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RESEARCH

Isolated restricted diffusion in glioblastoma: incidence, progression, and survival impact ‘

Jai Shankar[1] · Nikunj Patil[1] · Marco Ayroso[1] · Roman Marin[1] · Marc Del Bigio[1] · Marshall Pitz[1] · Jason Beiko[1] · Joseph Silvaggio[1] · Marco Essig[1] · Saranya Kakumanu[1] · Namita Sinha[1]

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Abstract

Background Glioblastoma (GB) is the most malignant primary brain tumor. Isolated restricted diffusion (IRD) is restricted diffusion outside the confines of enhancing tumor with no corresponding enhancement on post contrast study. The aim of our study was to prospectively assess the incidence of IRD in GB patients, determine how often these foci proceed to contrast enhancement on follow up, and analyze the survival pattern of patients with IRD.

Methods In a prospective pilot cohort study, consecutive adult patients (≥18 years old) suspected of having GB on initial MRI of brain, were included and screened for the presence of IRD. All images were independently analyzed by two experienced radiologists for inter-rater reliability. The survival pattern of patients with IRD was assessed with Cox-regression and Kaplan-Meier curve analysis.

Results Of the 52 patients (median age- 63 years; male-63.5%) included, 21% (11 of 52) exhibited foci of IRD. Inter-rater agreement on the diagnosis of IRD foci was fair (kappa = 0.29) between the two readers. Among the 11 patients with IRD, only 7 (64%) showed enhancement in the IRD focus on imaging at a median follow up time of 110 days. The Kaplan Meier analysis revealed a significant decrease ($p = 0.035$) in the survival among patients with IRD focus.

Conclusion In conclusion, IRD foci were seen in 21% of patients with GB, with 64% of these demonstrating enhancement at the IRD focus on follow up imaging. A shorter survival was associated with IRD foci.

Keywords Brain tumour · Gliobralstoma · Magnetic resonance imaging (MRI) · Isolated restricted diffusion (IRD) · Diffusion weighted imaging (DWI)

✉ Jai Shankar
shivajai1@gmail.com

Nikunj Patil
npatil@cancercare.mb.ca

Marco Ayroso
ayrosom@myumanitoba.ca

Roman Marin
roman.Marin@umanitoba.ca

Marc Del Bigio
Marc.Delbigio@umanitoba.ca

Marshall Pitz
MPITZ@cancercare.mb.ca

Jason Beiko
jbeiko@hsc.mb.ca

Joseph Silvaggio
jsilvaggio@hsc.mb.ca

Marco Essig
messig@hsc.mb.ca

Saranya Kakumanu
skakumanu@cancercare.mb.ca

Namita Sinha
drnamitasinha1@gmail.com

[1] University of Manitoba, Winnipeg, Canada

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Background

Glioblastoma (GB) is the most malignant primary brain tumor, comprising 30% of all central nervous system tumors and 70% of primary malignant brain tumors [1]. Despite significant advances in understanding tumor pathogenesis, the median overall survival of patients has increased by only 3.3 months (from 11.3 to 14.6) over 25 years, emphasizing the need for additional biomarkers, including imaging biomarkers, to identify disease progression and improve decision-making and management [2, 3].

Current imaging standards for detecting and assessing tumor progression in GB patients rely on changes in contrast-enhancing abnormalities on brain magnetic resonance imaging (MRI) [4]. These abnormalities are examined to estimate prognosis and guide management [4]. Advanced MRI techniques, such as MR spectroscopy and perfusion, has been proposed for characterizing GB [1]. However, these techniques require complex post-processing of the images and can be challenging to interpret. MRI features have been suggested to be associated with isocitrate dehydrogenase (IDH) mutation status but the association of diffusion-weighted imaging (DWI) findings with IDH status in GB remains unclear [5, 6]. Isolated restricted diffusion (IRD), defined as restricted diffusion outside the confines of enhancing tumor with no corresponding enhancement on post contrast study, has been observed in retrospective studies, but with inconsistent results [7, 8].

The aim of our study was to prospectively assess the incidence of IRD in GB patients, determine how often these foci proceed to contrast enhancement on follow up, investigate the molecular alteration associated with GB showing IRD and analyze the survival pattern of patients with IRD.

Method

A prospective pilot cohort study was conducted on patients diagnosed with GB, approved by the University of Manitoba research ethics board (File number- H2021:246), in accordance with the Declaration of Helsinki. Informed consent was waived by our ethics board. Consecutive adult patients (≥ 18 years old) suspected of having GB based on initial MRI of brain, between December 2021 and March 2023, were included and screened for the presence of IRD. These patients underwent MRI of the brain on 1.5 T magnet (Siemens Somatom) using a standard brain tumor protocol, including- T1-, T2, FLAIR, DWI, Post gadolinium T1 axial, and MPRAGE images (supplemental material). The reader estimated the location of the tumor within the brain lobes by identifying the site of maximum tumor volume. Multifocality was diagnosed based on post-contrast T1 images, which revealed multiple enhancing foci separated by non-enhancing brain tissue. The tumor volume was calculated on post-gadolinium MPRAGE images, utilizing a volumetric tracing method within the software provided by the vendor. The extent of resection was assessed through post-operative MRI conducted within 48 h following the surgery.

The IRD (Fig. 1) was defined as a focus, whether single or multiple, exhibiting restricted diffusion, and lacking enhancement on the post contrast T1 images. All images were independently analyzed by two experienced radiologists, both with expertise in brain MRI analysis, to identify the presence of IRD. Inter-rater reliability was calculated, and any disagreements regarding the presence of IRD were resolved by consensus.

Patients with IRD were prospectively assessed for the feasibility and safety of targeted biopsy from this focus. The decision to proceed with targeted biopsy was made based on the location and surgical approach deemed safe and feasible by our neurosurgeon, with the primary goal of avoiding any undue risk for research purpose. Eligible patients were approached for consent for possible biopsy for research purposes. All patients underwent surgical resection or biopsy as per standard of care. The extent of surgical resection was defined by the readers as either gross total resection, partial resection, or biopsy only.

The biopsy samples obtained underwent standard of care histopathological examination and molecular testing to establish the pathological and molecular diagnosis of these tumors. The neuropathologic examination was conducted and reviewed by neuropathologists (NS and MRB). Initial histologic evaluation included examination of Hematoxylin and Eosin-stained sections followed by immunohistochemical studies for GFAP, mutant IDH1 R132H, ATRX, and p53. Sequencing was performed only in IDH1 R132H negative GB who are younger than 55 years as elderly patients were considered IDH-wildtype if immunohistochemistry for mutant IDH1 R132H was negative as per WHO classification 2021 [8]. Mutations in IDH1/2, TP53, TERT promoter, PTEN, BRAF V600E, and Histone (H3 F3 A, H3 F3B, HIST1H3B) genes were tested using a mass spectrometry-based assay (Agena Mass ARRAY Neuropathology Panel at Calgary Laboratory Services). Some of the glioblastoma could not be classified by standard molecular testing (immunohistochemistry and mass array or limited NGS panel). In those cases, methylation analysis was pursued for definitive diagnosis, which was performed at National Institutes of Health, Bethesda MD. All CNS neoplasms with a diagnosis of IDH-wild type glioblastoma were included in this study. O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation was determined by pyrosequencing.

All patients underwent further management with radiotherapy and consideration of concurrent temozolomide

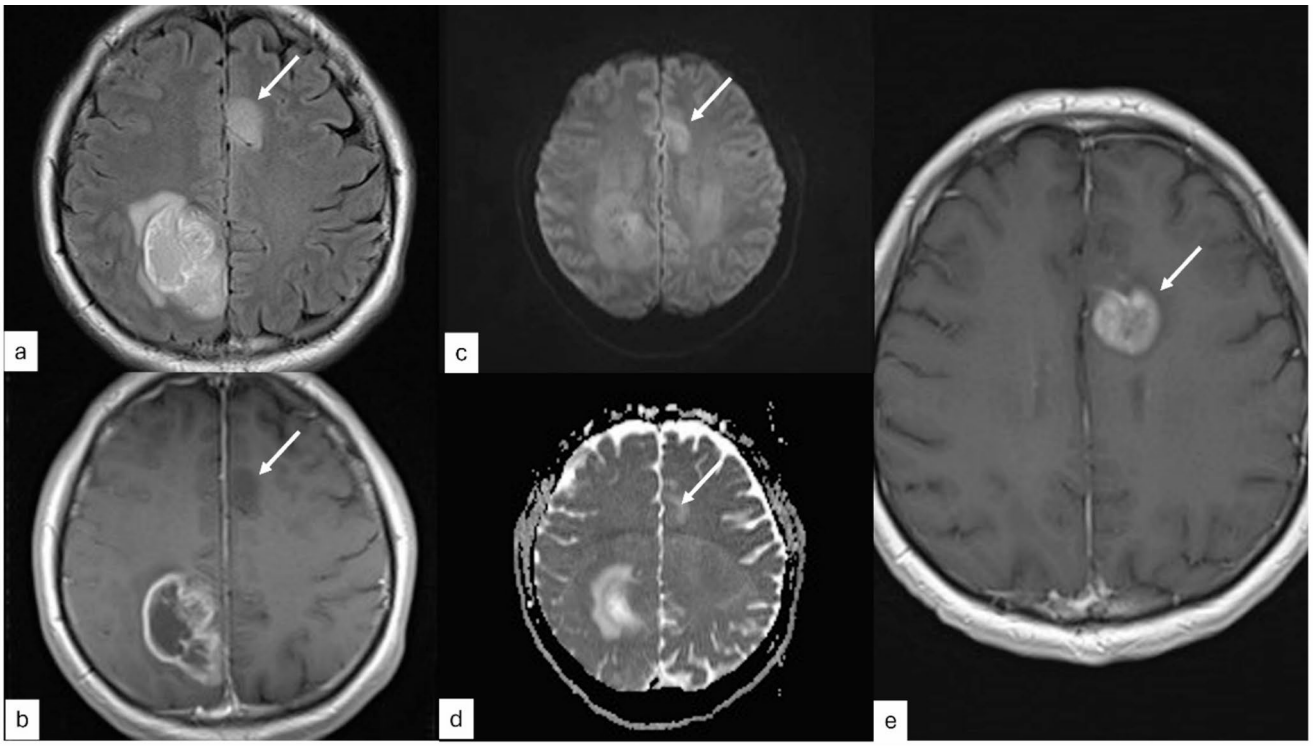

Fig. 1 This patient had heterogenous enhancing necrotic (a and b) glioblastoma seen in the right frontal-parietal lobe on the diagnostic imaging. This also showed the isolated restricted diffusion (IRD) focus (arrows) in the left medial frontal lobe, showed no enhancement on post contrast T1-weighted images (b) and showed mildly restricted diffusion (c and d). Follow up MRI of the same patient 3 months later, showed enhancement in the region of IRD on the post gadolinium T1-weighted images (e)

followed by adjuvant temozolomide according to institutional standard. Clinical follow-up occurred at least monthly and MRI every 8–12 weeks based on clinical status. Tumor recurrence was detected during follow up MRI scans. Follow up assessments were included up to the end of the study in October 2023.

Statistical analysis

The incidence of IRD was assessed as simple proportion. Additionally, the proportion of patients in whom IRD progressed to contrast enhancement was calculated. Molecular results from biopsy were assessed using descriptive analysis. The survival pattern of patients with IRD was assessed with Cox-regression and Kaplan-Meier curve analysis. Assuming that some patients will die in the early follow up period irrespective to best possible management, a landmark analysis [11] was used to determine the landmark time on follow up to exclude the patient who died before this time points. 3 time-points of 45, 75 and 90 days were tested using Kaplan-Meier curve analysis to select the optimal landmark time. To minimize the number of patients excluded, 75 days was found to be the optimal landmark time for this study. This excluded 5 patients, one of these died from post-surgical complications and the other 4 did not receive any chemo or radiotherapy. Inter-rater reliability was assessed using Cohen's kappa. All statistical analyses were conducted in R-studio (R version 4.3.2, RStudio, PBC, Boston, MA) using the tidyverse package for data management and survminer for figures [12–15].

Results

A total of 52 patients (median age- 63 years; male-33, 63.5%) were included in the study. Table 1 provides a summary of the demographics, baseline clinical characteristics, and outcome of study population. Approximately 21% (11 of 52) of patients with GB exhibited foci of IRD. Patients with and without IRD were comparable, except for those with IRD, who had a significantly larger tumor volume ($p = 0.025$) and showed a trend towards shorter survival ($p = 0.082$). Inter-rater agreement on the diagnosis of IRD foci was fair (kappa = 0.29) between the two readers. Two patients showed more than one focus of IRD.

Among the 11 patients with IRD, only 7 (64%) showed enhancement in the IRD focus on imaging at a median follow up time of 110 days (interquartile range [IQR] 92–217 days). The median survival time for patients who died by the end of study was 128 days (IQR 73–194 days), while the

Table 1 Demographics, baseline clinical characteristics, and outcome of study population

	Total (n = 52)	Non-IRD (n = 41)	IRD (n = 11)	P-value
Age, median (IQR), y	63 (56–68)	63 (59–68)	60 (50–68)	0.234
Sex, n (%)				0.472
Male	33 (63.5)	25 (61.0)	8 (72.7)	
Female	19 (36.5)	16 (39.0)	3 (27.3)	
Death, n (%)	17 (32.7)	11 (26.8)	6 (54.5)	0.082
Extent of resection, n (%)				0.105
Gross total resection	28 (53.8)	25 (61.0)	3 (27.3)	
Partial resection	7 (13.5)	4 (9.8)	3 (27.3)	
Biopsy only	17 (32.7)	12 (29.3)	5 (45.5)	
Tumor volume, mean±SD, cc	34.9±32.2	29.8±27.9	54.0±40.8	0.025
Multifocality, n (%)	20 (38.5)	16 (39.0)	4 (36.4)	0.916
Chemotherapy, n = 51 (%)	41 (80.4)	32 (80.0)	9 (81.8)	0.893
Radiotherapy, n = 51 (%)	47 (92.1)	36 (90.0)	11 (100)	0.275
Molecular markers, n (%)				
IDHwt	51/51 (100)	40/40 (100)	11/11 (100)	
ATRX retained	51/51 (100)	40/40 (100)	11/11 (100)	
TP53 mutation	9/27 (33.3)	8/22 (36.4)	1/5 (20.0)	0.861
TERT promoter mutation	20/20 (100)	15/15 (100)	5/5 (100)	
MGMT promoter methylation	22/47 (46.8)	17/36 (47.2)	5/11 (45.4)	0.918
GB location, n (%)				0.892
Frontal	30 (57.7)	22 (53.7)	8 (72.7)	
Corpus callosum	3 (5.8)	2 (4.9)	1 (9.1)	
Parietal	14 (26.9)	11 (26.8)	3 (27.3)	
Temporal	19 (36.5)	15 (36.6)	4 (36.4)	
Occipital	4 (7.7)	4 (9.8)	0 (0)	
Thalamus	4 (7.7)	3 (7.3)	1 (9.1)	

IRD- Isolated restricted diffusion; IQR- Inter-quartile range; n= number; SD- standard deviation; cc =cubic centimeter IDH- isocitrate dehydrogenase; ATRX- alpha-thalassemia/mental retardation syndrome X-linked; TP53- tumor protein 53; TERT- Telomerase reverse transcriptase gene; MGMT- O6-methylguanine-DNA-methyltransferase promoter

median follow-up time for those alive at the end of study was 144 days (IQR 119–320 days).

Table 2 presents the overall probability of survival at 1 year, which was lower in patients with IRD compared to those who did not have IRD. Among those with IRD, the probability of survival was lower in males and those with multi-focal GB. Note that all patients with IRD received radiotherapy.

Cox regression analysis was conducted to explore the association of survival of GB patients, first focusing on

Table 2 Median survival time and at 1 year time point survival by IRD status and characteristics

	Survival probability at t = 1 year (%) (95% C.I.)	
	Non IRD (n = 41)	IRD (n = 11)
Overall	64.9 (49.3–85.4)	43.3 (19.6–95.6)
Sex		
Male	64.3 (44.3–93.2)	34.3 (11.2–100)
Female	65.6 (44.6–96.5)	66.7 (30.0–100)
Chemotherapy		
Yes	73.7 (56.0–96.9)	47.6 (22.0–100)
No	40.0 (18.7–85.5)	50.0 (12.5–100)
Radiotherapy		
Yes	68.2 (51.6–90.2)	43.3 (19.6–95.6)
No	50.0 (18.8–100)	-
Focality		
Uni-focal	80.4 (64.4, 100)	50.0 (22.5–100)
Multi-focal	37.6 (16.3, 86.9)	37.5 (8.4–100)

IRD- isolated restricted diffusion; C.I.- confidence interval

IRD presence alone, and then controlling for other risk factors including age (scaled at 10-year intervals), tumor volume, first line chemotherapy, radiotherapy, and multifocality (Fig. 2). For patients with IRD focus alone, there was a non-significant (p = 0.29) 1.8 (95% CI 0.65–4.91) times increase in hazard of death. However, in the multivariable analysis, patients receiving chemotherapy experienced an 87% decrease in the hazard of death (p < 0.001). Patients with an IRD focus had a 3.2-fold increase in the hazard of death compared to those without IRD (p = 0.043). Multifocal tumor presence was associated with a 3-fold increase in the hazard of death (p = 0.044). These findings highlight the significance of IRD presence, chemotherapy, and tumor multifocality as prognostic factors in GB patient survival.

The Kaplan Meier analysis (Fig. 3) at a landmark time of 75 days revealed a significant decrease (p = 0.035) in the survival was observed among patients with IRD focus. This indicates that beyond the initial 75 days, there was a notable difference in survival between patients with and without IRD focus.

Discussion

Our study represents one of the first prospective investigations highlighting the role of IRD focus as an imaging biomarker influencing the probability of survival of patients with GB. Patients with IRD focus demonstrated significantly decreased survival compared to those without, particularly beyond 75 days post-diagnosis. Furthermore, our study revealed a decreased hazard ratio associated with chemotherapy, signifying its significant contribution in survival. Conversely, an increased hazard ratio for mortality

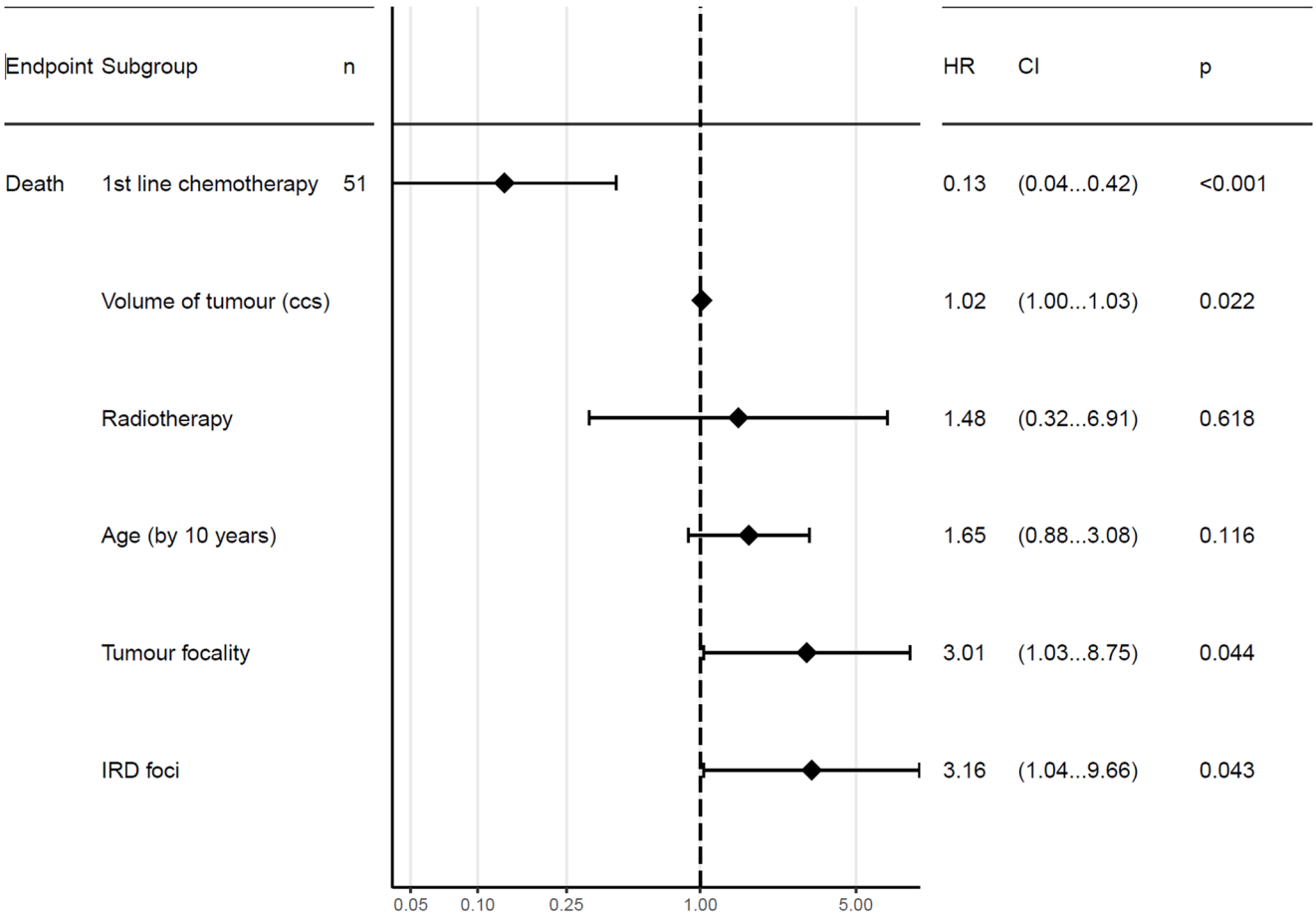

Fig. 2 Forest plot displays the results of Cox-proportional hazard ratio analysis, illustrating the significant survival benefits associated with various risk factors in GB patients including those on chemotherapy and increased hazard of death for those with IRD focus and those with multi-focal tumor. HR- hazard ratio; CI- confidence interval; ccs- cubic centimeters

was observed with multifocal tumors, emphasizing their substantial role in elevating the risk of death in GB patients.

Previous studies have described IRD focus in GB, noting that it often precedes enhancement on post-gadolinium studies [7, 9]. The reported incidence of IRD has ranged from 13 to 39% in retrospective studies [7, 9]. In our prospective study, we found IRD foci in 21% of patients with GB. While previous retrospective studies have suggested potential survival benefit in patients with IRD, our study provided robust evidence showing that GB patients with IRD foci had significantly shorter survival rates [7]. Therefore, IRD foci may serve as an imaging biomarker indicative of a worse prognosis in GB patients. It is important to note that ours is the first study after the re-classification of gliomas by the World Health Organization, reemphasizing the value of IRD focus in GB patients [15].

Enhancement on follow up images has been reported in the range of 25 to 96.3% in previous studies, typically occurring after an average of 144 to 120 days respectively [7, 9]. In our study, we observed enhancement in the IRD focus on follow up in 64% of patients, with an average time interval of 110 days, consistent with findings from other studies. This consistent observation suggests that IRD foci may indeed represent a precursor to enhancing tumor formation. However, confirmation of this hypothesis would require targeted biopsy from the IRD foci. Although, we planned to conduct targeted biopsy during standard of care surgery or biopsy, ethical considerations prevented its implementation due to the additional risks associated with biopsying yet-to-be proven GB foci solely for research purposes, as deemed by our neurosurgeons.

One of the advantages of IRD detection is its simplicity, as it does not require additional image sequences or complicated post-processing. However, despite this advantage, these foci of IRD are often overlooked in most institutions, or if identified, they are not recognized as foci of GB. Consequently, targeted tumor management strategies typically do not include these foci, leading to untreated or untargeted areas that serve as sites for early recurrence. By acknowledging these IRD foci on diagnostic MRI imaging as potential tumor foci, a significant delay can be avoided in targeted treatment. Our study demonstrated that GB patients with

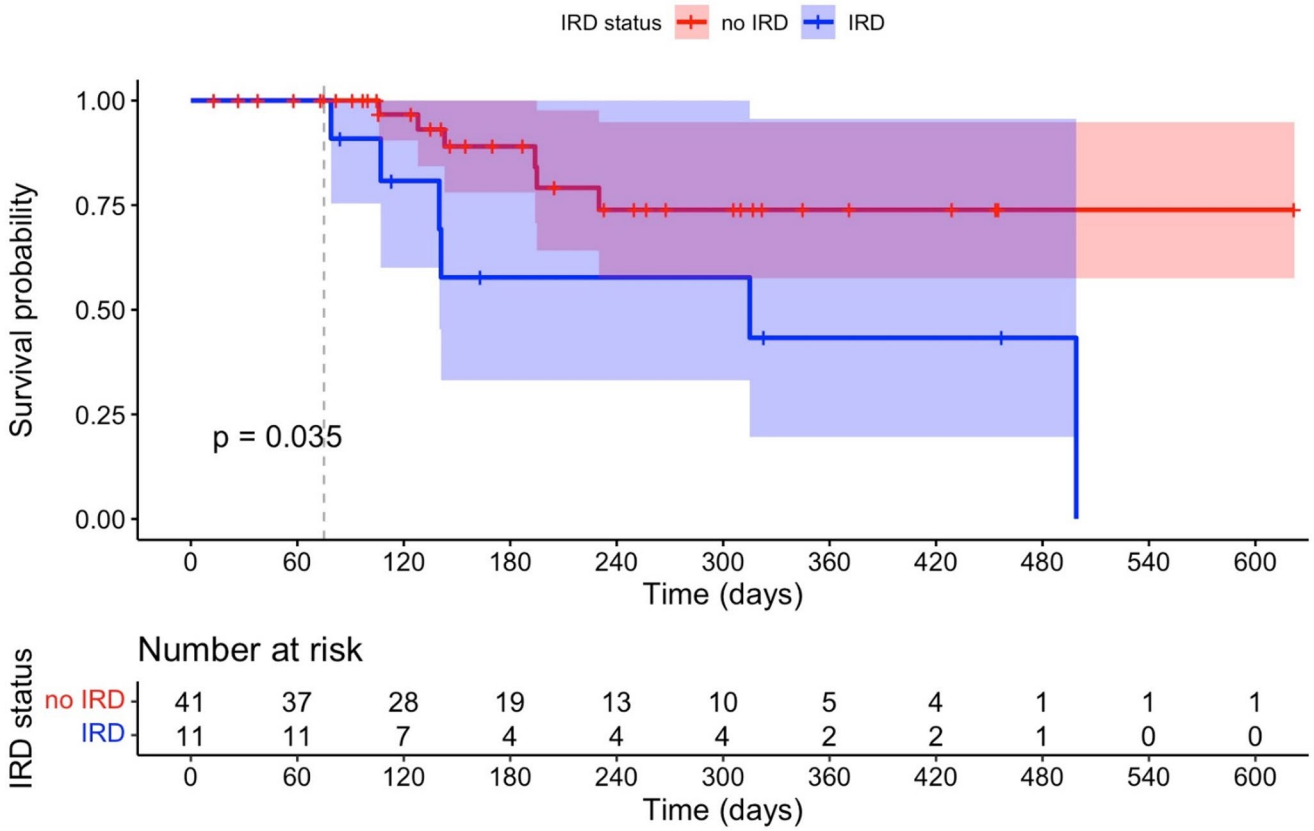

Fig. 3 The Kaplan Meier curve depicts survival of patients with GB with and without IRD demonstrates a significant survival benefit in those without IRD beyond the first 75 days of the study period. IRD- isolated restricted diffusion

IRD focus on their diagnostic MRI had a 3.6-fold increase in hazard of death ($p = 0.041$) relative to those without IRD focus, underscoring the importance of recognizing and addressing these foci in clinical practice.

Multiple molecular alterations have been studied for their association with the biological behaviour of GB including mutations in various genes [10]. However, in our study, none of these molecular markers were found to be significantly associated with GB with IRD foci.

Despite being the first prospective study on IRD focus in GB patients, our study had limitations including a small sample size and short follow up duration. We did only minimal molecular analysis as per our institutional standard. Additionally, we were unable to conduct targeted biopsy from IRD focus and we only utilized molecular markers used in routine clinical care. Further studies should explore a broad range of molecular markers to investigate any potential association with IRD foci. Extent of resection was not significantly associated with overall survival of the patients in our study, likely due to the small sample size.

Conclusion

In conclusion, IRD foci were seen in 21% of patients with GB, with 64% of these demonstrating enhancement at the IRD focus on follow up imaging. Importantly, we observed an increased hazard of death associated with IRD foci. These findings underscore the potential significance of IRD foci as an imaging biomarker for predicting prognosis in GB patients. Further research is warranted to validate these findings and explore the clinical implications of IRD detection in the management of GB.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00234-025-03672-4.

Author contributions Statement of authorship- JS- conceptualized, secured funding, conducted the study, collected data, and wrote the first draft; NS- conceptualized, secured funding, conducted the study, collected data, and editing of manuscript; MA- helped collect data, did statistical analysis and reviewed the final draft; RM- Collected the data, second reader for the MRIs and reviewed the final draft; NP- Collected the data and reviewed the final draft; MRD– neuropathology diagnoses and editing of manuscript; MP, SK, JB, JS- collaborator and editing of manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical statement The study was approved by the University of Manitoba research ethics board (File number- H2021:246), in accordance with the Declaration of Helsinki. Informed consent was waived by our ethics board.

Competing interests The authors declare no competing interests.

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