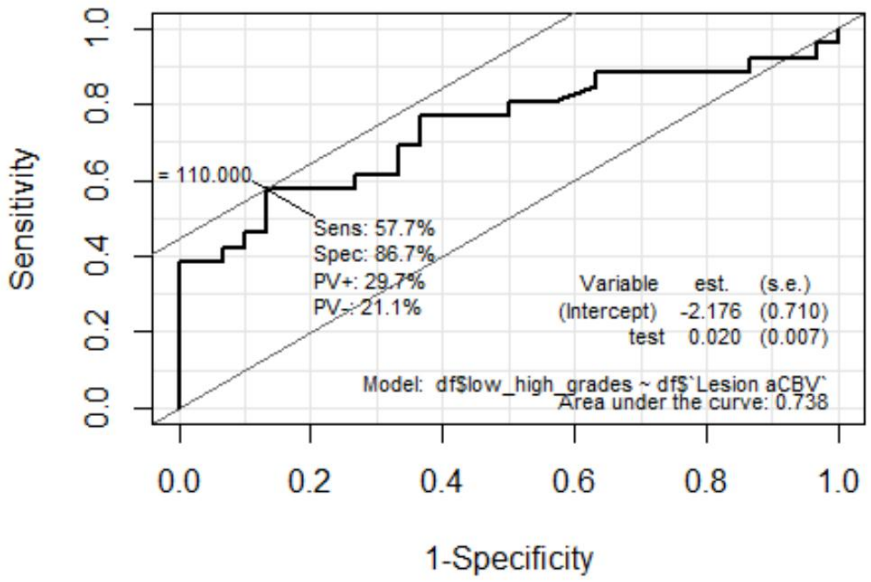

Fig 1.7: ROC graph for aCBV

Characteristic	Low, N = 30	High, N = 26
naCBV	LGG	HGG
Mean (SD)	0.9 (0.3)	1.8 (0.7)
Median (IQR)	0.9 (0.7, 1.1)	1.7 (1.4, 2.0)

Table 1.2 Normalised aCBV characteristics of low and high grade glioma.

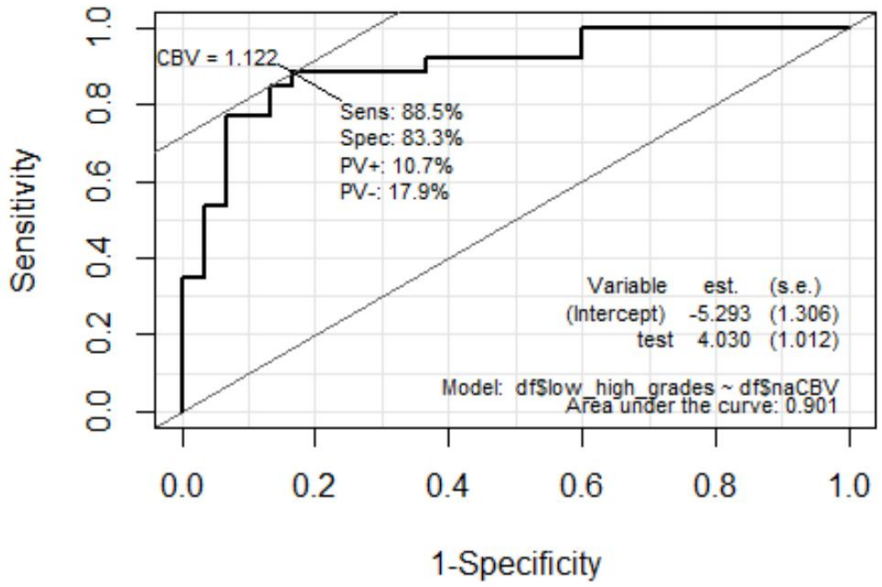

Fig 1.8: ROC graph for naCBV

The rCBV calculated from T2*DSC in in low grade gliomas (LGG) is 1.3 (+/-1.1) as compared to 2.9 (+/- 1.6) in high grade gliomas (HGG), while the normalised rCBV (nCBV) value in LGG is 0.90 (+/-0.4) as compared to 2.0(+/-0.8) in HGG. The cutoff value of rCBV for differentiating LGG from HGG is 1.84 with sensitivity of 73.1 % and specificity of 80 % and AUC of 0.796 .The cutoff value of normalised CBV (nCBV) for differentiating LGG from HGG is 1.254 with sensitivity of 88.5 % and specificity of 90 % and AUC of 0.913

Characteristic	Low, N = 30	High, N = 26
Lesion CBV	LGG	HGG
Mean (SD)	1.3 (1.1)	2.9 (1.6)
Median (IQR)	1.0 (0.6, 1.8)	2.8 (1.8, 3.9)

Table 1.3 Relative CBV (rCBV)

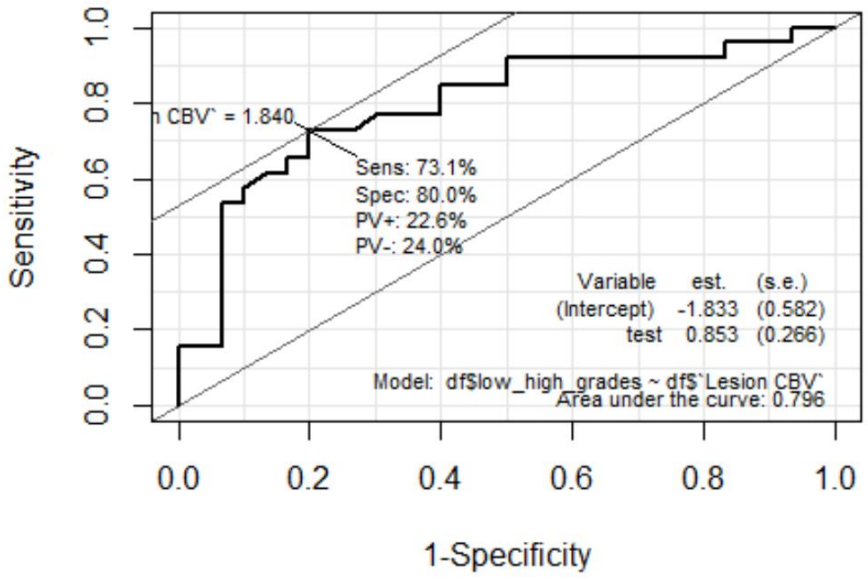

Fig 1.9 ROC graph for rCBV

Characteristic	Low, N = 30	High, N = 26
nCBV	LGG	HGG
Mean (SD)	0.9 (0.4)	2.0 (0.8)
Median (IQR)	0.8 (0.6, 1.0)	1.9 (1.5, 2.4)

Table 1.4 Normalised relative CBV (nCBV)

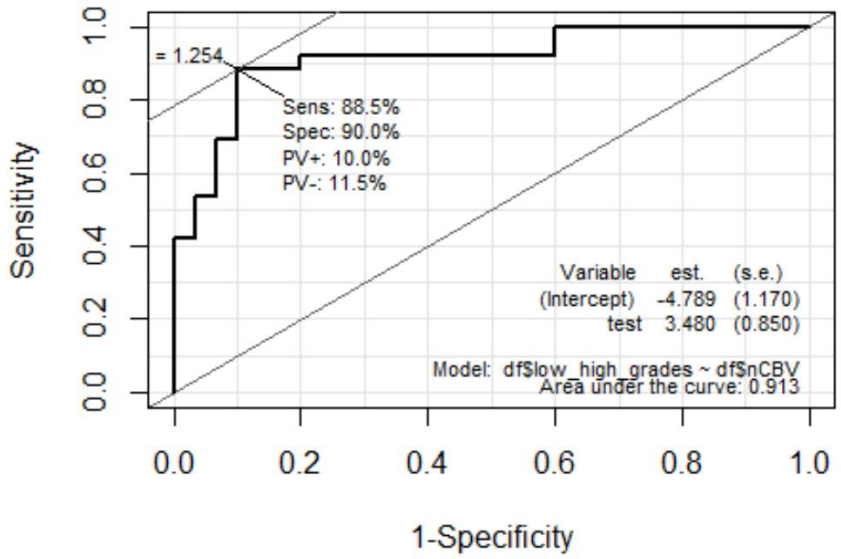

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Fig 2.0 ROC graph for nCBV

The rCBF calculated from T2*DSC in in low grade gliomas (LGG) is 10.7 (+/-11.2) as compared to 19.3 (+/-11.2) in high grade gliomas (HGG), while the normalised rCBF (nCBF) value in LGG is 0.80 (+/- 0.4) as compared to 1.9 (+/- 0.9) in HGG. The cutoff value of rCBV for differentiating LGG from HGG is 12.23 with sensitivity of 76.9 % and specificity of 73.3 % and AUC of 0.753 . The cutoff value of normalised nCBF for differentiating LGG from HGG is 1.31 with sensitivity of 76.9 % and specificity of 93.3 % and AUC of 0.895 .

Lesion CBF	LGG	HGG
Mean (SD)	10.7 (11.2)	19.3 (11.2)
Median (IQR)	6.6 (4.3, 13.4)	19.2 (12.8, 24.9)

Table 1.5 CBF

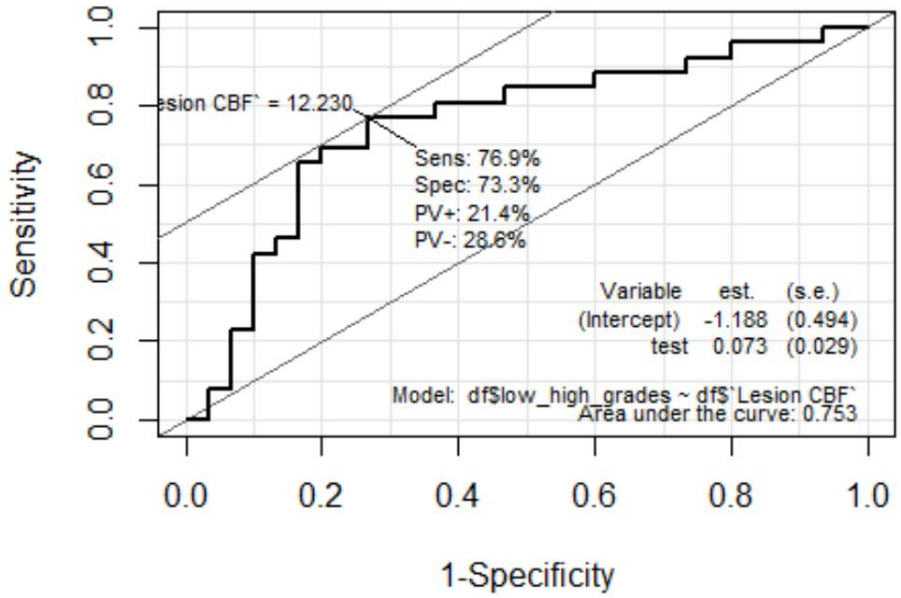


Fig 2.1 ROC graph for rCBF

nCBF	LGG	HGG
Mean (SD)	0.8 (0.4)	1.9 (0.9)
Median (IQR)	0.8 (0.6, 1.0)	1.8 (1.3, 2.2)

Table 1.6 Normalised CBF

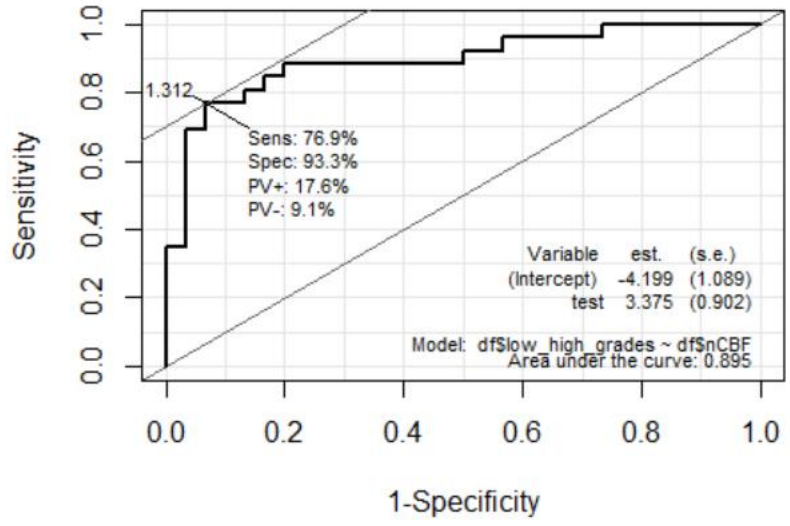


Fig 2.2 ROC graph for nCBF

The MTT calculated from T2*DSC in in low grade gliomas (LGG) is 8.4 (+/-2.9) as compared to 9.7 (+/-4.9) in high grade gliomas (HGG). The cutoff value of MTT for differentiating LGG from HGG is 10.09 with sensitivity of 38.5 % and specificity of 90.0 % and AUC of 0.52 .

Lesion MTT	LGG	HGG
Mean (SD)	8.4 (2.9)	9.7 (4.9)
Median (IQR)	7.8 (6.8, 8.8)	7.4 (6.6, 11.0)

Table 1.6 Mean transit time (MTT)

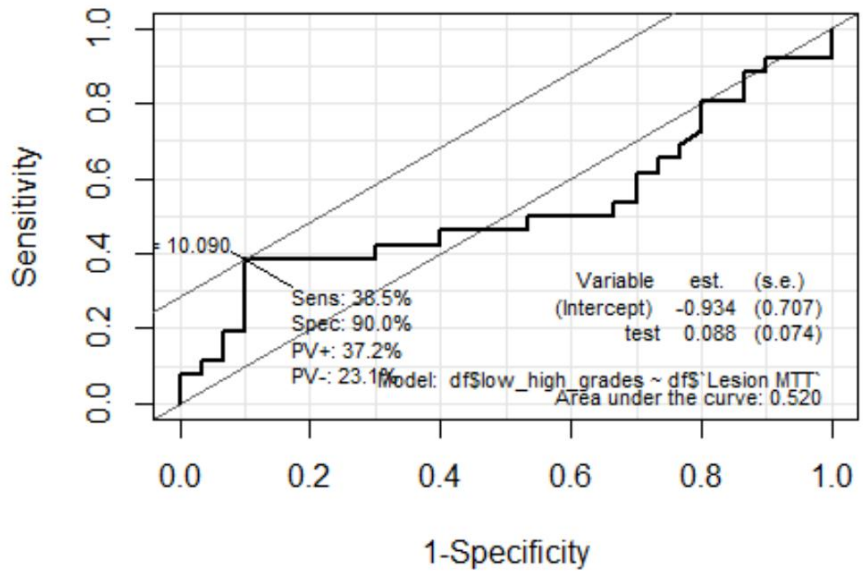


Fig 2.3 ROC graph for MTT

The TTP calculated from T2*DSC in in low grade gliomas (LGG) is 29.2 (+/-4.5) as compared to 31.3 (+/-4.7) in high grade gliomas (HGG). The cutoff value of TTP for differentiating LGG from HGG is 32.34 with sensitivity of 46.2 % and specificity of 86.7 % and AUC of 0.678 .

Lesion TTP	LGG	HGG
Mean (SD)	29.2 (4.5)	31.3 (4.7)
Median (IQR)	27.9 (26.2, 31.4)	31.1 (28.3, 34.6)

Table 1.7 Time to peak (TTP)

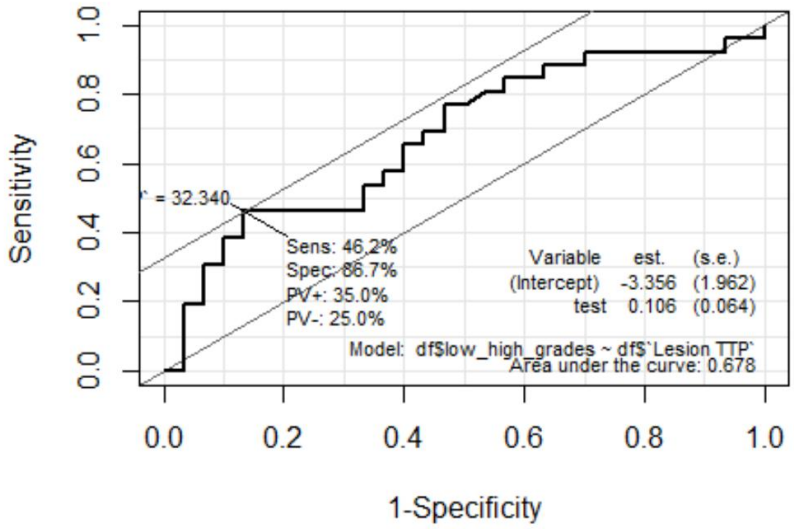


Fig 2.4 ROC graph for TTP

The Tmax calculated from T2*DSC in in low grade gliomas (LGG) is 2.4 (+/-1.2) as compared to 3.5 (+/-3.1) in high grade gliomas (HGG). The cutoff value of MTT for differentiating LGG from HGG is 3.170 with sensitivity of 46.2 % and specificity of 80.0 % and AUC of 0.644 .

Lesion Tmax	LGG	HGG
Mean (SD)	2.4 (1.2)	3.5 (3.1)
Median (IQR)	2.1 (1.5, 3.1)	2.8 (2.0, 3.8)

Table 1.8 T max

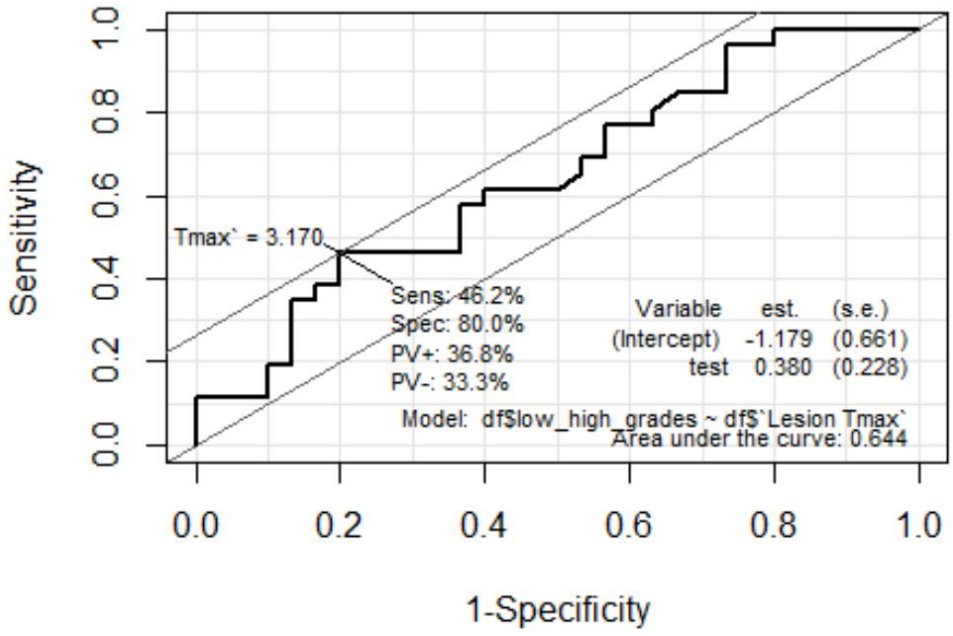


Fig 2.5 ROC graph for Tmax

The K-trans calculated from T1 DCE in in low grade gliomas (LGG) is 0.1 (+/-0.1) as compared to 0.2 (+/-0.1) in high grade gliomas (HGG). The cutoff value of K-trans for differentiating LGG from HGG is 0.115 with sensitivity of 61.5 % and specificity of 80.0 % and AUC of 0.715 .

Ktrans lesion	LGG	HGG
Mean (SD)	0.1 (0.1)	0.2 (0.1)
Median (IQR)	0.1 (0.1, 0.1)	0.1 (0.1, 0.2)

Table 1.9 Ktrans

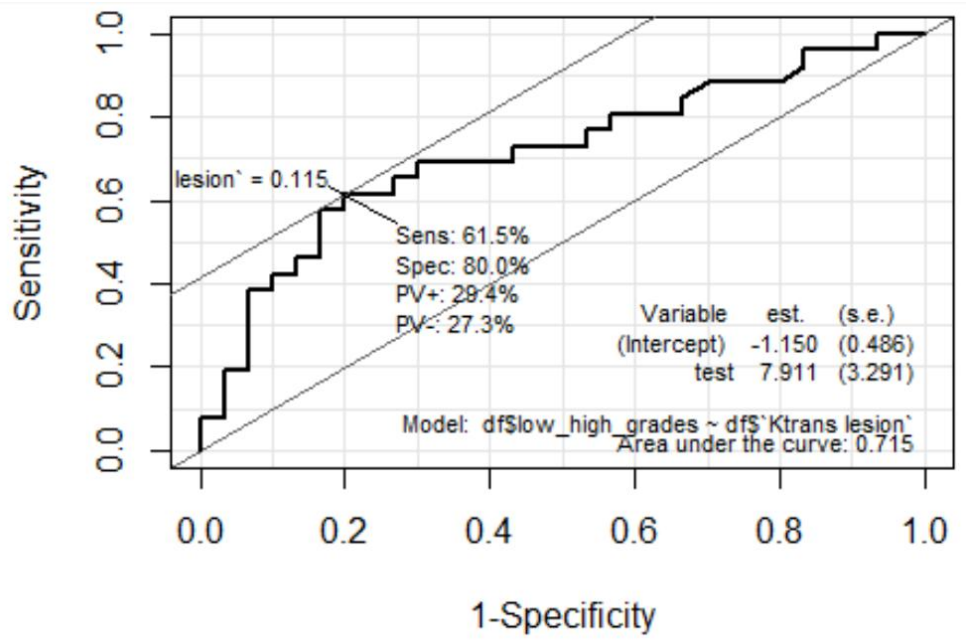


Fig 2.6 ROC graph for K-trans

The Ve calculated from T1 DCE in in low grade gliomas (LGG) is 0.1 (+/-0.1) as compared to 0.2 (+/-0.2) in high grade gliomas (HGG). The cutoff value of Ve for differentiating LGG from HGG is 0.04 with sensitivity of 73.1 % and specificity of 50.0 % and AUC of 0.573 .

Lesion Ve	LGG	HGG
Mean (SD)	0.1 (0.1)	0.1 (0.2)
Median (IQR)	0.0 (0.0, 0.1)	0.1 (0.0, 0.1)

Table 2.0 Lesion Ve

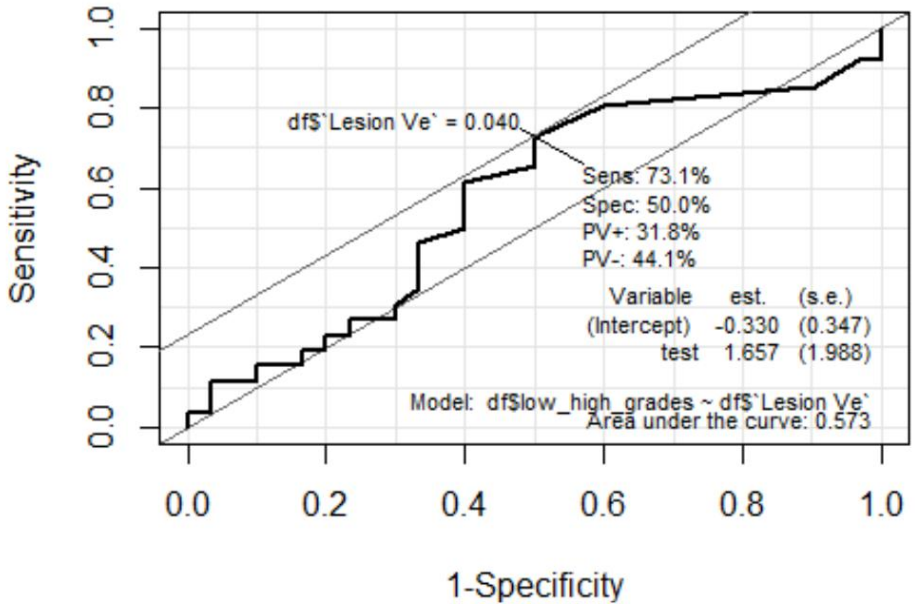


Fig 2.7 ROC graph for Ve

The IAUGC calculated from T1 DCE in in low grade gliomas (LGG) is 0.0 (+/-0.0) as compared to 0.1 (+/-0.1) in high grade gliomas (HGG). The cutoff value of K-trans for differentiating LGG from HGG is 0.06 with sensitivity of 42.3 % and specificity of 86.7 % and AUC of 0.668 .

Lesion IAUGC	LGG	HGG
Mean (SD)	0.0 (0.0)	0.1 (0.1)
Median (IQR)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)

Table 2.1 Lesion IAUGC

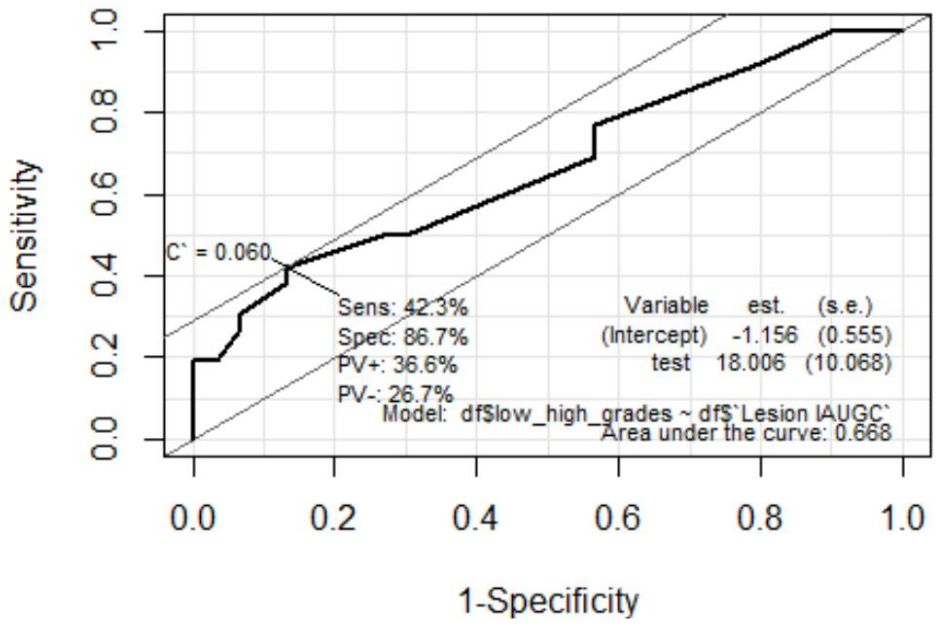


Fig 2.8 ROC graph for IAUGC

On evaluating the correlation between various variables we have found that naCBV and nCBV are showing very high correlation (Pearson's correlation coefficient value of 0.94) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

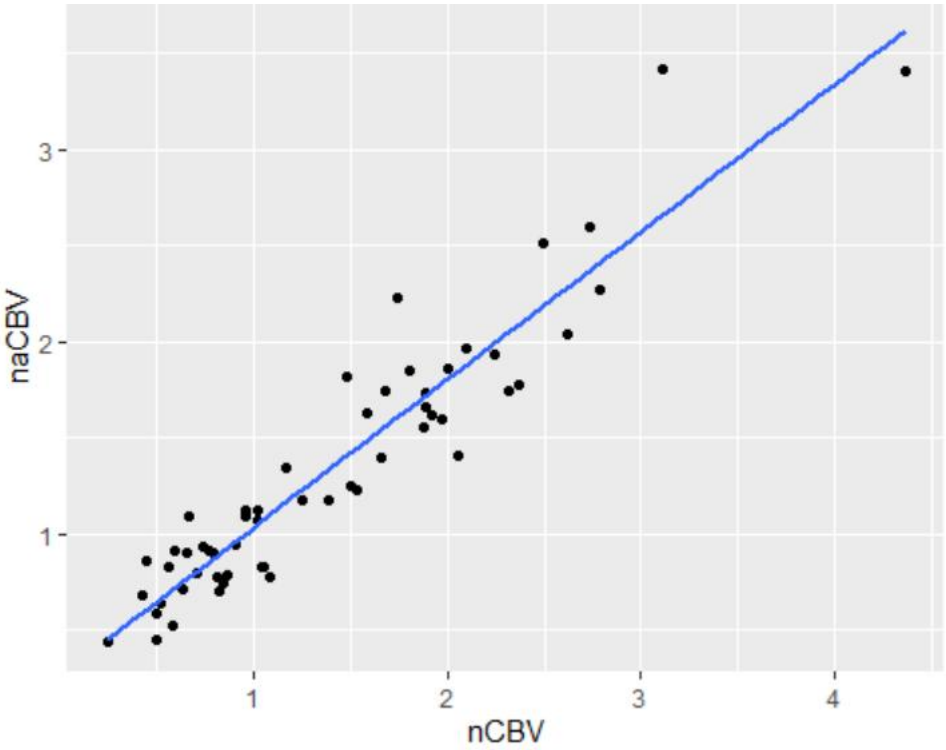


Fig 2.9 Scatter plot showing correlation between nCBV and naCBV.

Similarly on evaluating the correlation between nCBV and nCBF we have found that nCBV and nCBF are very highly correlated (Pearson's correlation coefficient value of 0.919) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

Similarly on evaluating the correlation between naCBV and nCBFwe have found that naCBV and nCBF are very highly correlated (Pearson's correlation coefficient value of 0.876) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

On evaluating correlation between naCBV and K-trans we have found that there is low correlation (Pearson's correlation coefficient value of 0.473) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

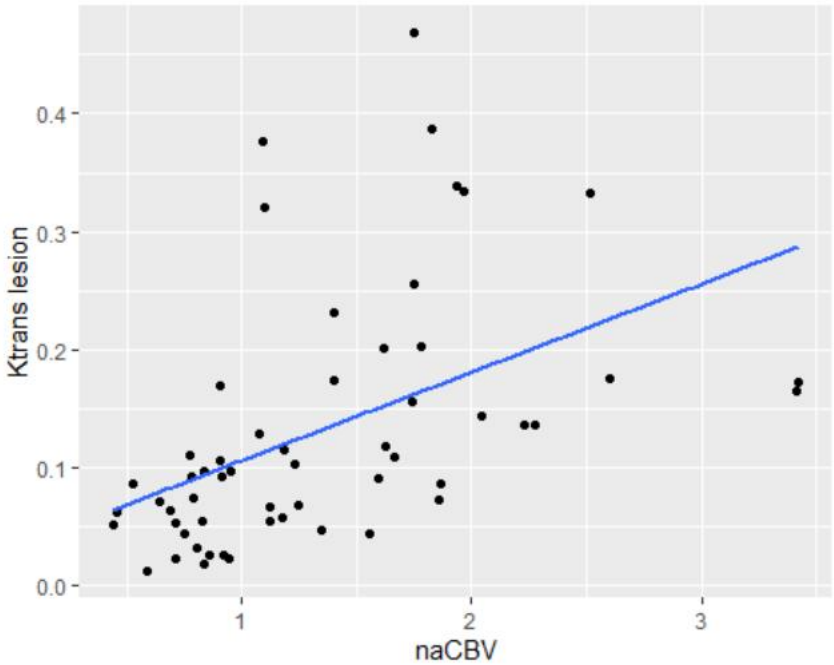


Fig 3.0 Scatter plot showing correlation between K-trans and naCBV

Between nCBV and K-trans again there is low correlation (Pearson's correlation coefficient value of 0.450) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

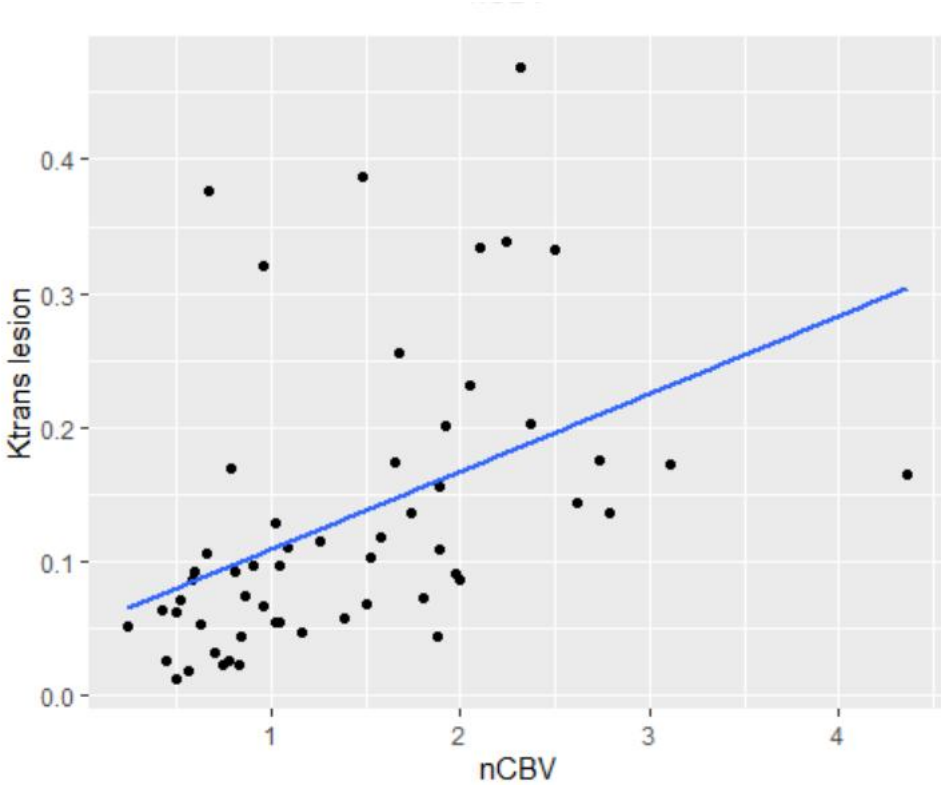


Fig 3.1 Scatter plot showing correlation between K-trans and nCBV.

Between nCBF and K-trans again there is low correlation (Pearson's correlation coefficient value of 0.354) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

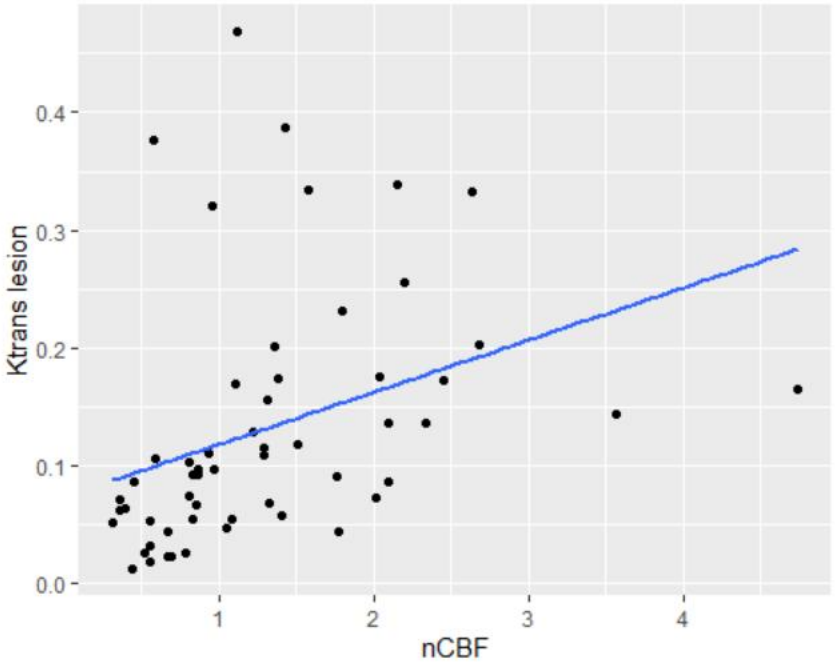


Fig 3.2 Scatter plot showing correlation between K-trans and nCBF

Similarly we have found that there is low correlation between naCBV with CBF with values of Pearson's correlation coefficient being 0.49 .

Between nCBV and CBF there is moderate correlation (Pearsons correlation coefficient value of 0.55) and the correlation is statistically significant at 95% confidence interval ($p<0.05$).

When we have used absolute values of aCBV and rCBV the correlation between these values is not strong which may be due to lot of intrinsic confounding factors during acquisition and processing. However when we have normalised aCBV and rCBV using contralateral white matter as internal control the correlation between normalised values of aCBV and rCBV turned out to be significant.

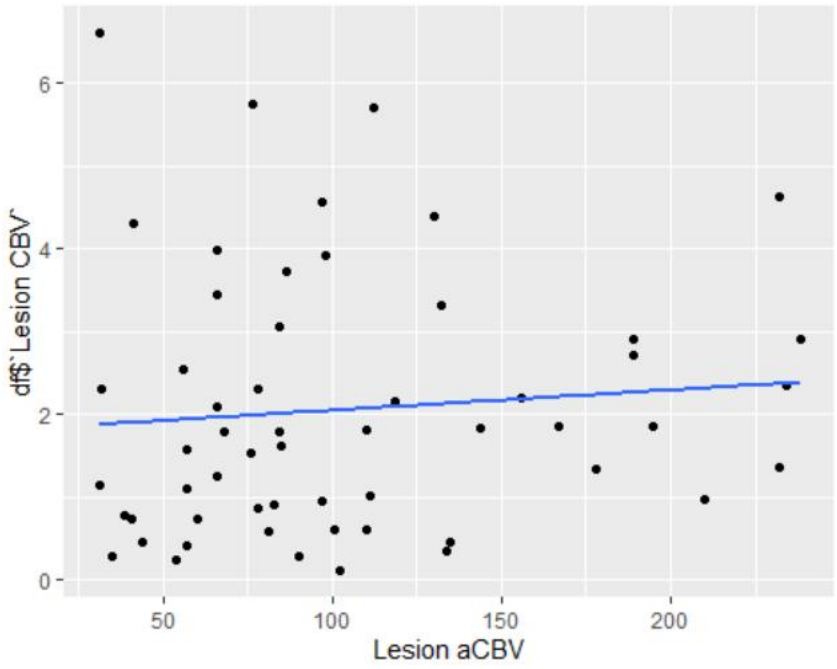


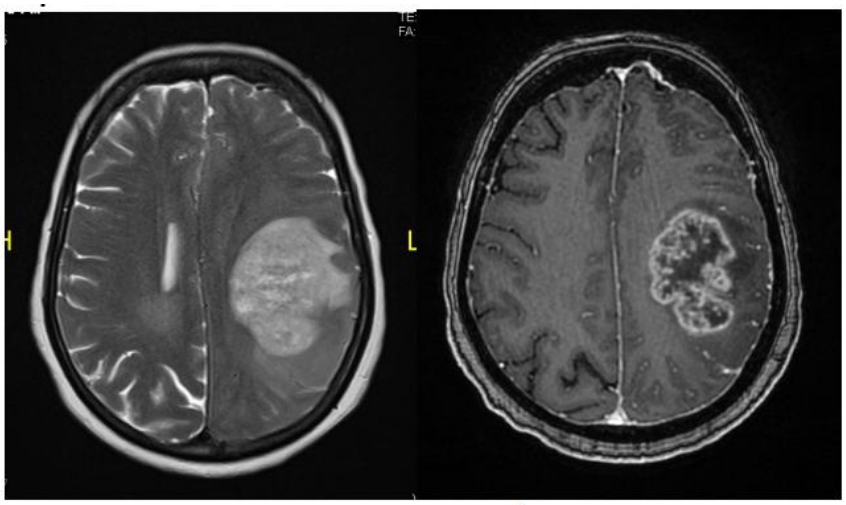
Fig 3.3 Scatter plot showing correlation between rCBV and aCBV.

Rest of the perfusion parameters are also compared and we could not find any significant correlation between those parameters with naCBV, nCBV and K-trans.

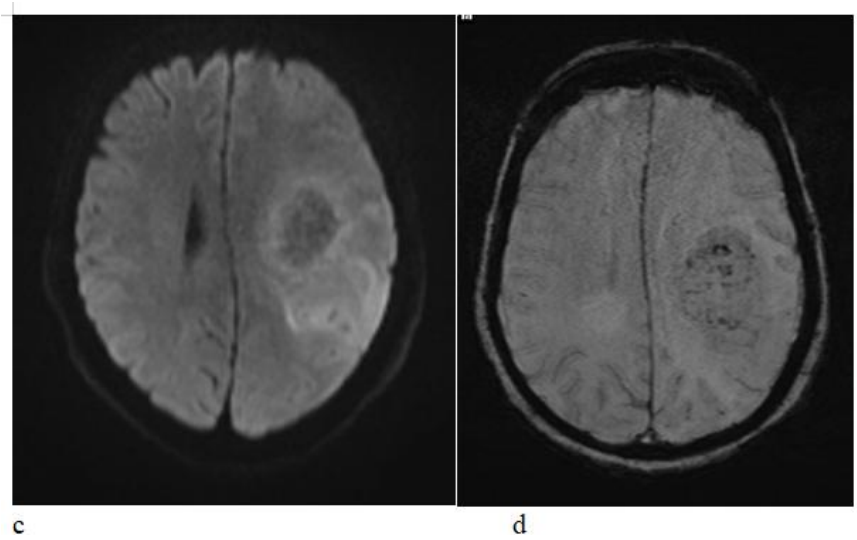
The images are analysed by two independent observers with adequate imaging experience and high inter-observer agreement (kappa of 0.87).

REPRESENTATIVE CASES

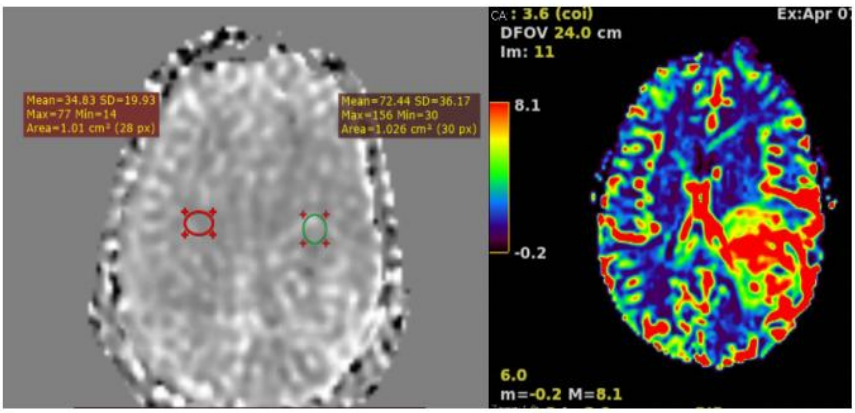
Case 1 : 66 year old female presented with headache of 3 months duration with associated episodes of vomiting. No neurological deficits.



a b



c d



e f

Fig 3.4 Case 1: MRI scan shows image T2WI axial image (top row left) shows a heterogeneous hyperintense lesion in left fronto parietal region which on post contrast T1WI (Image a) shows predominantly peripheral enhancement shaggy wall and irregular inner margins. On DWI(Image c-middle row left) at b=1000, images there is heterogeneous restriction of diffusion noted, on SWI (Image d-middle row right) the lesion shows few foci of blooming within suggestive of hemorrhagic foci. On ASL perfusion imaging(image e- bottom row left) the aCBV map shows normalised aCBV value of 2.86 and T2*DSC generated normalised rCBV(image f- bottom row right) having value of 2.52. On histopathological evaluation it was Glioblastoma WHO grade IV- IDH wild type.

Case 2: 53 year old female presented with headache of 02 years duration with seizures. No neurodeficits.

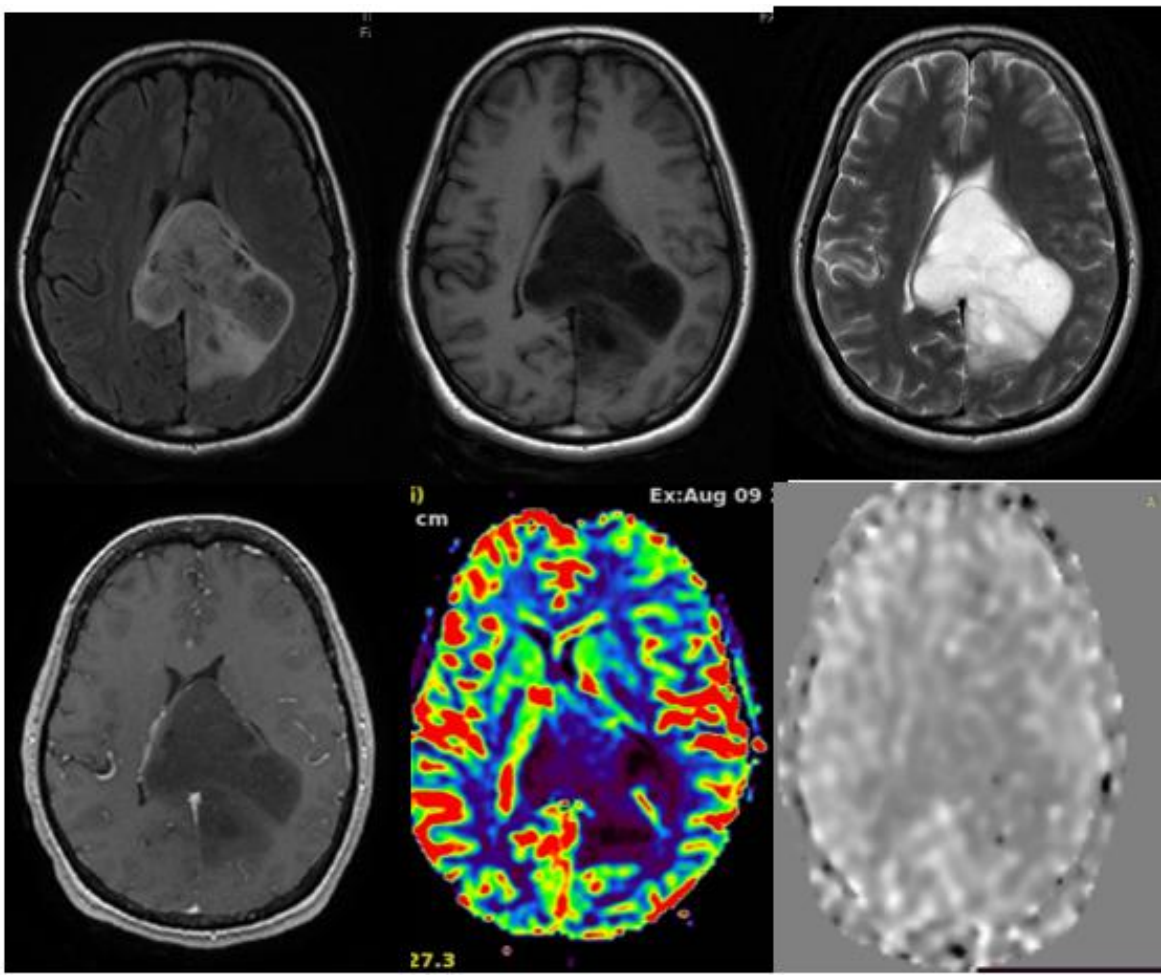


Fig 3.5 Case 2: MRI scan shows FLAIR axial image (top row left) with a heterogeneous hyperintense lesion in splenium of corpus callosum with extension on either side of midline. It appears hyperintense on T2WI(top row right) and hypointense on T1WI(top tow middle) with T2/FLAIR mismatch. On perfusion imaging the lesion shows reduced perfusion. The values of normalised T2*DSC perfusion rCBV(bottom row middle) in the region is 0.76. ASL perfusion (bottom

row right) normalised aCBV in the region of tumor is 0.70 which corresponds to the T2 DSC perfusion imaging.

On histopathological evaluation it turned out to be diffuse astrocytoma WHO grade II-IDH mutant

Case 3: 46 year old male presented with unprovoked seizures and paraesthesias of right lower limb. No other neurodeficits.

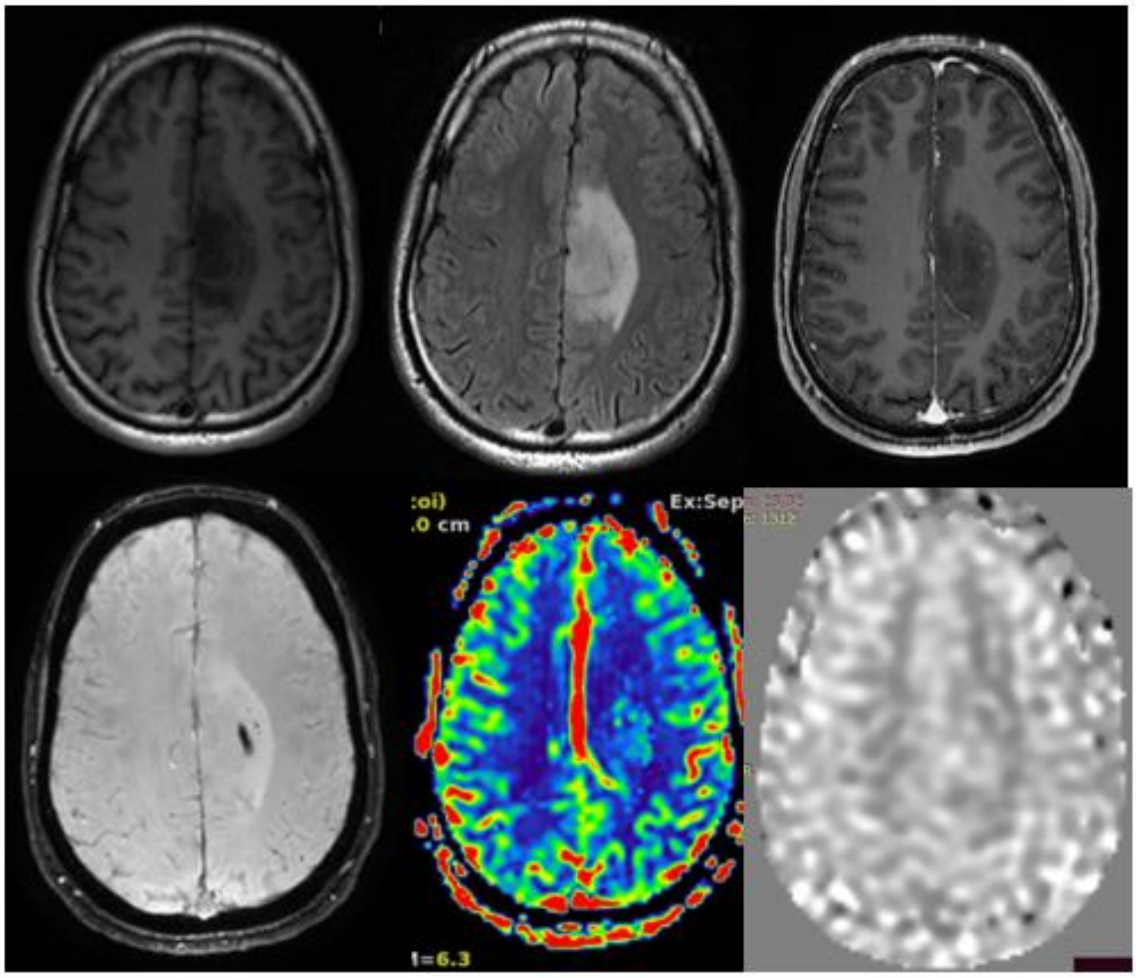
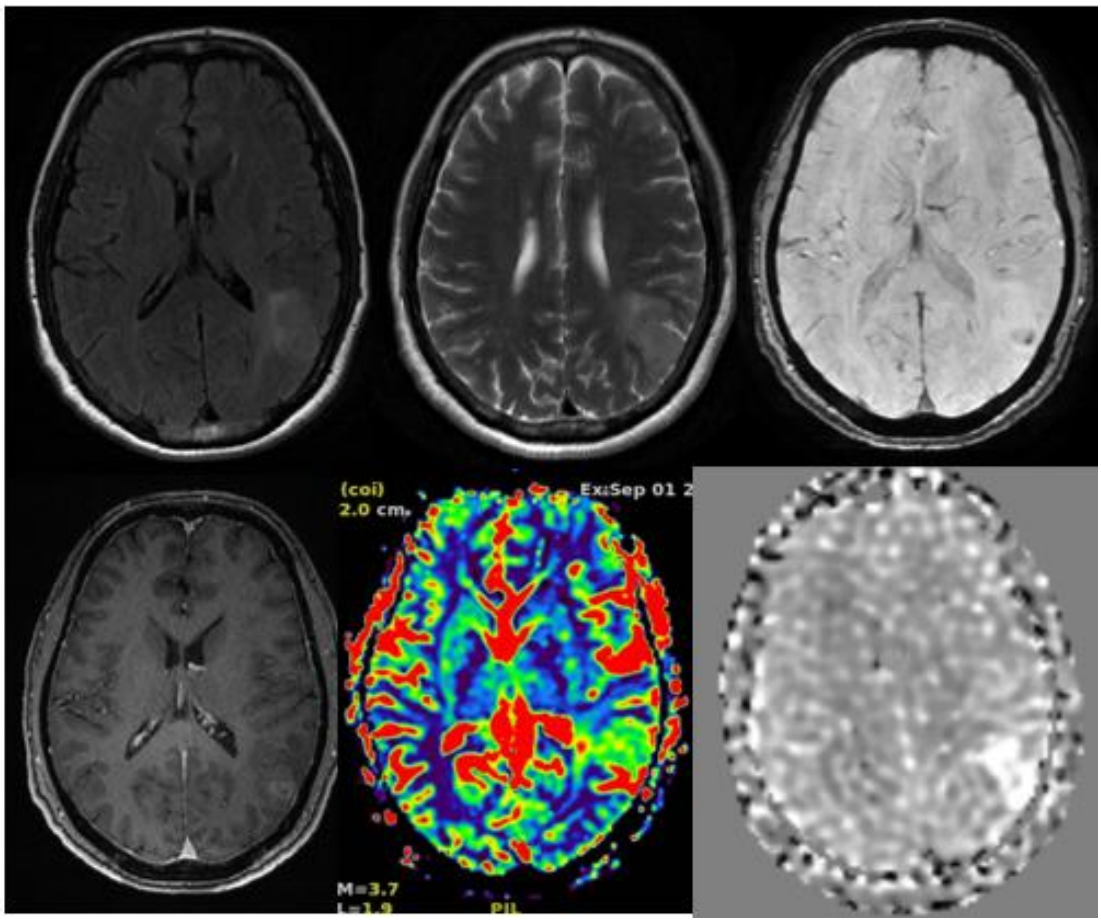


Fig 3.6 Case 3: MRI scan shows FLAIR axial image(top row middle) with a hyperintense lesion in left para central lobule and cingulate gyrus. It shows few foci of nodular enhancement within on T1WI post contrast images (top row right) and blooming on SWI(bottom row left). On T2 DSC perfusion imaging the lesion shows increased perfusion. The values of normalised rCBV(bottom row middle) in the region is 1.46. Normalised aCBV (bottom row right) in the region of tumor is 1.40

On histopathological evaluation it was Alaplastic astrocytoma WHO grade IV-IDH mutant.

Case 4: 61 year old male presented with unprovoked complex partial seizures of 3 months duration and headache of 06 months duration. No neurodeficits.



Case 3.7 Case 4: MRI scan shows FLAIR axial image(top row left) with a hyperintense lesion in left inferior parietal lobule. It is hyperintense on T2WI(top middle image). It shows few foci of blooming on SWI(top row right) and on post contrast TIWI (bottom row left) enhancement within. On perfusion imaging the lesion shows increased perfusion. The values of normalised rCBV in the region is 1.86. Normalised aCBV in the region of tumor is 2.10 which corresponds to the T2*DSC perfusion imaging. On histopathological evaluation it was Alaplastic astrocytoma WHO grade III-IDH mutant.

DISCUSSION:

Glial neoplasms of brain contribute significantly to morbidity and mortality and incur a huge loss in terms of productivity and quality of life. Despite best treatment options and management the average survival post surgery continues to be sub optimal both in terms of 5 year survival and disease free interval. Recently updated WHO CNS 2021 classification of brain tumors has heavily stressed on molecular and genetic parameters in characterising grades of glial tumors. With the progress in technology, discovery of newer genetic/molecular markers and planning of treatment strategy based on these factors these classifications are bound to undergo periodic change. We have based our study on WHO CNS tumor classification of 2016 in which gliomas are divided into high (Histopathological grades 3and 4) and low grades (histopathological grades 1 and 2). Now with the advent of new CNS tumor classification of 2021 there is slight change in classification in which gliomas are classified as diffuse adult type gliomas and its three sub-types which are defined by genetic, molecular and histological basis.

Despite the advancement in diagnosis and treatment there is delayed diagnosis in a significant number of cases due to presentation at advanced stage with involvement of critical structures. After years of deliberation and studies it is recommended that management of brain glial neoplasm needs to be team approach with specialists across various fields like neurosurgeons, neuropathologists and geneticist neuro-radiologists, neuropsychiatrists and rehabilitation experts forming a treatment group.

One of the main limitations in management and which is also reason of poor outcome is delayed confirmed diagnosis despite early imaging diagnosis owing to lack of tissue diagnosis because of fear of complications of morbid surgery and apprehension

towards brain biopsy. Even after surgery there is a need for continuous monitoring to look for recurrence and differentiate recurrence from the post operative post radiation changes.

One of the feasible options for diagnosis of the glial neoplasms and their possible characterization into various grades is by way of imaging. Multiple perfusion imaging studies done in the past have proved the utility of imaging in diagnosis and characterization of glial neoplasms with fair diagnostic accuracy confirmed subsequently on histopathological correlation.

Perfusion parameters have a fair degree of diagnostic accuracy for grading glial neoplasm. In the literature there are studies which have proven efficacy of T2*DSC perfusion by showing difference between high and low grade glioma and with good histopathological correlation.

ASL being non invasive in nature and being a non Gadolinium contrast based perfusion technique, the perfusion parameters of ASL can be calculated repeatedly in the eventuality of motion artefact or sub optimal scan due to other factors. This is also of immense benefit in patients with compromised renal function in which case contrast administration may be detrimental to the patient.

One of the most important aspect in glioma imaging is perfusion imaging which have a fair degree of diagnostic accuracy for grading glial neoplasm[75]. Studies in the past have proven efficacy of T2*DSC perfusion parameters especially rCBV and rCBF in differentiating high grade(histological grade 3 and 4) and low grade (histological grade 1 and 2) glioma and with good histopathological correlation[76]. Similarly, ASL perfusion parameter CBF shows good correlation in grading glial neoplasm as compared to T2 DSC perfusion of CBV and CBF[77],[78],[79],[80],[81]. One of the parameters of ASF perfusion which has never been used before for grading glial neoplasms is

arterialized cerebral blood volume using ASL (aCBV). In this study we have used aCBV for grading glial brain neoplasms and we have found that aCBV could be of immense clinical utility since ASL being non invasive, does not require gadolinium administration[82],[83],[84]. Moreover, we have seen in various studies that T2*DSC perfusion derived rCBV is a better predictor of tumor grading as compared to various other parameters of T2 DSC perfusion studies on glial brain neoplasms[85],[86]. T2*DSC derived rCBV has also shown its usefulness and is in concurrence to various other modalities like PET and SPECT in grading glial neoplasm[87].

In calculating aCBV, both the CBF and arterial transit time of ASL perfusion are taken into account which might show better correlation with tumor grading because aCBV represents arterialised volume of blood in tumor tissue which may be similar to rCBV calculated by T2 DSC perfusion imaging.

In this study, we have calculated arterialised CBV of glial neoplasms from ASL perfusion and compared it with other perfusion techniques like T1 DCE and*T2 DSC using various quantitative parameters like rCBV, rCBF, K-trans, Ve, MTT, Tmax, IAUGC and so on.

T2*DSC derived rCBV has already shown its usefulness with various other techniques like PET and SPECT in grading glial neoplasm across various studies. But with the contrast MRI perfusion techniques like T1 and T2*DSC there is always a concern of Gadolinium deposition in brain and its long term consequences which till date is not known. Moreover, in certain situations where there is a need to repeat the study due to motion artifacts or poor quality data due to other factors repeated administration of Gadolinium adds to further concern. So there is always a need to search for a robust imaging biomarker which would obviate the need for gadolinium

contrast administration and if needed can be repeated any number of times without any concerns.

This study was undertaken with an intent to explore the potential and evaluate the utility of arterialized CBV(aCBV) of ASL perfusion in terms of diagnostic utility as compared to T1 DCE and T2*DSC derived perfusion parameters. In the extensive literature search and to the best of our knowledge we could not find any study evaluating the role of aCBV derived from ASL perfusion in grading glial neoplasms. This study is unique where we have evaluated ASL perfusion derived aCBV as a novel imaging biomarker for grading glial neoplasms as compared to T1 DCE derived K-trans, Ve and T2 DSC derived perfusion parameters rCBV and rCBF.

In the study, ASL derived aCBV has shown significant statistical correlation with T2 DSC derived rCBV with Pearson correlation coefficient of 0.94 (at 95% confidence interval, $p<0.05$), for grading glial neoplasms. One important observation which we have noted is that when we used normalised aCBV (naCBV) for defining a cut off between high-grade and low-grade glial neoplasm as compared to arterialized CBV alone, there is significant improvement in sensitivity and mild reduction in specificity of naCBV. The sensitivity and specificity for cutoff between low and high grade glioma using normalised aCBV (naCBV) is 88.5 and 83.3 as compared to aCBV which has sensitivity and specificity of 57.7% and 86.7% respectively. So, by using normalised values of perfusion parameters we tend to standardize these parameters using an internal control in the form of contralateral normal white matter. This leads to higher sensitivity with good specificity in grading glial neoplasms. Similarly, the cut-off values derived statistically for differentiating glial neoplasm into high vs low grade is 1.12 for T2 DSC derived normalised aCBV and is 1.24 for ASL perfusion derived normalised rCBV (at 95% confidence interval).

Similarly, we have assessed other perfusion parameters for grading glial neoplasms and we found that T2 DSC derived normalised rCBF also has very strong correlation with normalised aCBV(ASL) as well as normalised CBV(T2 DSC).

One important observation we have noted is that while using normalised aCBV (naCBV) for defining a cut off between high grade and low grade glial neoplasm as compared to arterial CBV there is significant improvement in sensitivity and mild reduction in specificity. The sensitivity and specificity for cutoff between low and high grade glioma using naCBV is 88.5 and 83.3 as compared to aCBV which has sensitivity and specificity of 57.7% and 86.7% respectively. So while using normalised values of perfusion parameters we tend to standardize the parameter using an internal control in the form of contralateral white matter. This leads to higher sensitivity and specificity in grading glial neoplasms.

Similarly, we have assessed other perfusion parameters for grading glial neoplasms and we found that nCBF also has very strong correlation with nCBV and naCBV. While studying correlation between naCBV and K-trans it showed low correlation statistically (Pearson's correlation coefficient value of 0.473) and also between nCBV and K-trans there is low statistical correlation (Pearson's correlation coefficient value of 0.450). One possible explanation for this poor correlation between T2DSC and T1 DCE perfusion parameters could be related to different aspects of tumor vascularity analysed by these parameters. While CBV and CBF related parameters account of blood volume and its flow rate in the tumor, these parameters assesses neo-vasculature which indirectly assesses the tumor vascularity. On the other hand K-trans is a perfusion parameter assessing the leakiness of vasculature and thus the degree of contrast extravasation into EES. So in a way, as tumor has both these components the amount of vascularity and its leakiness gives uniqueness to a tumor. Now despite best

of pharmakokinetic models we have not been able to delineate how these two parameters i,e. tumor neovascularity and its leakiness correlate in vivo and what grade of leakiness determines the true behaviour of tumor. The neovascular leakiness calculated using K-trans has shown its utility in monitoring response to chemotherapy drugs after chemotherapy initiation.

In this study, we have used parameters based on ASL perfusion which uses magnetically labeled water as diffusible endogenous tracer and we have attempted to study the correlation between ASL perfusion and T2*DSC parameters. Another important fact is since water is freely diffusible and it may not be limited to EES alone, so the amount of labeled water gaining entry into the tumor cells is not completely traceable because of loss of signal as time passes.

If by some mechanism we could prolong the tagging duration, we may also study the internal micro milieu of the tumor just by tracing the magnetically tagged water. At present due to limitations in hardware and available software we may not be able to stretch that far but we hope that in the near future it may be possible. So ASL perfusion derived parameters have a great potential to study tumor micro-kinetics as well. The only limitation which ASL might face is lack of availability of robust standardized models but with the progress in technological advancement it should not be a limitation in the near future.

SUMMARY AND CONCLUSION:

Arterial spin labeling derived arterialised CBV (aCBV) is a novel imaging parameter which has showing statistically significant correlation with T2 DSC derived CBV in its ability to grade glial brain neoplasms. aCBV being non invasive and with a distinct advantage of it being a non Gadolinium contrast based perfusion parameter has a potential to become perfusion parameter of choice in individuals requiring repeated scans and in individuals with contraindication for Gadolinium administration. This study is unique and first of its kind to the best of our knowledge where arterialised CBV (aCBV) is used for grading glial neoplasms of brain with strong statistical correlation with CBV derived from T2 DSC perfusion. We could not find any significant correlation between aCBV and K-trans in this study. Similarly, other studies can also be planned for evaluating utility of aCBV in tumor recurrence post radiotherapy and chemotherapy cases which can also help in understanding the micro-milieu of glial neoplasms and its pharmakokinetis.

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ANNEXURES

APPENDIX A

PATIENT INFORMATION SHEET

TITLE OF THE STUDY: ***Role of multi delay arterial spin labelling (e ASL) as a novel imaging biomarker for magnetic resonance imaging (MRI) grading of glial neoplasms as compared to T1 dynamic contrast perfusion and T2 dynamic contrast susceptibility perfusion techniques.***

Name of Institute where study undertaken: Sree Chitra Tirunal Institute of Medical Sciences & Technology(SCTIMST).

Study number: **IEC/1601 dated 09-12-2020**

Based on your symptoms, MRI brain will be done for you in the form of "Tumor protocol". It does not involve radiation. Basic MRI brain sequences along with contrast images and MR perfusion will be done. It will help usto characterize your lesion better if found any. It will also help usto predict future course of action.

You are being requested to participate in a study to evaluate the diagnostic utility of multi delay arterial spin labelling (eASL) as compared to other perfusion parameters. Participating in this study, in which only data from the investigations you have undergone for your treatment will be used, will in no way influence treatment decisions.

You will not feel any pain or discomfort during the MRI procedure. Though MRI machines generates some sound during scanning but it can be tolerated. If you are not able to tolerate the noise of MRI you will be provided with ear plugs. You will also be given Injection Gadolinium during the procedure. Though it a safe MRI contrast, in rare cases some people may have allergic reaction to the contrast.

This test is vital in diagnosis of your condition and is also the means of follow up after treatment if planned subsequently.

If you take part what will you have to do?
• For this study, we'll be using some of the data like history and other clinical details, Imaging details, treatment, outcome of the procedure, delayed follow up clinical and radiological regarding your disease and treatment which you undergo in this hospital.
• No additional cost will be incurred /no additional drugs will be used and there are no additional risks as a part of the research.
• Analysis of this data generated by your imaging is likely to give more understanding of
this disease and treatment, for the benefit of future generation. You understand that strict

confidentiality will be maintained.

Can you withdraw from this study after it starts?
Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What will happen if you develop any study related injury?
This study only involves imaging and thus we do not expect any injury to happen to you but if you do develop any side effects or problems due to the study, these will be treated at this institute by the experienced team of medical professionals. We are unable to provide any monetary compensation, however.

Will you have to pay for the study?
The study will only involve an additional sequence apart from the regular imaging protocol which will be followed in the natural process of your workup at this institute and no extra cost will be borne by you for this particular study.

What happens after the study is over?
You may or may not benefit from this study, after the study we will be able to assess the utility of clinical and angiographic outcome predictors in high risk carotid stenting and which will further guide us in choosing the patients for the carotid artery stenting procedures; it may thus benefit other patients with similar illness.

Will your personal details be kept confidential?
The results of this study may be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, please ask
Dr. Krishan Pratap Singh, Senior Resident; Dept of Neuroimaging & Interventional Neuroradiology (tel: 9637111504/9451746188) or email: drkpss009@gmail.com

or contact

Mala Ramanathan
IEC member secretary (tel: 0471-2524263)

Dr Krishan Pratap Singh (Principal Investigator)
Signature .....…

APPENDIX B

CONSENT FORM

TITLE OF THE STUDY: Role of multi delay arterial spin labelling (e ASL) as a novel imaging biomarker for magnetic resonance imaging (MRI) grading of glial neoplasms as compared to T1 dynamic contrast perfusion and T2 dynamic contrast susceptibility perfusion techniques.

Study number: IEC/1601 dated 09-12-2020

Participant's name: …… Date of Birth / Age (in years): ……..

I , Son/daughter of (Please tick boxes) In case of minor I……. F/O, M/O, G/O give my full and free consent in respect of……. for the MRI examination after having duly understanding and agree for the MRI protocol as explained to me.
Declare that I have read the above information provide to me regarding the study: "*Role of multi delay arterial spin labeling (e ASL) as a novel imaging biomarker for magnetic resonance imaging (MRI) grading of glial neoplasms as compared to T1 dynamic contrast perfusion and T2 dynamic contrast susceptibility perfusion techniques.*" and have clarified any doubts that I had.

• I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights.
• I also understand that study investigators will be using some of the data like history and other clinical details, Imaging details (CT/MRI/ CTA /MRA), outcome of the procedure, delayed follow up clinical and radiological, regarding the disease and treatment which I undergo in hospital.
• I also understand that no additional cost will be incurred /no additional drugs will be used and there are no additional risks as a part of the research.
• I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access.
• I understand that my identity will not be revealed in any information released to third parties or published.
• I voluntarily agree to take part in this study.
• I received a copy of this signed consent form.
Name:
Signature:
Date:Name of witness:
Relation to participant

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied. I have discussed the research project with the participant and explained to him or her in nontechnical terms all of the

_____

information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the participant to ask questions and that all questions asked were answered.

_____ _____

Name and Signature of Person Obtaining Consent

If there is any clarification sought by the participant : Please contact IEC member Secretary (study independent contact person), email: mala@sctimst.ac.in
Tele No : 0471- 2524234

APPENDIX C

PROFORMA

TITLE: Role of multi delay arterial spin labeling (e ASL) as a novel imaging biomarker for Magnetic resonance imaging based (MRI) grading of glial neoplasms as compared to T1 dynamic contrast perfusion and T2 dynamic contrast susceptibility perfusion techniques.

Investigator analysing the study:……
….

1. Patient ID(Anonymised for study):
2. Age:
3. Gender:

4. Comorbidities/ Risk factors:
    a. Diabetes Mellitus Yes/No
    b. Hypertension Yes/No
    c. Dyslipidemia Yes/No
    d. Cardiac disease Yes/No

5. Contraindications for MRI: *Tick Applicable*
    a. Renal dysfunction [ ]
    b. Metallic Implants [ ]
    c. Cardiac pacemakers [ ]
    d. Any other electronic device [ ]
    e. Claustrophobia [ ]

6. Clinical features:

	mptoms	s/No
	adache	
	miting	
	izures	
	cal weakness/paralysis	
	it disturbances	
	havioral changes	
	emory disturbances	

7. Duration of symptoms :

8. Clinical signs

MMSE	
Cranial Nerve Deficits	
Motor deficits	
Sensory deficits	
Reflexes	
Cerebellar signs	
Gait	

9. Follow up cases:

Time since last follow up	
Fresh symptoms	
Duration	
Revised surgery	

10. Imaging findings:

	Location	Finding/Signal changes
TWI		
T2WI		
FLAIR		
DWI/ADC		
GRE/SWI		
T1 DCE		
T2DSC		
eASL		

11. Perfusion Imaging comparison

Perfusion technoque	rCBV/aCBV	rCBF/CBF	MTT/ATT
T1DCE			
T2DSC			
eASL			

Surgery performed :
Near total / Subtotal/ Partial decompression

12. Histopathological findings

a. Grade

b. Genetic makeup of tumor(if available) and IHC

c. Overall Diagnosis

APPENDIX D

IEC PERMISSION LETTER

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेंद्रम - 695 011, केरल, भारत
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
TRIVANDRUM - 695 011, KERALA, INDIA
(एक राष्ट्रीय महत्व का संस्थान, विज्ञान एवं प्रौद्योगिकी विभाग, भारत सरकार)
(An Institution of National Importance, Department of Science and Technology, Government of India)
टेलीफॉन नं./Telephone No.: 0471-2443152 फैक्स/Fax: 0471-2446433, 2550728
ई-मेल/E-mail: sct@sctimst.ac.in वेबसाइट/Website: www.sctimst.ac.in

Institutional Ethics Committee
(IEC Regn No. ECR/189/Inst/KL/2013/RR-16)

SCT/IEC/1601/DECEMBER-2020 09.12.2020

Krishan Pratap Singh
Senior Resident
Department of IS & IR
SCTIMST, Thiruvananthapuram

Dear Dr. Krishan Pratap Singh,

Thank you for submitting documents related to your proposal titled "**ROLE OF MULTI DELAY ARTERIAL SPIN LABELLING (E ASL) AS A NOVEL IMAGING BIOMARKER FOR MAGNETIC RESONANCE IMAGING (MRI) GRADING OF GLIAL NEOPLASMS AS COMPARED TO T1 DYNAMIC CONTRAST PERFUSION AND T2 DYNAMIC CONTRAST SUSCEPTIBILITY PERFUSION TECHNIQUES** (IEC/1601)" to the IEC for review.

The following documents were reviewed:

1. List of documents for IEC
2. Covering letter addressed to the IEC, SCTIMST dated 20.10.2020 by the PI forwarded by HOD
3. Check list
4. TAC Approval Letter
5. IEC Application Form
6. Project Proposal
7. Proforma
8. Information Sheet in English
9. Information Sheet in Malayalam
10. Consent Form in English
11. Consent Form in Malayalam
12. Assent Form in English
13. Assent Form in Malayalam
14. CV of Dr. Krishan Pratap Singh with MCI number
15. CV of Dr Kesavadas C TCMC number
16. CV of Dr Bejoy Thomas with TCMC number
17. CV of Dr Krishna Kumar with TCMC number
18. CV of Dr Deepti AN with TNMC number

The following members of the Students Sub-Committee of the Institutional Ethics Committee participated in the discussions held between August 23-October 29, 2020 at the offices and residences of the members

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. R V G Menon	M Tech, PhD	Male	Lay Person (Chairman)	No
2.	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
3.	Dr. Kala Kesavan. P	MBBS, MD	Female	Basic Medical Scientist	No
4.	Smt. Sathi Nair	MA (English Literature)	Female	Lay Person	No
5.	Dr. Rema M. N	MD	Female	Basic Medical Scientist	No
6.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
7.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision
The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

APPENDIX E

PLAGIARISM CHECK REPORT

Ouriginal by Turnitin

Document Information

Analyzed document	Thesis draft final - Krishnapratap.doc (D142261857)
Submitted	7/21/2022 6:19:00 AM
Submitted by	C.Kesavadas
Submitter email	kesav@sctimst.ac.in
Similarity	1%
Analysis address	kesav.sctims@analysis.urkund.com

Sources included in the report

SA	**All India Institute of Medical Sciences Bhopal / THESIS - PERFUSION MRI IN BRAIN TUMORS.docx** Document THESIS - PERFUSION MRI IN BRAIN TUMORS.docx (D110790006) Submitted by: amit.library@aiimsbhopal.edu.in Receiver: amit.library.aiimsb@analysis.urkund.com	1
W	URL: https://radiopaedia.org/articles/cerebral-blood-volume-cbv?lang=us Fetched: 2/11/2022 12:47:12 PM	1
SA	**All India Institute of Medical Sciences Bhopal / THESIS - ROLE OF ADVANCED MRI IN INTRA-AXIAL BRAIN LESION.docx** Document THESIS - ROLE OF ADVANCED MRI IN INTRA-AXIAL BRAIN LESION.docx (D89270306) Submitted by: amit.library@aiimsbhopal.edu.in Receiver: amit.library.aiimsb@analysis.urkund.com	4
W	URL: https://radiologykey.com/mr-perfusion-imaging-asl-t2-weighted-dsc-and-t1-weighted-dce-methods/ Fetched: 7/21/2022 6:19:36 AM	1
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