



Review Article

Blood-brain barrier permeability following conventional photon radiotherapy – A systematic review and meta-analysis of clinical and preclinical studies

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ABSTRACT

Radiotherapy (RT) is a cornerstone treatment strategy for brain tumours. Besides cytotoxicity, RT can cause disruption of the blood–brain barrier (BBB), resulting in an increased permeability into the surrounding brain parenchyma. Although this effect is generally acknowledged, it remains unclear how and to what extent different radiation schemes affect BBB integrity. The aim of this systematic review and meta-analysis is to investigate the effect of photon RT regimens on BBB permeability, including its reversibility, in clinical and preclinical studies. We systematically reviewed relevant clinical and preclinical literature in PubMed, Embase, and Cochrane search engines. A total of 69 included studies (20 clinical, 49 preclinical) were qualitatively and quantitatively analysed by meta-analysis and evaluated on key determinants of RT-induced BBB permeability in different disease types and RT protocols. Qualitative data synthesis showed that 35% of the included clinical studies reported BBB disruption following RT, whereas 30% were inconclusive. Interestingly, no compelling differences were observed between studies with different calculated biological effective doses based on the fractionation schemes and cumulative doses; however, increased BBB disruption was noted during patient follow-up after treatment. Qualitative analysis of preclinical studies showed RT BBB disruption in 78% of the included studies, which was significantly confirmed by meta-analysis ($p < 0.01$). Of note, a high risk of bias, publication bias and a high heterogeneity across the studies was observed. This systematic review and meta-analysis sheds light on the impact of RT protocols on BBB integrity and opens the discussion for integrating this factor in the decision-making process of future RT, with better study of its occurrence and influence on concomitant or adjuvant therapies.

Abbreviations: BBB, Blood-brain barrier; RT, Radiotherapy; MRI, Magnetic resonance imaging (MRI); LC-MS, Liquid chromatography-mass spectrometry; CT, Computed tomography; PET, Positron emission tomography; NMR, Nuclear magnetic resonance; NSCLC, Non-small cell lung cancer; AVM, Arteriovenous malformations; WBRT, Whole-brain RT; SRS, Stereotactic radiosurgery; EB, Evans Blue.

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Introduction

Homeostasis of the central nervous system is sustained by the blood–brain barrier (BBB), also known as the neurovascular unit, that protects the brain tissue from potential harmful pathogens or substances. The BBB has a restricted permeability due to being both (1) a physical barrier formed by tight junctions between the endothelial cells that are surrounded by pericytes and a basal membrane and (2) a functional barrier where ATP-binding cassette efflux transporters have the potential to pump a large spectrum of molecules from the extravascular interstitium back into the blood stream [1]. These anatomical and functional features result in the exclusion of large substances (greater than 500 Da) [2] and over 98% of all small molecules from the brain, amongst which are chemotherapeutics and targeted therapies [3]. Hence, the BBB limits the overall treatment efficacy in brain malignancies because of the reduced, if not absent, drug delivery into the brain parenchyma [4,5].

Meanwhile, radiotherapy (RT), after maximal safe surgery, is still a cornerstone for the treatment of brain tumours such as high-grade gliomas and diffuse midline gliomas. While conventional photon radiation therapy has been applied as a treatment modality for roughly 50% of all cancer patients [6], technological advances, like image-guided RT or different particle radiations (electron, proton, neutron beams) have improved the specificity of the treatment modality and enabled better and precise radiation treatment of the tumours while sparing the healthy tissue [7]. Despite this technical progress, large volumes of the functioning brain tissue have to be radiated due to the highly infiltrative nature of most primary brain tumours [8]. Due to low radio-sensitivity of certain tumours [9], often high dosages are needed to achieve the maximal anti-tumour effect, which also cause damage to the surrounding normal tissue. Vascular endothelial cells are one of the most radio-sensitive cells and consequently the brain vasculature is prone to be affected by radiation [10].

There is evidence that BBB integrity is altered after the application of RT leading to both reversible and irreversible tissue damage for the patient. Whereas early brain damage caused by radiation is mostly reversible, later, more chronic injuries, manifesting at the earliest three months after treatment, can cause (sometimes severe) problems for the patient [11]. It is assumed that cellular and vascular responses of the BBB upon RT are mediated by astrogliosis and endothelial ultrastructural changes [12]. These changes to the BBB can eventually lead to seizures, brain inflammation and leaky vessels causing haemorrhage and/or stroke [13–15]. Furthermore, it has been postulated that mostly RT with cumulative doses between 20 and 30 Gy increases BBB permeability [16], however the actual impact of RT protocols (fractions, frequency) on BBB integrity remains to be elucidated, in order to support decision-making with regard to the prevention of toxicity and the use of concomitant chemotherapeutic therapies. To the best of our knowledge, there is no clear consensus on to what extent radiation doses and fractionation schemes affect BBB integrity. Subsequently, it is unknown to what extent confounding factors, such as the patients' clinical picture, interplay in the evaluation of RT-induced effects on BBB permeability. In addition, ascertainment of the kinetics of BBB opening can be helpful to decide on dosing and timing for drugs that are not expected to cross an intact BBB.

The aim of this study is therefore to provide a thorough review of clinical and preclinical studies that have ascertained the effect of conventional photon RT on BBB permeability and its reversibility following different RT regimens. A systematic review of all available clinical and preclinical literature was performed, in three different search engines. Data were processed by qualitative analysis and meta-analysis to statistically assess the extent of BBB disruption following photon radiation in comparison to a non-irradiated control group.

Methodology

Data sources and literature search

A literature search was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement [17]. To identify all relevant publications, systematic searches in the bibliographic databases PubMed, Embase, and The Cochrane Library (via Wiley) were performed on April 24th, 2020, without any restrictions on publication date. Search terms were based on two key words; “Radiotherapy” and “Blood-brain barrier” and included controlled terms (MeSH in PubMed and Emtree in Embase), as well as free text terms. The full search strategies for all databases can be found in the [supplementary data](#), Table S1, S2, and S3.

Study selection and in- and exclusion criteria

All abstracts from the search were screened and assessed for their relevance in this study. Upon inclusion and abstract screening, full articles were examined based on the in- and exclusion criteria, see Table S4. To emphasize, the articles were screened for conventional photon RT, indicated as “RT” in the rest of the article. For both screening levels all studies were evaluated by two independent investigators.

Risk of bias of individual studies and publication bias assessment

Risk of bias was determined by the Cochrane Risk of Bias tool for the clinical studies [18] and by the SYRCLE Risk of Bias tool for the pre-clinical studies [19]. Parameters chosen were based on the objectives of this review and the characteristics of all included studies. “Other bias” includes all other potential sources of bias, not included in the pre-defined parameters. Scoring of the studies was performed by two independent investigators until a unanimous result was achieved. Risk of bias graphs were established by Review Manager 5.3 (The Cochrane Community, the Nordic Cochrane Centre: Copenhagen, 2014) [20]. To estimate publication bias, a funnel plot was created in Rstudio [21] with the Metaviz package [22] and an Eggers test was performed [23]. Possible missing studies were imputed using the trim-and-fill method [24].

Data collection

For the qualitative and quantitative analyses, studies were classified based on 1) the disease type of the subjects (clinical only), 2) the pre-clinical model (preclinical only), 3) the type of radiation used, 4) the biological effective dose (BED) (≤ 50 Gy, 50–100 Gy and ≤ 100 Gy), 5) the readout technique that measured BBB disruption, and 6) the time-point(s) which BBB disruption was measured (follow-up time). To allow for better interstudy comparison and analysis, per study both the RT fractionation scheme and cumulative radiation dose were used to calculate the BED based on the linear-quadratic formula by Fowler et al (1989) [25] with an α - β of 3 (patients with solid tumours and animals) or 10 (patients with AVM and leukaemia). The timing of the occurrence of BBB disruption by RT, described in the clinical studies was classified based on the radiation injury classification of Greene-Schloesser et al (2012) [26], as follows: 1) acute effects (within one month), 2) early delayed effects (within 1–6 months), and 3) late delayed effects (after 6 months of radiation) [26]. For preclinical studies the classification of Wei et al (2016) and Collins et al (2017) was used: 1) acute effects (within 4 weeks), 2) early delayed effects (within 4–12 weeks), and (3) late delayed effects (after 12 weeks) [27,28]. For the quantitative analyses, data was extracted from the studies either directly or using ImageJ [29] as a digital ruler for the figures.

Statistical analysis

Analyses were conducted using Review Manager 5.3 software (The Cochrane Community) [20]. The effect of RT on BBB permeability was assessed based on continuous variables found in the included studies, as described in Table S4. Only studies containing a treatment and control group were eligible for meta-analysis. In the meta-analysis, random-effect models were applied because of anticipated heterogeneity [30] between studies with inverse-variance weighting to obtain the summary effect size. The summary effect measurement was calculated as the standard mean difference (SMD) with the corresponding 95% confidence interval (CI). Because most studies were composed of multiple experimental groups, the effect measurement was calculated at group level (instead of study level). Forest plots were generated based on the following parameters: 1) animal model, 2) BED, 3) read-out technique of BBB disruption and 4) follow-up time after radiation for all included quantitative preclinical studies. Subgroup analysis was applied if groups contained at least five studies or more. Heterogeneity was calculated by means of the dispersion index of effect sizes I^2 . Publication bias was studied using Funnel plots, the Egger method and trim-and-fill analysis.

Results

Search results

A total of 4883 unique studies were screened, of which 215 studies deemed eligible (Fig. 1). After full text assessment, 20 clinical studies and 49 preclinical studies could be included for qualitative analysis. The

meta-analysis encompassed 29 preclinical studies. The remaining 20 preclinical studies were excluded because of missing information regarding the effect size, group size, or any procedural information. None of the clinical studies could be included in the meta-analysis, since none of these studies included randomized control groups.

Description of the included studies

The 20 relevant clinical studies were published between 1979 and 2018 (Table 1). Of these 20 studies, four included patients diagnosed with arteriovenous malformations (AVM) [31–34], four described patients suffering from haematological cancers [35–38], one focused on nasopharyngeal cancer [39], six included primary brain tumour patients [40–46] and four studies included patients diagnosed with brain metastases of other cancer types [47–50]. The 49 preclinical studies were published between 1964 and 2019, consisting of different animal models (Table 2). The majority of the studies, i.e., 27 (55%), investigate BBB disruption in rats [51–77]. In addition, eleven (22%) studies use mice [12,78–87], five involve rabbits [88–92], two include dogs [93,94], two describe monkeys [95,96], one article studied the effects of RT on the BBB in sharks [97] and one in pigs [98].

Risk of bias and reported quality

The risk of bias for individual studies was assessed separately for clinical and preclinical studies. For clinical studies, a high or unclear risk of bias was found for seven of the twelve scoring criteria (Fig. S1). Conversely, a low risk of 75%, 75%, 60% and 55% was scored for the

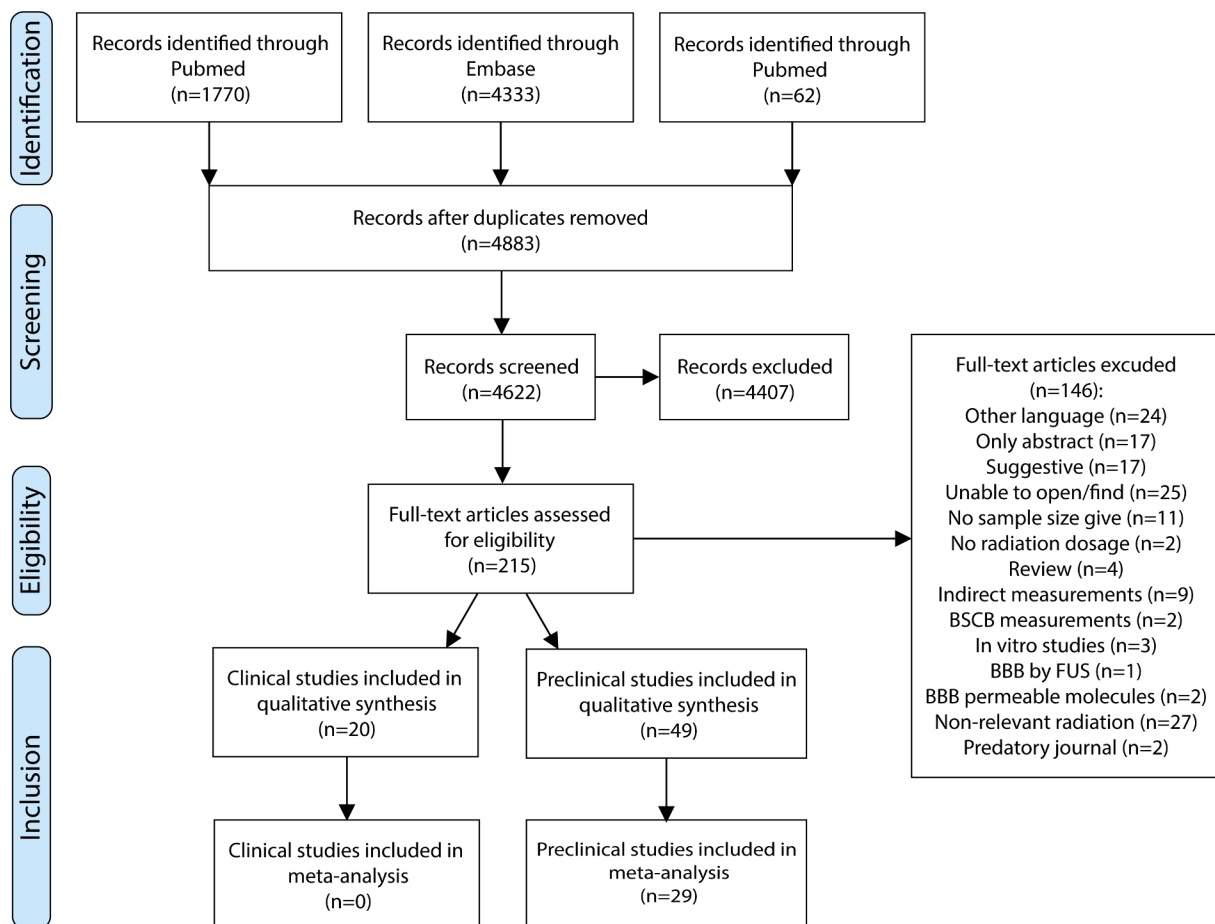


Fig. 1. PRISM flow chart of study selection. After selection and filtering of a total of 4883 studies, 215 studies were included in this study, of which 20 clinical studies were evaluated for inclusion in the subsequent qualitative analysis and no clinical study was suited for meta-analysis. 49 preclinical studies were qualitatively analysed, of which 29 studies were included in the meta-analysis.

Table 1

Clinical studies included in the qualitative synthesis and the key parameters of interest in this review article.

	Author, year	Disease type	Type of radiotherapy	Number of fractions	Fraction dose	Total dose	Biological Effective Dose	Readout technique	Age	Time	BBB disruption
1	Lim et al, 2018	Supratentorial glioblastoma	RT	?	?	45 or 61.1 Gy	?	MRI	Adults	4.2 months	Yes
2	Okawa et al, 2018	Brain metastases (NSCLC)	WBRT	?	?	30 Gy	?	LC-MS	Adults	3 weeks	Yes
3	Teng et al, 2017	Brain metastases	WBRT/SRS	Single, 10 or 15	3 or 2.5 Gy	15, 18, 24, 30, or 37.5 Gy	37.5, 39, 46.9, 50.4 or 81.6 Gy	MRI	Adults	1 month	Yes
4	Fang et al, 2015	Brain metastases (NSCLC)	WBRT	10	3 Gy	30 Gy	39 Gy	LC-MS	Adults	29 days	No
5	Farjam et al, 2015	Low-grade glioma	Conformal or intensity-modulated RT	28 – 33	1.8 Gy	50.4–59.4 Gy	59.5–70.1 Gy	MRI	Adults	18 months	Yes
6	Moraes et al, 2015	AVM	RT/SRS	?	?	10–22.5 Gy	?	MRI/CT	Adults	greater than 6 months	Unclear
7	Parkhutik et al, 2012	AVM	SRS	Single dose	–	24 Gy	216 Gy	MRI	Adults	63 months	Unclear
8	Cao et al, 2009	Low-grade glioma	Conformal RT	28 – 33	1.8 Gy	50.4–59.4 Gy	59.5–70.1 Gy	MRI	Adults	6 months	Unclear
9	Matulewicz et al, 2006	Glioma (high and low grade)	RT	30	2 Gy	60 Gy	72 Gy	NMR spectroscopy	Adults	24 months	Unclear
10	Tu et al, 2006	AVM	SRS	Single dose	–	18–20 Gy	126–153.3 Gy	Electron microscopy	Adults/children	64 months	Yes
11	Wu et al, 2006	Glioblastoma	RT	28	1.6 Gy	45 Gy	52.2 Gy	MRI/CT	?	5–10 days	No
12	Cao et al, 2005	High grade glioma	Conformal RT	35	2 Gy	70 Gy	84 Gy	MRI	Adults	6 months	Unclear
13	Levegrün et al, 2004	AVM	SRS	Single dose	–	19 Gy	139.3 Gy	MRI	Adults/children	26.8 months	Yes
14	Chan et al, 1999	Nasopharyngeal carcinoma	RT	?	?	66–71.2 Gy	?	MRI	Adults	4.4 years	Yes
15	Riccardi et al, 1998	Acute leukemia	RT	10 or 12	1.8 Gy	18 or 24 Gy	28.8 or 38.4 Gy	LC-MS	Children	24 h	No
16	Ott et al, 1991	Intracranial lymphoma	RT	?	?	30 or 40 Gy	?	PET	Adults	21 weeks	No
17	Riccardi et al, 1991	Acute leukemia	RT	?	?	18 or 24 Gy	?	LC-MS	Children	1 year	No
18	Qin et al, 1990	Intracranial tumors	RT	15 or 20	2 Gy	30 or 40 Gy	36 or 48 Gy	CT	?	8 months	Unclear
19	Jarden et al, 1985	Brain metastases	WBRT	6 or 10	2, 3, or 4/6 Gy	20, or 30 Gy	24, 34 or 44.4 Gy	PET	Adults	72 h	No
20	Seshadri et al, 1979	Acute leukemia/non-hodgkin's lymphoma	RT	12 or 16	1.5 or 2 Gy	24 Gy	36 or 40 Gy	LC-MS	Adults/children	48 h	No

Abbreviations: NSCLC, non-small cell lung cancer; AVM, arteriovenous malformations; RT, radiotherapy; WBRT, whole-brain RT; SRS, stereotactic radiosurgery; MRI, magnetic resonance imaging; LC-MS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; CT, computed tomography; PET, positron emission tomography.

categories “Incomplete outcome data”, “Selective reporting”, “Reliability of outcome measurements” and “Timing similarity of outcome assessment” respectively, while no other bias is found. Preclinical studies were assessed with a high or unclear risk of bias for six of the ten scoring criteria (Fig. S2). A low risk of bias was assigned in 63%, 63% and 67% of the studies for the scoring criteria: “Baseline characteristics”, “Incomplete outcome data” and “Selective outcome reporting”, respectively. Three studies were found to have a high risk of “Other bias” for the following reasons: 1) “Missing statistics”, 2) “Missing group sizes”, and 3) “Fluctuating follow-up times”.

Effect of RT on BBB permeability

Clinical data - qualitative analysis

Of the 20 clinical studies that investigated BBB integrity after RT, fifteen (75%) were performed in adults, three [32,33,38] (15%) in both adult and paediatric patients, and two [35,36] (10%) studied the effect of RT on the BBB in children only (Table 1). Seven out of the 20 studies (35%) reported alterations of BBB permeability [32,33,39–41,47,50], six studies (30%) observed a shallow but unclear effect

[31,34,42–44,46], whereas seven (35%) do not detect any effect [35–38,45,48,49] (Fig. 2A).

Disease type

Two of the six studies including glioma and two of the four studies with patients suffering brain metastases observed BBB permeability after treatment (Fig. 2B). None of the studies in which patients were treated with RT for a haematological disease reported any alteration of BBB integrity after treatment. Causal links between the application of radiation and the impact on the BBB in brain tumours and exposed non-tumour tissue, could not be established because of the design of the clinical studies.

Biological effective dose

In only one of the six studies [47], in which patients were treated with a BED of ≤ 50 Gy [47], and two of the six studies [41,47] with a BED of 50–100 Gy, an increase in BBB permeability was observed (Fig. 2C). Two of these studies, Farjam et al (2015) [41] (yes) and Cao et al (2009) [44] (unclear), noticed a peak in permeability at 1–1.5 months, which was reversed over time. Interestingly, in both studies

Table 2

Preclinical studies included in the qualitative synthesis and the key parameters of interest in this review article.

	Author, year	Preclinical model	Type of radiotherapy	Number of fractions	Fraction dose	Total dose	Biological Effective Dose	Readout technique	Time	BBB disruption	meta-analysis
1	Jost et al, 2019	Rats	RT	6 fractions	5 Gy	30 Gy	45 Gy	MRI	2 days	Yes	Yes
2	Yoshida et al, 2018	Mice	RT	Single dose	–	60 Gy	420 Gy	EB extravasation	1 week	Unclear	Yes
3	Constanzo et al, 2017	Rats	Gamma knife RT	Single dose	–	10, 37, or 100 Gy	20, 173.9, 1100 Gy	MRI	140 days	Yes	Yes
4	Kalm et al, 2017	Mice	RT	Single dose	–	8 Gy	14.4 Gy	Radioactive brain uptake	72 h	Yes	Yes
5	Zhou et al, 2017	Rats	RT	Single dose	–	6 Gy	9.6 Gy	Immunocytochemistry	24 h	Yes	Yes
6	Murrell et al, 2016	Mice	WBRT	2	10 Gy	20 Gy	40 Gy	MRI	36 days	No	Yes
7	Ngen et al, 2016	Mice	RT	Single dose	–	80 Gy	720 Gy	MRI	2 weeks	Yes	Yes
8	Tamborini et al, 2016	Mice	WBRT	Single dose	–	2 Gy	2.4 Gy	Immunohistochemistry	48 h	Yes	Yes
9	Tong et al, 2016	Mice	RT	Single dose	–	10 Gy	20 Gy	EB extravasation	48 h	Yes	yes
10	Fan et al, 2015	Rats	WBRT	Single dose	–	22 Gy	70.4 Gy	MRI or EB extravasation	2 h	Yes	Yes
11	Zhang et al, 2015	Mice	RT	Single dose	–	20 Gy	60 Gy	EB extravasation	4 weeks	Yes	Yes
12	Cheng et al, 2014	Rats	Gamma knife RT	Single dose	–	60 Gy	420 Gy	EB extravasation	24 weeks	Yes	Yes
13	Jin et al, 2014	Rats	RT	2	3 Gy	6 Gy	7.8 Gy	EB extravasation	28 days	Yes	Yes
14	Lampron et al, 2012	Mice	WBRT	Single dose	–	10 Gy	20 Gy	Immunohistochemistry	7 days	No	No
15	Guan et al, 2011	Rats	X-knife RT	Single dose	–	20 Gy	60 Gy	CT perfusion imaging	5 days	Yes	Yes
16	Khatiri et al, 201	Rats	RT	Single dose	–	10 or 20 Gy	20 or 60 Gy	LC-MS	6 h	Yes	No
17	Zhou et al, 2011	Rats	RT	4 or 8	5 Gy	20 or 40 Gy	30 or 60 Gy	EB extravasation	12 weeks	Yes	Yes
18	Liu et al, 2010	Rats	WBRT	Single dose	–	15 Gy	37.5 Gy	EB extravasation	24 h	Yes	Yes
19	Wilson et al, 2009	Mice	RT	Single dose	–	20 Gy	60 Gy	Intravital microscopy	48 h	Yes	Yes
20	Ernst-Stecken et al, 2007	Rats	SRS	2–4	10 Gy	20, 30, or 40 Gy	40, 60 or 80 Gy	MRI/CT	16 weeks	Yes	No
21	Yuan et al, 2006	Mice	RT	20	2 Gy	40 Gy	48 Gy	Intravital microscopy	180 days	Yes	Yes
22	Kaya et al, 2004	Rats	WBRT	Single dose	–	18 Gy	50.4 Gy	EB extravasation	24 h	Yes	Yes
23	Yuan et al, 2003	Rats	RT	Single dose	–	20 Gy	60 Gy	Intravital microscopy	96 h	Yes	Yes
24	Mima et al 1999	Rats	RT	Single dose	–	25 Gy	87.5 Gy	Immunohistochemistry	5 days	Yes	No
25	Fike et al, 1998	Dogs	Interstitial RT	Single dose	–	20 Gy	60 Gy	CT	2–8 weeks	Yes	No
26	Karger et al, 1997	Rats	SRS	Single dose	–	20, 30, 40, 50, or 100 Gy	60, 120, 200, 300, 1100 Gy	MRI	19 months	Unclear	No
27	Kamiryo et al, 1996	Rats	Gamma knife RT	Single dose	–	50, 75, or 125 Gy	300, 637.5, 1687.5 Gy	EB extravasation	12 months	Unclear	No
28	Miot et al, 1995	Pigs	RT	Single dose	–	40 or 60 Gy	200, 420 Gy	MRI/EB extravasation	180 days	Yes	No
29	Nakata et al, 1995	Rats	RT	Single dose	–	20, 40, or 80 Gy	60, 200, 720 Gy	Immunohistochemistry	30 days	Unclear	No
30	Omary et al, 1995	Rats	Gamma knife RT	Single dose	–	120 Gy	1560 Gy	MRI	4 weeks	Yes	No
31	Krueck et al, 1994	Rats	WBRT	Single dose	–	15 or 25 Gy	37.5 or 87.5 Gy	MRI	48 h	Yes	Yes
32	Rubin et al, 1994	Rats	RT	Single dose	–	60 Gy	420 Gy	MRI	24 weeks	Yes	Yes
33	d'Avella et al, 1992	Rats	WBRT	20	2 Gy	40 Gy	48 Gy	Radioactive brain uptake	3 weeks	Yes	Yes
34	Lo et al, 1992	Rabbits	RT	Single dose	–	60 Gy	420 Gy	MRI	10 weeks	Unclear	Yes
35	Gobbel et al, 1991	Dogs	Interstitial RT	Single dose	–	20 Gy	60 Gy	CT	6 weeks	Yes	No

(continued on next page)

Table 2 (continued)

	Author, year	Preclinical model	Type of radiotherapy	Number of fractions	Fraction dose	Total dose	Biological Effective Dose	Readout technique	Time	BBB disruption	meta-analysis
36	Lo et al, 1991	Rabbits	RT	Single dose	–	15 or 30 Gy	37.5 or 120 Gy	MRI	8 months	Yes	No
37	Bezek et al, 1990	Rats	RT	Single dose	–	25 Gy	87.5 Gy	Radioactive brain uptake	7 days	Unclear	No
38	Delattre et al, 1989	Rats	RT	Single dose	–	3 Gy	3.9 Gy	Radioactive brain uptake	3 h	Yes	Yes
39	Spence et al, 1987	Rats	Whole body RT	Single dose	–	20 Gy	60 Gy	Radioactive brain uptake	2 days	No	Yes
40	Kourtopoulos et al, 1983	Rabbits	RT	Single dose	–	10 Gy	20 Gy	Chemical brain uptake	90 min	Yes	Yes
41	Levin et al, 1979	Rats	RT	Single dose, 3, 5, 10, or 25	2 or 4 Gy	2, 4, 7, 10, 12, 20, 25, 30 Gy	2.4, 5.6, 11.9, 12, 16.8, 20, 24, 36 or 87.5 Gy,	Radioactive brain uptake	24 h	Unclear	No
42	O'neill et al, 1977	Monkeys	RT	Single dose	–	35 Gy	157.5 Gy	EB extravasation	22 weeks	Yes	No
43	Blomstrand et al, 1975 (1)	Rabbits	RT	Single dose	–	30 Gy	120 Gy	EB extravasation	4 months	Yes	No
44	Blomstrand et al, 1975 (2)	Rabbits	RT	Single dose	–	30 Gy	120 Gy	EB extravasation	1 week	Yes	No
45	Olsson et al, 1975	Rats	RT	Single dose	–	300 Gy	9300 Gy	EB extravasation	9 days	Yes	No
46	Tanaka et al, 1975	Monkeys	RT	Single dose	–	35 Gy	157.5 Gy	Radioactive brain uptake	40 weeks	Yes	Yes
47	Olsson et al, 1972	Sharks	RT	Single dose	–	10, 35, 50, 107, 200, 250 or 300 Gy	20, 157.5, 300, 1251.9, 4200, 6500, 9300 Gy	EB extravasation	28 months	No	No
48	Bulat et al, 1966	Rats	WBRT	Single dose	–	9 or 80 Gy	17.1, 720 Gy	Chemical brain uptake	24 h	No	Yes
49	Nair and Roth, 1964	Mice	RT	Single dose	–	80 or 115 Gy	720, 1437.5 Gy	Radioactive brain uptake	120 h	Yes	No

Abbreviations: EB, Evans blue.

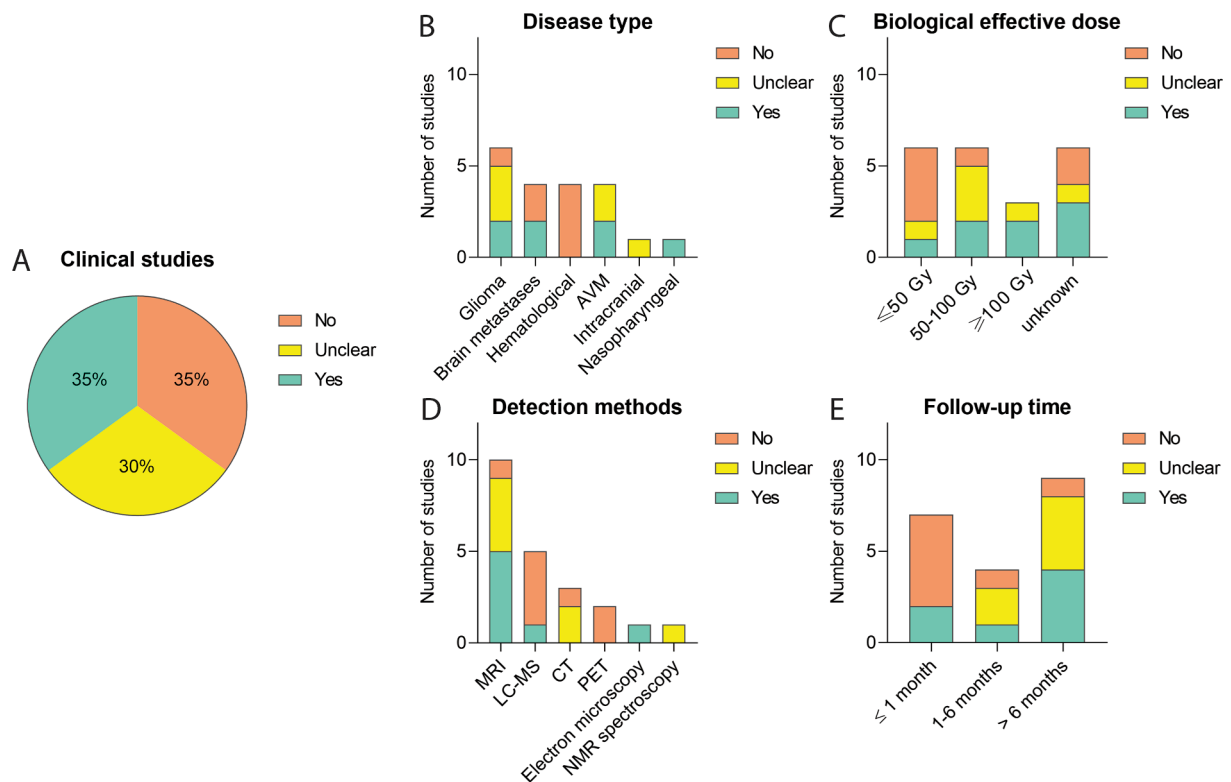


Fig. 2. Effect of RT on BBB permeability in clinical studies - qualitative analysis. Analysing clinical studies, the absence or presence of RT-induced BBB permeability was evaluated (A) and subgrouped by disease type (B), Biological effective dose (C), BBB disruption detection method (D), and duration of patient follow-up (E), showing differential effects.

patients with low-grade glioma were irradiated, with the same fractionation scheme, cumulative dose, BED, and the same read-out technique was applied. The three studies with a BED of ≥ 100 Gy used a single-dose radiation in patients suffering from AVM where RT is used for stereotactic radiosurgery; Tu et al (2006) [32] and Levegrün et al (2004) [33] reported a clear BBB disruption, while Parkhutik et al (2012) [31] only observed this in part of the patients. For the remaining six studies [34,35,37,39,40,50] no BED could be calculated, because details on the fractionation scheme applied were not reported. Of these six studies, three did observe an increased BBB permeability. Two of these studies, Chan et al (1999) [39] and Lim et al (2018) [40] used a cumulative dose between 61 and 80 Gy observed increased BBB breakdown after radiation. Looking at the fractionation scheme Farjam et al (2015) [41] was the only one of five studies with a fraction dose below 2 Gy that observed a significant increase in BBB permeability after 1 month of radiation, while Cao et al (2009) [44] reported temporal changes in the vascular volumes and Gd-DTPA signal in the cerebral tissue. One of the six studies with a 2 Gy fraction scheme observed a clear increase BBB permeability after RT, in contrast to Cao et al (2005) [43] who noticed only a BBB permeability difference close to the tumour. Qin et al (1990) [46] noted a change but also observed recovery 8 months after radiation; the authors indicated that acute effects can be reversible and do not necessarily result in permanent damage. Remarkably, Jarden et al (1985) described 6 Gy per fraction without any BBB alterations, albeit in combination with dexamethasone [49].

Detection method

Five [33,39–41,47] of the ten studies using magnetic resonance imaging (MRI) measuring the enhancement/extravasation of gadolinium-DTPA observed a clear change in BBB permeability. Other studies used a more indirect technique to detect BBB disruption. One [50] of the five studies using liquid chromatography–mass spectrometry

(LC-MS) to detect drugs in cerebrospinal fluid as a surrogate marker of BBB disruption noticed a change (Fig. 2D). Fang et al (2015) [48] treated patients with Gefitinib but did not observe an effect despite a high cumulative radiation dose (40 Gy vs 30 Gy). Tu et al (2006) [32] detected an alteration in BBB integrity by electron microscopy, while studies using computed tomography (CT) or positron emission tomography (PET) imaging did not detect any effect. Even with a high cumulative dose of 60 Gy, Matulewicz et al (2006) [42], who used nuclear magnetic resonance (NMR) spectroscopy, could not draw any clear conclusion but observed oscillations of choline-containing compounds over time, which might be indicative of BBB disruption and repair processes.

Follow-up time

Two of the seven clinical studies (29%) investigating an acute effect in BBB permeability after radiotherapy (Fig. 2E) reported changes after three and four weeks [47,50]. Of the four studies studying early delayed effects, only Lim et al (2018) observed a clear increase in BBB permeability [40], while Cao et al (2009) [44] and Cao et al (2005) [43] were unclear in their conclusion but both documented a peak of BBB permeability for radiation doses greater than 40 and in the range 20 to 40 Gy respectively. Late delayed effects of radiation on the BBB is found in four of nine studies [32,33,39,41].

Preclinical data – Qualitative analysis

With respect to the qualitative analysis of the 49 preclinical studies, 38 (78%) reported a clear difference in BBB permeability after RT, six (12%) detect an unclear effect, and five (10%) did not observe an effect (Fig. 3A).

Animal model

In 55% of the preclinical studies, rats were examined as animal

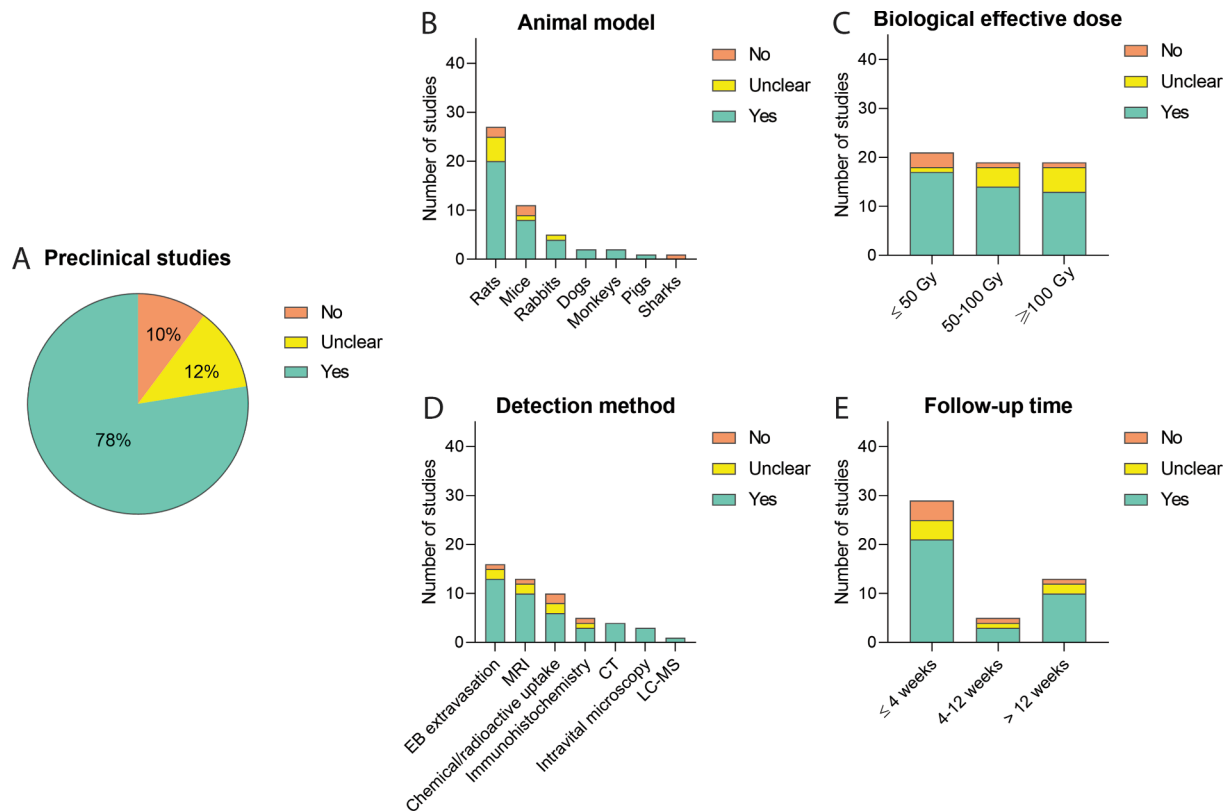


Fig. 3. Effect of RT on BBB permeability in preclinical studies - qualitative analysis. Analysing preclinical studies, the absence or presence of RT-induced BBB permeability was evaluated (A), and subgrouped by animal model used (B), Biological effective dose (C), BBB disruption detection method (D), and duration of follow-up (E), showing differential effects.

model. Of these rat studies 74% reported that radiotherapy influenced the BBB permeability (Fig. 3B). Other animal species were less frequently used, but permeability changes were also observed in mice, rabbits, dogs, monkeys, and pigs, but not for sharks. Olsson et al (1972) concluded that the shark brain is not a suitable model because of its radio-resistant properties [97]. Of note, Spence et al (1987) [66] and Bulat (1966) [67] reported an absence of effect on BBB integrity disturbances in rats after a short follow-up time of 24 and 48 h. In addition, in two mouse studies, no changes were observed in BBB permeability; Murrell et al (2016) [83] and Lampron et al (2012) [78] noticed no changes in BBB permeability with a cumulative dose of 20 and 10 Gy respectively.

Biological effective dose

Most of the preclinical studies (81%) with a BED of ≤ 50 Gy reported an increase of BBB permeability. Comparable effects were observed in studies using a BED of 50–100 Gy (74%) and ≥ 100 Gy (68%), see Fig. 3C. Although an overall comparable BED was used, clinical studies relatively used a higher cumulative dose compared to preclinical studies, whereas in clinical studies BBB permeability was observed to lesser extent than in preclinical studies (35% vs 78%). Additionally, the bulk of the preclinical studies (84%) used a single dose fraction for the irradiation of the animals, with 76% of these studies observing an enhanced BBB permeability. When multiple fractionations were applied, the animals received 2 or up to 10 Gy per fraction, with a cumulative dose of 6 or up to 40 Gy. Levin et al (1979) [75], applied fractions of 2 or 4 Gy with a cumulative dose up to 30 Gy and detected some permeability changes, whereas Murrell et al (2016) [83] did not observe any change after two fractions of 10 Gy with a cumulative dose of 20 Gy.

Detection method

Unlike clinical studies, animal research more easily allowed for post-mortem observations and the usage of multiple detection methods (Fig. 3D). Interestingly, two of the five studies that did not observe a BBB integrity issue were analysed by Evans blue extravasation or immunohistochemistry [78,97], in which brain surgery and processing was necessary to acquire the results. When comparing clinical and preclinical studies that use MRI, the results are relatively similar.

Follow-up time

For each follow-up time (acute, early delayed, and late delayed) most preclinical studies observed a relatively equal occurrence of increased BBB permeability: 72%, 60% and 77%, respectively (Fig. 3E). In contrast, in clinical studies an increase in BBB permeability was predominantly reported as late delayed effect.

Preclinical data – Meta-analysis

The 29 preclinical studies included in the meta-analysis showed a significant effect of radiation on BBB permeability between irradiated animals (radiotherapy group) and non-irradiated animals (control group): -9.92 [$-11.89, -7.95$] ($n = 29, p < 0.01$) (Fig. S3). However, heterogeneity was high ($I^2 = 98\%$).

Preclinical model

In the studies included in the meta-analysis, in both mice (34%) and rats (55%), RT significantly increased BBB permeability (mice SMD -10.97 [$-15.77, -6.18$], $n = 10, p < 0.01$; rats- SMD 8.79 [$-11.24, -6.70$], $n = 16, p < 0.01$) (Fig. S4). Subgroup analysis did not show any significant difference between effect estimates in mice and rats ($p = 0.26$). Heterogeneity in both subgroups was high ($I^2 = 98\%$ and $I^2 = 98\%$) in mice and rats, respectively. Moreover, the studies using monkeys and rabbits were excluded from the meta-analysis because data was insufficient to create a subgroup, i.e., group size lower than five.

Biological effective dose

A significant effect of radiation on BBB permeability was found in all the three subgroups of the BED, ≤ 50 Gy, 50–100 Gy and ≥ 100 Gy: -8.14 [$-10.60, -5.69$] ($n = 15, p < 0.01$), -11.66 [$-15.97, -7.35$] ($n = 11, p < 0.01$), and -6.63 [$-9.53, -3.74$] ($n = 8, p < 0.01$) (Fig. S5). No significant difference of these effects is found between the three subgroups ($\text{Chi}^2, p = 0.16$). In addition, a substantial heterogeneity was found in all three subgroups: $I^2 = 98\%$, $I^2 = 99\%$, and $I^2 = 97\%$, indicating a low similarity between studies with similar BEDs.

Detection method

Subgroup analysis of the detection methods: Evans blue extravasation, MRI (gadolinium-DTPA) and extravasation of radioactive tracers (Fig. S6), showed a significant increase in BBB permeability after radiation: -23.75 [$-34.82, -12.69$] ($n = 7, p < 0.01$), -6.77 [$-9.44, -4.10$] ($n = 8, p < 0.01$) and -8.81 [$-14.36, -3.26$] ($n = 5, p < 0.01$), respectively. Comparison of the effect estimates between subgroups showed a significant difference in BBB permeability ($p = 0.01$), which could be ascribed to a difference between the sensitivity of Evans blue extravasation and MRI ($\Delta\text{SMD} = 16.98, 95\% \text{ CI } -25.38, -8.59$) as a detection method. Heterogeneity was high in each subgroup: $I^2 = 98\%$, $I^2 = 97\%$ and $I^2 = 98\%$ for Evans blue, MRI, or radioactive tracers, respectively.

Follow-up time

The onset of BBB permeability after radiation therapy was explored with acute, early delayed, and late delayed categories and a significant increase in BBB permeability is observed in all follow-up time categories: -7.75 [$-9.65, -5.85$] ($n = 25, p < 0.01$), -6.06 [$-8.83, -3.29$] ($n = 7, p < 0.01$) and -5.79 [$-9.07, -2.52$] ($n = 5, p < 0.01$), respectively (Fig. S7). For each category, a high heterogeneity was found: $I^2 = 98\%$ ($I^2 = 97\%$, and $I^2 = 94\%$, respectively). No significant difference between these follow-up time categories was found ($p = 0.46$).

Publication bias

Potential publication bias was assessed for the outcome of BBB permeability upon RT in the 29 preclinical studies included in the meta-analysis (Fig. S8). Asymmetry observed in the funnel plot suggests the presence of publication bias, which was confirmed by Egger's regression line. The funnel plot indicates that studies with small cohorts favouring negative results were missing in the publication record. Trim-and-fill analysis resulted in the addition of 13 extra predicted studies (black dots), with a new total calculated effect, indicating an overestimation of the effect size. Despite this overestimation, the effect of radiation on BBB permeability remains significant.

Discussion

Conventional photon RT is a therapeutic cornerstone in brain cancer and it is commonly postulated that this treatment modality alters BBB permeability [16], a key protective component to maintain brain tissue homeostasis. The downside of a dysfunctional BBB is that the brain tissue is more exposed to blood-borne proteins, waste products and pathogens, potentially resulting in a variation of neurological disorders such as neuroinflammatory reactions and neurodegenerative diseases [99–101]. On the other hand, in the context of neurological diseases and brain cancer, an increased BBB permeability can have therapeutic advantages, whereby drugs that normally have limited access to the brain parenchyma are able to better reach the diseased brain [4]. To illustrate, new upcoming techniques such as focused ultrasound, aim to increase the BBB permeability locally and transiently for the extravasation of drugs into the brain parenchyma for the treatment of neurodegenerative diseases such as Alzheimer's Diseases and primary brain tumours as well as brain metastases [102–104]. In scope of the safety of patients and their (concomitant and adjuvant) treatment, it is crucial to monitor,

evaluate and control the extent of BBB permeability caused by RT. However, the factors leading to BBB alteration remain to be better understood and a thorough analysis of the evidence on RT-induced BBB disruption thus far has been lacking. This systematic review and meta-analysis therefore explored the contribution of these determinants in the state-of-the-art literature. Based on a qualitative analysis of relevant literature and by performing a meta-analysis, we conclude from pre-clinical and clinical studies that photon radiotherapy indeed enhances the permeability of the BBB, although the low level of data-reporting and likely occurrence of publication bias of the included studies, limits the strength of these conclusions.

For better comparison between studies, the BED was calculated. Whereas clinical studies mostly observed BBB permeability upon RT at ≥ 100 Gy, preclinical studies display an overall effect in each of the BED categories. Most of the preclinical studies used a single dose instead of a multiple fractionation scheme which is mainly used in the clinical studies. The three included clinical studies using a single dose (18–24 Gy) observed an increase in BBB permeability, while this effect was observed in only 20% of the preclinical studies applying fractionation protocols at a respectively equal cumulative dose. Another point of interest: laboratory animals are generally given a higher BED given compared to the patients in the clinical studies. Recently, the use of proton therapy as an alternative to conventional photon therapy is gaining popularity. Proton beam RT may possibly overcome the effects of RT at the BBB to some extent, as proton therapy is characterized by the highest energy deposition at the point of interest without an exit dose, hereby lowering the dose in the surrounding healthy tissue [105]. Now an emerging option for paediatric patients, it may herewith reduce long term side effects at the developing brain. However, to our knowledge, there is still a limited data on the effects of proton therapy on BBB permeability, we were unable to analyse this in this systematic review and meta-analysis. Concerning the follow-up time, in clinical studies, increased BBB permeability was often observed after six-months, which could be explained by radio-necrosis [106]. In meantime a significant effect was reported in all post-RT time subcategories in the pre-clinical studies. Both clinical and preclinical studies mentioned a peak in permeability a few months after radiation, followed by BBB restoration afterwards [41,56]. Furthermore, a correlation between longer permeability and increased radiation doses was observed [44,58,72]. Over the years, the techniques to measure BBB permeability have improved and are more refined in their measurements. Older clinical studies mostly did not detect any BBB permeability change while over the years MRI became more the standard and studies using this technique found the opposite effect. Older preclinical studies mostly relied only on the extravasation of Evans blue for visible conformation of increase permeability and reported mostly negative results, but on the other hand more recent studies using Evans blue measured that BBB permeability is increased after RT.

The disease type, stage and/or use of different pharmacological agents are also thought to cause alterations of the BBB and are therefore likely to be confounding factors in the assessment of RT-induced BBB alteration. For instance, glioblastoma often exhibits areas of increased BBB permeability at diagnosis, which is progressive in advanced stages of the disease [107]. This permeability not only occurs along the disease course, but is also often characterized, at a specific disease stage, by a spatial intra-patient heterogeneity owing to the anarchic formation of a blood-tumour barrier in the case of certain brain cancers [108]. Noteworthy, also inter-patient heterogeneity induces a significant variability in the interpretation of permeability data in BBB studies. For example, clinical studies exploring haematological malignancies did not observe any permeability indicating the fact that underlying diseases may influence the extent of BBB permeability, confounded that the cumulative dose of RT in these studies was lower. Moreover, most of the patients in the included clinical studies were treated with additional medication, which could have further compromised or restored BBB integrity [37,38]. There is evidence that certain pharmacological agents exert an

effect on BBB functioning and structure, for example inducing BBB permeability by efflux transporter inhibition [109], or by reinforcement, as reported in studies including dexamethasone [49]. Dexamethasone is often prescribed to reduce cerebral edema [110] in brain malignancies by initiating the glucocorticoid receptor-mediated signalling, ultimately leading to strengthening and restoration of BBB integrity [111]. It is often assumed that the juvenile brain (especially that of infants) is more permeable than the adult brain, even though animal and clinical studies [112–114] observed well-developed tight junctions and similar activity of transporters. As the juvenile brain is still in development, it can be hypothesized that it is more prone to damage and collateral effects [115]. Of the five clinical studies included, three enrolled paediatric patients but observed no effect on BBB permeability after radiation [35,36,38].

Our systematic review indicates that preclinical studies reported more RT-induced BBB permeability than clinical studies: 78% vs 35%, respectively. Besides the parameters investigated in this review several more other reasons can explain more the discrepancy: first, preclinical studies are designed and performed in a more controlled fashion, thus potentially reducing group variability and, in turn, increase statistical power and ultimately find significant differences. Second, preclinical studies give access to more readout modalities, which allows for multiparametric ascertainment and cross-validation of disease hallmarks, e.g., albumin extravasation into the brain parenchyma. In contrast, clinical studies mostly use MRI and/or LC-MS of CSF. However, preclinical protocols often require animal anaesthesia using agents that induce haemodynamic changes, e.g., isoflurane-induced vasodilation and increase in blood flow, which is directly sensed by the endothelial barrier, and may activate pathways that potentially modify BBB integrity likely to generate experimental biases [116]. Nonetheless, clinical modalities, such as MRI, CT and PET, can be performed in patients without resorting to the administration of anaesthetics. The ascertainment of BBB leakiness in clinical MRI protocols mainly uses gadolinium chelates whose extravasation, according to their high molecular weights, is mediated by tight junctions at a specific disease stage, thus only reflecting the status of the physical BBB; however, BBB leakiness for smaller sized molecules is also mediated by its functional counterpart, mainly including transcytosis, which may be upregulated in the course of specific diseases but cannot be measured by conventional MRI protocols. Drug-PET imaging after RT, using radioisotopes such as ^{11}C or ^{18}F for small molecule drugs and ^{89}Zr for monoclonal antibodies allows for visualization of enhanced brain uptake of these compounds. Based on subgroup analysis of the pre-clinical studies, Evans blue extravasation shows a significant increase in BBB permeability compared to MRI and radioactive compounds, which have a similar effect size and seem to be more related. Conversely, in case of Evans blue extravasation the results are obtained from post-mortem tissues, while MRI and radioactive compounds are acquired in real-time, explaining this discrepancy. The (pre)clinical studies using MRI show similar results, which might indicate that this technique is more reliable to determine BBB disruption in both humans and animals. More preclinical research is therefore needed to study the effect of RT on BBB disruption for small to large sized molecules.

In our meta-analysis, no clinical subgroup was eligible for further processing owing to the absence of non-irradiated control groups. All the eligible subgroups, *i.e.*, including at least five studies, showed a significant RT-induced BBB permeability. One of the excluded subgroups concerns monkeys, which are particular interesting due to their intracranial vessel structure close to humans [117]. However, the only non-human primate study exploring RT-induced BBB permeability reported non-significant differences in BBB permeability. The allocation of the follow-up time for the animal models was solely based on rats [27,28], which might have influenced the outcome of all the other models and was not specifically established for radiation effects.

Importantly, the majority of the clinical and preclinical studies scored either a high or unclear risk of bias, affecting the reliability of the data, but also can cause an over- or underestimation of the results [118].

In case of the clinical data, bias can be caused by certain ethical considerations, for example patients can be excluded due to deviant baseline characteristics, which can cause an overestimation of the results. Lastly, a potential publication bias was detected, and may also explain the difference found between clinical and preclinical studies in our qualitative analyses where the percentage of studies reporting an effect of RT on BBB permeability was higher for the preclinical studies. Nevertheless, the trim-and-fill analysis confirmed RT-induced BBB disruption.

Conclusion and future perspectives

This systematic review and meta-analysis of the literature demonstrates that RT influences BBB permeability, although our findings show that suboptimal study designs and a publication bias in the selected studies may be the source of an overestimation of the extent of BBB permeability induced by RT. Worth mentioning, the robust comparison of the variables between the studies for qualitative and quantitative analysis makes it even more difficult for any hard conclusions.

Future preclinical and clinical studies using novel readout modalities should therefore be focused on fully elucidating the extent and timing of BBB opening induced by RT. These considerations will be key to adjust and guide treatment planning in treatment regimens that include RT to the brain. The effect of RT on the BBB in patients can be studied in more detail and longitudinally during and after radiotherapy, using advanced MRI and PET studies. Drug imaging with PET after RT, will provide more insight on possible RT-induced enhancement of drug delivery to the brain, avoiding toxicity and optimizing concomitant and adjuvant treatment strategies for an optimal therapeutic index [119].

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ctro.2022.04.013>.

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