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The Blood-Brain Barrier in Bipolar Disorders: A Systematic Review

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Abstract

Background: Bipolar disorders (BD) are chronic, debilitating disorders. The blood-brain barrier (BBB) has been increasingly investigated in BD. This systematic review aimed to assess the available evidence on the relationship between BD and markers of BBB dysfunction.

Methods: A systematic search in PubMed, Embase, PsycINFO, CINAHL and Web of Science was run where the primary outcomes were BBB markers such as S100B, albumin ratio, matrix metalloproteinase (MMP), cell adhesion molecule (CAM), and tight junction proteins. Techniques included blood, cerebrospinal fluid (CSF), post-mortem, genetic and imaging methods in BD compared to healthy controls.

Results: 55 studies were identified, 38 of which found an association between BD and markers of BBB dysfunction. 16/29 studies found increased blood/CSF albumin ratio, S100B, CAMs or MMP levels in BD participants compared to controls. 5/19 post-mortem studies found increased levels of chondroitin sulphate proteoglycans, intercellular CAM, neurexin or claudin-5 mRNA in distinct locations throughout the brain in BD compared to controls. One imaging study identified extensive BBB leakage in 30% of BD participants, compared to 0% in controls.

Limitations: The diversity in methodologies used in the included studies makes direct comparison of results challenging. Furthermore, imaging methods are the gold standard, but only one study used them. Other markers are only indicative of BBB permeability.

Conclusions: This review suggests an association between BD and BBB dysfunction. Further research is needed to provide definite answers considering the existing literature’s limitations, and to clarify whether this association provides a pathogenic mechanism, or is an epiphenomenon of BD.
1. Introduction

Bipolar disorders (BD) are chronic and highly debilitating psychiatric disorders which affect an estimated 45 million people worldwide (James et al., 2018). The aetiology of BD is still poorly understood but is conceptualised as a multifactorial process in which the environment and genetics play a role (Rowland & Marwaha, 2018). The involvement of the blood-brain barrier (BBB), a highly selective, protective physical barrier that separates the central nervous system (CNS) from the peripheral circulation, has been increasingly investigated in the aetiology of BD. The BBB is critical to ensure a controlled environment within the CNS (Abbott, Rönnbäck & Hansson, 2006), maintaining a specific ionic environment that favours appropriate neuronal activity and shields the brain tissue from potentially toxic materials carried in the circulation (Abbott, 2010). The BBB is comprised of a single layer of microvascular endothelial cells, bound by tight junctions, with discontinuously placed pericytes on the luminal side (Abbott et al., 2010). This structure is then enclosed by the basal membrane and astrocytic end-feet projections, surrounding their surface (see Figure 1A). This final complex, together with the transporters and ion channels expressed by the cells, forms a tightly sealed barrier separating the blood from the cerebral tissue (Abbott et al., 2010). (For further details on the structure and function of the BBB, see Abbott et al., 2010; Lawther et al., 2011; Patel & Frey, 2015).

Recent models propose that dysfunction of the BBB leads to increased permeability and reduced protection of the brain, allowing peripheral damaging substances into the cerebral tissues, such as albumin, fibrinogen and many xenobiotics (Hindle et al., 2017; Profaci, Munji, Pulido, & Daneman, 2020; Sanchez-Cano, Hernández-Kelly, & Ortega, 2021). This, in turn, may activate inflammatory responses which can disrupt healthy brain function (Patel & Frey, 2015) (see Figure 1B).
Although there is well-established evidence for altered BBB function in many neurological disorders, such as epilepsy, multiple sclerosis, and dementia (Gorter, Van Vliet, and Aronica, 2015; Burgmans, Van de Haar, Verhey, and Backes, 2013; Pinheiro, 2016), the literature about the role of BBB dysfunction in psychiatric disorders is still growing. There is accumulating animal and human evidence for the disruption of the BBB and neuroinflammation in major depressive disorders (MDD) (Dudek et al., 2020; Medina-Rodriguez & Beurel, 2022). Given the phenomenological similarities of depression and BD, there has been a growing interest in the associations that might exist between the BBB and BD (Zhao et al., 2022). Pollak et al. (2018) reviewed the relation between BBB integrity and psychosis, concluding that disruption in the BBB could be contributing to psychosis due to neuronal and synaptic dysfunction, increased permeability into brain tissue and disrupted glutamate homeostasis. A recent review by Futtrup et al. (2020) identified evidence suggesting a potential link between BBB pathology and several mental health disorders. The present systematic review sought to specifically and comprehensively synthesize relevant studies investigating the role of the BBB in BD using a wide range of markers and methodologies.
2. Methods

2.1. Design

This study was a systematic review with a narrative synthesis, conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). The study protocol was registered with the UK National Institute for Health Research PROSPERO international prospective register of systematic reviews (ID, CRD42021228324). The review sought to include studies which measured BBB structure and function in people with BD and that compared findings to a healthy participant comparator group.

2.2. Included studies

Studies were included if they assessed BBB function or structure in participants of any age or sex, who met ICD or DSM criteria for BD (including BD type I, type II and not otherwise specified) and compared findings to a matched healthy participant control group. Only studies written in English were included. Exclusion criteria included studies which included participants with a diagnosis for cyclothymia. We excluded studies which included participants with a diagnosis for cyclothymia, given the uncertainty about the diagnostic reliability of cyclothymia (Perugi et al., 2017). Furthermore, given the number of published human BBB studies in bipolar disorders available, we decided to exclude animal studies and studies of major depressive disorder to ensure that we could adequately cover the breadth of the human BBB BD evidence base in this review.

2.3. Outcome measures

Markers of BBB structure or function in people with BD compared to healthy participants was the primary outcome measure. BBB status was measured using a) blood- and CSF-based measures such as the CSF:serum albumin ratio (QAlb), lymphocyte concentration, serum concentration of brain-blood barrier associated proteins, b) post-
mortem histological assays, c) neuroimaging studies, and d) studies of BBB-relevant genetic polymorphisms. We based our search strategy on those used in previous reviews of the BBB, extracting the markers that we deemed most reliable and relevant to BBB function.

2.4. Data sources and searches

A systematic literature search for published studies was performed using PubMed, MEDLINE, PsycINFO, CINAHL, EMBASE and Web of Science from inception until 01/08/2023. The search was conducted by two independent reviewers following the same protocol, included studies were compared and disputes resolved by consensus. All studies included in the review were evaluated against pre-defined inclusion criteria by two of the review authors (CW and FM). Any disparities were addressed by reaching agreement via an additional review author (PS). No disagreements came up between the reviewers. The full search strategy can be found below:

Search Strategy

#1: Depression OR major depressive disorder OR depressive disorder OR bipolar disorder OR mania OR manic symptoms

#2: blood brain barrier OR BBB OR blood-brain barrier OR blood cerebrospinal fluid barrier OR BCSFB OR s100b OR S100 Calcium Binding Protein beta Subunit OR albumin OR QAlb OR fibrinogen OR plasminogen OR matrix metalloproteinase OR MMP OR tissue inhibitor of metalloproteinase OR TIMP OR cell adhesion molecule OR selectin OR ICAM OR VCAM OR glycocalyx OR syndecan OR heparan sulphate OR chondroitin sulphate OR hyaluronan OR glial fibrillary acidic protein OR GFAP OR p-glycoprotein OR ATP Binding Cassette Transporter, Subfamily B, Member 1 OR choroid plexus
2.5. Data extraction
For each study, the following information was extracted where available: study type, sample size, intervention components, type of BD, mood state during sample collection, medications used, whether BD participants were in/outpatients, measure of BBB function and outcomes. Only data relating to BD and healthy control groups were extracted.

2.6. Quality assessment
The Newcastle-Ottawa scale was used to evaluate the quality of included case-control studies (Wells et al., 2021) and the Joanna Briggs Institute (JBI) Checklist for Analytical Cross-Sectional Studies (Joanna Briggs Institute, 2017) was used to evaluate cross-sectional studies. Risk of bias tables indicating the quality of included studies were created. See supplementary tables 2 and 3, respectively.

3. Results
3.1. Study Selection and characteristics
The study selection process is shown in PRISMA flow chart (see Figure 2).

Supplementary Table 4 shows a summary of the characteristics of the studies included in this systematic review. All included studies were observational. 29 studies investigated blood/CSF markers, 19 studies used a post-mortem methodology, including Western blotting, quantitative polymerase chain reaction (PCR) and immunohistochemistry. 7 studies used a genetic approach and one study used magnetic resonance imaging (MRI) to assess the integrity of the BBB. The studies included heterogenous samples of people
with BD in terms of illness duration, severity, subtype, current affective state, and past and current medication use. Most BD participants were taking psychotropic medication at the time of the study.

3.2. **Blood/CSF biomarker results**

23 out of 29 studies included reported an association between blood or CSF markers of BBB dysfunction in BD; 18 found a difference between BD and healthy controls and 7 found a difference between people with a diagnosis of BD I vs BD II, or between BD manic/depression and remission groups (See Table 1).

**Table 1:** Summary of the results of Blood and CSF markers of BBB function in BD compared to healthy controls
<table>
<thead>
<tr>
<th>Molecule measured</th>
<th>Author, Date</th>
<th>Sample Size BD/HC (total)</th>
<th>BD Diagnosis</th>
<th>Illness phase</th>
<th>Effect Direction</th>
<th>QA</th>
</tr>
</thead>
<tbody>
<tr>
<td>QAlb ratio</td>
<td>Zetterberg et al., 2014</td>
<td>134/86 (220)</td>
<td>TI, TII and NOS</td>
<td>Not specified</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td>S100B + autoantibodies</td>
<td>Andreazza et al., 2007</td>
<td>85/32 (117)</td>
<td>TNS</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Jakobsson et al., 2014</td>
<td>133/86 (219)</td>
<td>TI, TII and NOS</td>
<td>Euthymia</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Jankovic &amp; Djordjijević, 1991</td>
<td>117/112 (781)</td>
<td>TNS</td>
<td>Not specified</td>
<td>▲</td>
<td>2/8</td>
</tr>
<tr>
<td></td>
<td>Tsai &amp; Huang, 2017</td>
<td>17/30 (47)</td>
<td>TI</td>
<td>Mania &amp; euthymia</td>
<td>-</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Machado-Vieira et al., 2002</td>
<td>20/20 (40)</td>
<td>TI</td>
<td>Mania</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Hidese et al., 2020</td>
<td>68/118 (384)</td>
<td>TI, TII</td>
<td>Not specified</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Karabulut et al., 2019</td>
<td>107/30 (137)</td>
<td>TNS chronic and early stages</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Schroeter et al., 2002</td>
<td>11/32 (52)</td>
<td>TNS</td>
<td>Mania</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Ormerod et al., 2022</td>
<td>346/814 (1762)</td>
<td>TI, TII and NOS</td>
<td>Not specified</td>
<td>▼</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Knorr et al., 2022</td>
<td>86/44 (130)</td>
<td>TI and TII</td>
<td>Mania, depression &amp; euthymia</td>
<td>-</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>Haenisch et al., 2014</td>
<td>17/46 (63)</td>
<td>TI and TII</td>
<td>Not specified</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Chaarani et al., 2013</td>
<td>20/20 (40)</td>
<td>TNS</td>
<td>Mania &amp; euthymia</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Södersten et al., 2014</td>
<td>263/155 (418)</td>
<td>TI, TII and NOS</td>
<td>Psychosis present or not</td>
<td>-</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>Frye et al., 2015</td>
<td>95/141 (288)</td>
<td>TI and TII</td>
<td>Depression</td>
<td>▲</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Reininghaus et al., 2016</td>
<td>112/80 (192)</td>
<td>TNS</td>
<td>Euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Rybakowski et al., 2013</td>
<td>54/29 (83)</td>
<td>TNS</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Saravanan et al., 2022</td>
<td>80/80 (160)</td>
<td>TI</td>
<td>Mania &amp; depression</td>
<td>▲</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>CAMs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Turan et al., 2014</td>
<td>75/50 (125)</td>
<td>TNS</td>
<td>Mania &amp; euthymia</td>
<td>▲</td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>Pantović-Stefanović et al., 2018</td>
<td>83/73 (156)</td>
<td>TI</td>
<td>Not specified</td>
<td>I▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Schaefer et al., 2016</td>
<td>83/73 (156)</td>
<td>TI and TII</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Reininghaus et al., 2016</td>
<td>112/80 (192)</td>
<td>TNS</td>
<td>Euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Ormerod et al., 2022</td>
<td>346/814 (1762)</td>
<td>TI, TII and NOS</td>
<td>Not specified</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Angrand et al., 2021</td>
<td>312/180 (492)</td>
<td>TNS</td>
<td>Mania, depression &amp; euthymia</td>
<td>▼</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Bai et al., 2014</td>
<td>130/130 (260)</td>
<td>TI and TII</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Ormerod et al., 2022</td>
<td>346/814 (1762)</td>
<td>TI, TII and NOS</td>
<td>Not specified</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>P-Selectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claudin</td>
<td>41/41 (82)</td>
<td>TI</td>
<td>Mania &amp; remission</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Hochman et al., 2023</td>
<td>21/28 (68)</td>
<td>TNS</td>
<td>Depression</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Zonulin</td>
<td>41/41 (82)</td>
<td>TI</td>
<td>Mania &amp; euthymia</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>Zengil et al., 2023</td>
<td>44/44 (88)</td>
<td>TI and TII</td>
<td>Mania, depression &amp; euthymia</td>
<td>▲</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>Cadherin-5</td>
<td>23/24 (80)</td>
<td>TNS</td>
<td>Not specified</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>AQP4 autoantibodies</td>
<td>25/30 (80)</td>
<td>TNS</td>
<td>Depression</td>
<td>-</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>GFAP mRNA</td>
<td>30/15 (45)</td>
<td>TNS</td>
<td>Not specified</td>
<td>▲</td>
<td>4/8</td>
</tr>
</tbody>
</table>

Associations reported represent comparison of marker levels between bipolar participants and healthy controls. BD Bipolar disorder; TI Type 1; TII Type 2; NOS Not otherwise specified; TNS type not specified;
MMP matrix metalloproteinase; mRNA messenger RNA; CAM cell-adhesion molecule; AQP4 aquaporin-4; GFAP glial fibrillary acidic protein, I ICAM, V VCAM; QAlb Cerebrospinal fluid/Serum quotient of albumin; QA Quality Assessment (score/total available; Newcastle Ottawa scale is scored out of 10, Joanna Briggs scale is scored out of 8). Other refers to a psychiatric diagnosis different from bipolar disorders included in the total sample size where the presented effect results do not include this group. These are typically major depressive disorder and/or schizophrenia.- No significant difference; ▲ Increase in the BD group compared to controls;▼ Decrease in the BD group compared to controls

3.2.1. QAlb

QAlb (CSF:serum albumin quotient) is the ratio of cerebrospinal fluid albumin to serum albumin. As albumin is exclusively synthesised in the liver, and in the healthy BBB is transported into the CNS only by passive diffusion, an increased QAlb indicates increased BBB and blood-CSF permeability (Mattsson, Blennow & Zetterberg, 2010). Only one study measured the QAlb in BD participants compared to healthy participants. Zetterberg et al. (2014) found QAlb was significantly higher in all BD groups (BD I, II and NOS) compared to controls ($p = 0.017$). Additionally, participants with BD I were found to have an elevated QAlb compared to participants with BD II ($p < 0.041$). QAlb levels also correlated with lifetime episodes of psychosis ($p = 0.026$) and was significantly increased in participants currently prescribed antipsychotic medication. When antipsychotic medication history was adjusted for, diagnosis and lifetime psychosis no longer had a significant effect. No difference in QAlb was found between BD participants not currently prescribed antipsychotics and healthy participants.

3.2.2. S100B

S100B is a protein which is predominantly synthesised and secreted by astrocytes. The normal concentration of S100B in blood is generally low and an increase in S100B serum concentration may indicate reduced BBB integrity (Arora et al., 2019; Strathmann, Schulte, Goerl & Petron, 2014). The presence of S100B autoantibodies has also been suggested to indicate a long-term reduction in BBB integrity (Marchi et al., 2013; Choi et al., 2016). However, there is also evidence of S100B being secreted from adipose tissue
11 studies measured S100B levels in people with BD and in healthy controls (see Table 1). Results of the blood-based S100B studies were mixed: Machado-Vieira et al. (2002) and Schroeter et al. (2002) compared participants with mania to age-matched healthy participants and found that S100B levels was significantly higher in mania ($p = 0.014$). Andreazza et al. (2007) found S100B levels to be significantly higher in mania ($p = 0.011$) and bipolar depression ($p = 0.004$) but was not altered in remission. Haenisch et al., (2014) found that S100B levels were significantly higher in BD participants compared to healthy controls ($p = 0.001$). Tsai & Huang (2017) reported no significant difference in S100B levels between BD participants and healthy controls but did find a significant reduction in S100B levels between BD participants when in a manic episode compared to remission. Knorr et al., (2022) also found no significant difference in S100B levels between BD participants and healthy controls whereas Ormerod et al., (2022) reported that S100B levels were significantly lower in BD participants vs healthy controls ($p = 0.001$). Karabulut et al. (2019) found that S100B levels were higher in participants with BD with a longer duration of illness. Neither of the S100B CSF studies (Jakobsson et al., 2014 and Hidese et al., 2020) found any significant difference between BD and healthy groups. Janković & Djordijević (1991) found that significantly more participants with BD (28.2%) had anti-S100B antibodies than controls (2.7%).

3.2.3. MMPs

Matrix metalloproteinases (MMPs) are enzymes that activate as a result of vascular endothelial damage and can lead to degradation of the extracellular matrix, including the proteins that form tight junctions (Van den Steen et al., 2002; Lischper et al., 2010; Feng et al., 2011). An increase in MMP concentration can potentially represent both a marker and a cause of BBB breakdown. Studies comparing MMP levels and/or expression in BD reported mixed results: 4 reported increased MMP levels in participants with BD compared to healthy controls (Frye et al.2015; Reininghaus et al., 2016; Rybakowski et
al., 2013) while 2 found no differences (Chiarani et al., 2013; Södersten et al., 2014). Among the significant associations Frye et al. (2015) found increased MMP-7 levels in BD compared to controls ($p = 0.009$), and also found increased levels in BD I participants compared to BDII. Saravanan et al., (2022) found that MMP-9 was increased in BD participants compared to healthy controls in both depressed ($p = 0.041$) and manic ($p = 0.001$) members of the BD group. Reininghaus et al. (2016) found that serum MMP-9 was increased in those with a longer duration of illness ($p = 0.006$).

### 3.2.4. ICAM and VCAM

Intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) are cell adhesion molecules which are involved in the regulation of leukocyte transport across the BBB. The endothelial cells that form the BBB upregulate expression of ICAM and VCAM in response to inflammation, thus increasing BBB permeability to leukocytes (Daneman, & Prat, 2015). 6 studies measured the serum concentration of cell adhesion molecules, and each reported an association between VCAM/ICAM levels and BD (Turan et al., 2014; Pantović-Stefanović et al., 2018; Schaefer et al., 2016; Reininghaus et al., 2016; Ormerod et al., 2022; Angrand et al., 2021). Schaefer et al. (2016) found that ICAM was significantly increased in people with BD compared to healthy controls regardless of the type of BD or the participant’s mood state. Reininghaus et al. (2016) found that overall, ICAM concentrations were increased in all BD groups studied ($p = 0.006$) and that BD participants with a longer duration of illness had higher ICAM concentrations compared to those with a more recent diagnosis. Pantović-Stefanović et al. (2018) reported elevated ICAM levels ($p = 0.021$), while also finding lower VCAM levels ($p = 0.000$) in BD compared to healthy controls. Turan et al. (2014) investigated ICAM and VCAM levels in participants with a first manic episode and found that both markers were significantly elevated in comparison to the same participants during subsequent remission, as well as compared to healthy controls ($p < 0.001$). Angrand et
al., (2021) found that both ICAM and VCAM were significantly lower in BD participants vs healthy controls ($p = 0.04, p = 0.0025$). Ormerod et al., (2022) found an increase in ICAM in BD participants vs controls ($p < 0.001$) but found no difference in levels of VCAM.

3.2.5. **Other markers**

Several studies have investigated other potential markers of BBB integrity in BD. Soluble P-selectin receptor (sP-selectin) has been implicated in contributing to BBB dysfunction (Kisucka et al., 2009; Jin et al., 2010) and Bai et al. (2014) found higher sP-selectin concentrations in BD ($p = 0.029$) however Ormerod et al., (2022) found no difference in sP-selectin concentration in BD compared to controls. Kılıç et al. (2020) found that claudin-5 and zonulin, both components of the tight junctions between the endothelial cells of the BBB (Sturgeon & Fasano, 2016; Greene, Hanley & Campbell, 2019) were elevated in BD I during mania and in remission compared to controls ($p < .001$). Zengil et al., (2023) also found that zonulin was elevated in BD compared to controls ($p < 0.001$) but found no difference between different phases of BD. Hochman et al., (2023) found that claudin-5 was elevated in BD compared to controls ($p = 0.03$). Smirnova et al. (2019) reported no difference between serum levels of cadherin 5, a transmembrane protein that forms the adherens junctions between the endothelial cells that form the BBB (Kuriyama et al., 2014), in BD compared to controls ($p = 0.45$).

3.2.6. **Effect of mood state on BBB Blood/CSF markers**

13 studies compared BBB dysfunction markers in BD participants in different mood states. 7 studies reported that markers of BBB dysfunction were significantly increased during manic or bipolar depressive episodes compared to euthymia (Andreazza et al., 2007; Tsai & Huang, 2017; Hidese et al., 2020; Turan et al., 2014; Rybakowski et al., 2012; Pantović-Stefanović et al., 2018; Saravanan et al., 2022). Hidese et al. (2020) found that CSF S100B levels were positively correlated with the severity of mania in BD,
while there was no correlation with depression severity. S100B was found to be increased in manic episodes compared to depressive and/or euthymic episodes (Andreazza et al., 2007; Tsai & Huang, 2017; Hidese et al., 2020). Increased ICAM as well as VCAM levels were also reported in manic episodes compared to remission ($p = 0.049$) (Turan et al., 2014). However, Pantović-Stefanović et al. (2018) found decreased levels in VCAM during mania compared to depression in BD ($p = 0.016$). MMP-9 levels were found to be increased during acute depression and remission after depression compared to mania, manic remission and healthy controls younger than 45 years of age (Rybakowski et al., 2013). Saravanan et al., (2022) found that MMP-9 levels were higher in mania compared to depression ($p = 0.008$) and correlated with severity of mania ($p = 0.043$). See Table 2.

Table 2. Summary of the results of Blood and CSF markers of BBB function across mood states
<table>
<thead>
<tr>
<th>Author, Date</th>
<th>Mood State Sample Size</th>
<th>Mood state</th>
<th>Molecule Measured</th>
<th>Effect Direction</th>
<th>QA score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreazza et al., 2007</td>
<td>Depressive: 21 Manic: 32 Euthymics: 32</td>
<td>Manic and depressed vs controls</td>
<td>S100B</td>
<td>▲</td>
<td>8/8</td>
</tr>
<tr>
<td>Tsai &amp; Huang, 2017</td>
<td>Manic (pre): 17 Remission (post): 11 Responders (post): 6 Total BD: 68 Other not specified</td>
<td>Manic pre vs post treatment *</td>
<td>S100B</td>
<td>▲</td>
<td>7/8</td>
</tr>
<tr>
<td>Hidese et al., 2020</td>
<td>Euthymic: 62 Manic: 33 Depressive: 9 Hypomanic: 2 Mixed: 1</td>
<td>Manic vs depressive vs euthymic</td>
<td>S100B</td>
<td>-</td>
<td>7/8</td>
</tr>
<tr>
<td>Karabulut et al., 2019</td>
<td>Manic: 20 Euthymic: 20</td>
<td>Manic pre vs post treatment *</td>
<td>MMP-9</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td>Chiarani et al., 2013</td>
<td>Manic: 24 Depressive: 30</td>
<td>Depression vs mania and remission *</td>
<td>MMP-9</td>
<td>▲</td>
<td>6/8</td>
</tr>
<tr>
<td>Saravanan et al., 2022</td>
<td>Depressive: 44 Manic: Not specified</td>
<td>Manic vs Depressive</td>
<td>MMP-9</td>
<td>▲</td>
<td>6/10</td>
</tr>
<tr>
<td>Turan et al., 2014</td>
<td>Manic: 50 Remission: 40</td>
<td>Manic vs remission *</td>
<td>ICAM &amp; VCAM</td>
<td>▲</td>
<td>5/8</td>
</tr>
<tr>
<td>Pantovic-Stefanovic et al., 2018</td>
<td>Manic: 39 Depressive: 44</td>
<td>Manic vs depressive</td>
<td>VCAM</td>
<td>▼</td>
<td>8/8</td>
</tr>
<tr>
<td>Schaefer et al., 2016</td>
<td>Manic: 20 Depressive: 21 Euthymic: 31</td>
<td>Manic vs depressive vs euthymic</td>
<td>ICAM</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td>Bai et al., 2014</td>
<td>Manic: 14 Depressive: 41 Euthymic: 75</td>
<td>Manic vs depressive vs euthymic</td>
<td>P-selectin</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td>Kiliç et al., 2020</td>
<td>Manic: 20 Euthymic: 21</td>
<td>Manic vs euthymic</td>
<td>Zonulin &amp; claudin</td>
<td>-</td>
<td>8/8</td>
</tr>
<tr>
<td>Zengil et al., 2023</td>
<td>Manic: 21 Depressive: 7 Euthymic: 16</td>
<td>Manic vs Depressive vs euthymic</td>
<td>Zonulin</td>
<td>-</td>
<td>7/8</td>
</tr>
</tbody>
</table>

MMP-9 matrix metalloproteinase-9; ICAM intercellular adhesion molecule; VCAM vascular cell adhesion molecule; QA Quality Assessment (score/total available; Newcastle Ottawa scale is scored out of 10, Joanna Briggs scale is scored out of 8). - No significant difference; ▲ Increase in the mood state group compared; * BD participants treated, measurements taken and compared are pre and post treatment.

3.3. Imaging studies

One study, Kamintsky et al. (2020), used dynamic gadolinium contrast-enhanced MRI to create quantitative maps of brain leakage and assigned 50 participants (36 BD and 14 controls) into extensive or normal BBB leakage groups. All 10 of the participants in the
extensive BBB leakage group (with over 12.2% of the brain affected by leakage) were people with BD, and all had insulin resistance. These BD participants displayed a diffuse, rather than a focal, pattern of leakage with 112 out of 124 brain regions having significantly higher leakage. This study also found that people with BD and extensive BBB leakage had higher rates of chronic illness with more frequent and/or severe manic/depressive episodes, defined by use of the affective morbidity index (AMI, rating the severity and length of manic/depressive episodes, Berghofer et al., 2008), patient interviews, detailed review of medical records, and analysis of daily mood ratings.

3.4. Post-mortem studies

All 19 studies (which included 889 participants) used q-PCR and/or immunostaining for the quantification of BBB markers including astrocyte markers (GFAP, S100B and ALDH1L1), tight junction proteins (claudin-5), cell adhesion proteins (ICAM, VAM and neurexin) and glyocalyx proteins (chondroitin sulphate proteoglycans). All the extracted analyses were from people with BD compared to healthy controls. Given that GFAP is only a reliable marker of reactive astrocytes (Sofroniew & Vinters, 2009), after careful discussion, it was decided that GFAP staining was not a reliable marker of the BBB. For this reason, the studies using this method initially included in the review are summarised for completeness in the Supplementary Table 1.

Cell adhesion molecules (ICAM and neurexin) were reported to be increased in the ACC ($p = 0.001$) and dorsolateral prefrontal cortex ($p = 0.01$) in people with BD compared to controls (Jenkins et al., 2016; Thomas et al., 2016). Greene et al., (2020) reported increased claudin-5 mRNA levels in the occipital cortex ($p = 0.05$) and cerebellum in people with BD ($p = 0.03$) which negatively correlated with age of illness onset and duration. Meanwhile, claudin-5 protein levels in the orbitofrontal cortex and hippocampus did not differ among the groups. An abnormality in translational process in BD cannot be concluded from this study because mRNA levels were measured in separate areas of
the brain to protein levels. Chondroitin sulphate proteoglycans levels, constituents of the endothelial glycocalyx, were increased in the lateral nucleus of the entorhinal cortex (EC) ($p = 0.04$), decreased in layer III of the EC ($p = 0.02$) as well as in the amygdala ($p < 0.03$) in BD (Pantazopoulos et al., 2010 and 2015) and this also correlated with lithium medication history. See Table 3.

Table 3. Tight junctions and cell adhesion molecule post-mortem studies: results summary of measures in BD compared to healthy controls.

<table>
<thead>
<tr>
<th>Author, Date</th>
<th>Sample Size BD/HC (total)</th>
<th>Method</th>
<th>Target measured</th>
<th>Brain region</th>
<th>Effect Direction</th>
<th>QA score (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greene et al., 2020</td>
<td>15/15 (60) Other: 30</td>
<td>Immuno-histochemistry</td>
<td>Claudin-5</td>
<td>Hippocampus</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Claudin-5 mRNA</td>
<td>OFC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jenkins et al., 2016</td>
<td>34/245 (279)</td>
<td>qRT-PCR</td>
<td>Neurexin-α mRNA</td>
<td>Occipital cortex</td>
<td>▲</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantazopoulos et al., 2015</td>
<td>20/29 (73) Other: 24</td>
<td>Immuno-histochemistry</td>
<td>Chondroitin sulphate proteoglycans containing glial cells</td>
<td>Amygdala</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basal N</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Accessory Basal N</td>
<td>▼</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medial N</td>
<td>▼</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Central N</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cortical N</td>
<td>▼</td>
<td></td>
</tr>
<tr>
<td>Pantazopoulos et al., 2010</td>
<td>11/15 (37) Other: 11</td>
<td>Immuno-histochemistry</td>
<td>Chondroitin sulphate proteoglycans</td>
<td>EC layer III</td>
<td>▼</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LN of Amygdala</td>
<td>▲</td>
<td></td>
</tr>
<tr>
<td>Thomas et al., 2004</td>
<td>15/15 (60) Other: 30</td>
<td>Immuno-histochemistry</td>
<td>ICAM</td>
<td>ACC</td>
<td>▲</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GM</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>WM</td>
<td>▲</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DLPFC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OFC orbitofrontal cortex, PFC prefrontal cortex, EC entorhinal cortex, LN lateral nucleus, ACC anterior cingulate cortex, DLPFC dorsolateral prefrontal cortex, N Nucleus, GM grey matter, WM white matter, qRT-PCR quantitative reverse transcription polymerase-chain reaction, mRNA messenger RNA, ICAM intercellular adhesion molecule. QA Quality Assessment measured using The Newcastle-Ottawa Scale (scored out of 10 point). Bipolar disorder type was not specified in any of the studies included in the table. Other refers to a psychiatric diagnosis different from bipolar disorders included in the total sample size where the presented effect results do not include this group. These are typically major depressive disorder and/or schizophrenia. - No significant difference; ▲ Increase in the BD group compared to controls; ▼ decrease in the BD group compared to controls.

A full description of the results is available in Supplementary Table 4.
3.5. Genetic studies

A handful of studies looked at BBB-relevant genetic loci in relation to BD prevalence. Rybakowski et al. (2009) examined 416 people with BD and 558 controls, and found the T-allele of the \textit{MMP-9} gene to be more common in BD than controls ($p = 0.02$). However, although significant, this association could be considered quite weak given the relatively large sample size in this study. In a subsequent study, no significant differences were found in the distribution of these polymorphisms across different BD lithium response groups (Rybakowski et al., 2011). Saravanan et al. (2022) found no significant difference in the frequency of the \textit{MMP9} rs17576 polymorphism between BD and controls ($p = 0.09$). However, they found a significant relationship between \textit{MMP9} levels and genotype. Notably, BD patients carrying at least one mutant allele had elevated \textit{MMP9} serum levels compared to controls with the same genetic variants (AG: $p < 0.001$; GG: $p = 0.022$). Moreover, the mutant allele was more common among patients with depressive symptoms in BD compared to controls ($p = 0.013$). There were no significant differences in the distribution of the \textit{MMP3} functional polymorphisms (Kucukali et al., 2009). For the P-glycoprotein polymorphisms, Turgut et al. (2009) found a deviation from equilibrium in the C3435T polymorphism, indicating a possible association between BD and p-glycoprotein structure ($p < 0.01$). Naumovska et al. (2017) found an uneven distribution of p-glycoprotein polymorphisms only in women, with the alleles 1236 and 2677 being potential risk polymorphisms for BD in women ($p < 0.05$). In the case of cell adhesion molecule genes, 22 significant risk genes were identified (O’Dushlaine et al., 2011)(Table 4). No associations were found with other factors apart from sex. More information on these studies is presented in Supplementary Table 4.
Table 4. BBB genetic markers results summary in BD compared to healthy controls

<table>
<thead>
<tr>
<th>Author, Date</th>
<th>Sample size</th>
<th>BD Diagnosis</th>
<th>Gene</th>
<th>Comparison</th>
<th>Overall result</th>
<th>QA score (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saravanan et al., 2022</td>
<td>141/121 (262)</td>
<td>TI</td>
<td>MMP9 rs 17576</td>
<td>BD vs HC</td>
<td>Depressed ▲ in G and GG</td>
<td>6</td>
</tr>
<tr>
<td>Naumovska et al., 2017</td>
<td>18/107 (161) Other: 36</td>
<td>TNS</td>
<td>ABCB1 rs 1128503 rs 2032582 rs 1045642</td>
<td>BD vs HC</td>
<td>▲</td>
<td>6</td>
</tr>
<tr>
<td>Rybakowski et al., 2011</td>
<td>BD: 109 ER: 26 PR: 55 NR: 20</td>
<td>TI and TII</td>
<td>MMP9 rs 3918242</td>
<td>BD vs HC</td>
<td>Lithium groups</td>
<td>6</td>
</tr>
<tr>
<td>Kuckali et al., 2009</td>
<td>141/121 (262)</td>
<td>TI</td>
<td>MMP3 rs 1171 5A/6A</td>
<td>BD vs HC</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Rybakowski et al., 2009</td>
<td>416/558 (974)</td>
<td>TI and TII</td>
<td>MMP9 rs 3918242</td>
<td>BD vs HC</td>
<td>▲ in T</td>
<td>5</td>
</tr>
<tr>
<td>Turgut et al., 2009</td>
<td>104/169 (273)</td>
<td>TNS</td>
<td>MDR1 C3435T</td>
<td>BD vs HC</td>
<td>▲ in CT</td>
<td>6</td>
</tr>
<tr>
<td>O’Dushlaine et al., 2011</td>
<td>4847/3587 (7393) Other: 3322</td>
<td>TNS</td>
<td>CAM TJ</td>
<td>BD vs HC</td>
<td>255▲*</td>
<td>5</td>
</tr>
</tbody>
</table>

BD Bipolar Disorder; HC healthy controls; TI Type 1; TII Type 2; TNS type not specified; MMP matrix metalloproteinase; ABCB1 ATP binding cassette subunit B1; MDR1 Multidrug resistance 1; CAM cell adhesion molecule; TJ tight junction; NOS Not otherwise specified; TNS type not specified; SNP single-nucleotide polymorphism; Quality Assessment measured using The Newcastle-Ottawa Scale (scored out of 10 point)- no significant deviation; * The number of different SNPs in genes which were differentially distributed in BD subjects; ▲ Increased odds ratio with given SNP allele or genotype compared to wild type, comparing the frequency in BD to HC. Allele and/or genotype could confer a risk of BD.

3.6. Quality assessment

Overall, the quality of the studies included in this systematic review is acceptable. Two case-control studies did not meet the minimum quality criteria and, therefore, possible bias arises when using these studies to compare results (Supplementary Table 2, studies 13 and 20). One cross-sectional study was deemed low quality, while the rest
received a moderate or good quality score (Supplementary Table 3). Refer to the supplementary materials for more details.
4. Discussion

The results of this systematic review provide converging evidence to suggest the involvement of BBB dysfunction in BD. While a synthesis of the literature is challenging given the heterogeneity in study methods, participants and design, there is some evidence for BBB dysfunction from serum-based and CSF-based, post-mortem, neuroimaging, and genetic studies. The most extensive and perhaps convincing evidence is from serum and CSF studies, where 25 out of 29 included studies found evidence in favour of an association between BD and markers of BBB disruption, including 22 studies reporting a difference in levels of BBB markers between participants with BD and healthy controls, and 7 studies reporting a difference in levels of BBB markers across mood states in BD.

Measurement of BBB permeability in the living human brain is challenging and perhaps for this reason most studies in this review include a proxy marker of the state of the BBB. Perhaps the most direct measure of permeability is the radiographic analysis of intracerebral BBB leakiness. Only one study of this measure in BD has been published and this reported increased BBB permeability in diffuse brain regions in people with BD compared to controls (Kamintsky et al., 2020). Furthermore, our review found only one study investigating QA1b in BD (Zetterberg et al., 2014), despite numerous studies using this measure in the schizophrenia/psychosis literature (Pollak et al., 2018). QA1b is a measurement of the integrity of the blood-CSF barrier, rather than a true measurement of BBB integrity. Similar to schizophrenia and MDD, elevated QA1b was also found in BD (Futtrup et al. 2020; Medina-Rodriguez & Beurel, 2022; Zetterberg et al., 2014). We would suggest that using neuroimaging and QA1b measures to investigate BBB and blood-CSF barrier function in BD merits further investigation.
Overall, studies of serum and CSF markers of BBB function in BD included in this review are at best suggestive of dysfunction. One reason for this is that in most cases BBB-specific cell types are not the only source of the measured proteins and so these findings could also be measuring damage to other cell types (e.g., S100B and astrocytic damage). However, our review did identify a promising emerging literature investigating claudin-5 levels in BD, which is a component of BBB tight junctions (Greene et al., 2020; Hochman et al., 2023; Kılıç et al., 2020).

From our review, the evidence for BBB dysfunction being associated with specific mood states in BD was unclear. Of the blood/CSF studies, only 13 of the 29 studies performed subgroup analysis between different mood states and the results were mixed. 7 found that markers of BBB permeability were increased during acute episodes of depression or mania compared to euthymia and/or healthy controls or that the level of BBB permeability markers was associated with the severity of the acute episode. Increased S100B has been associated with depressive disorders (Medina-Rodríguez & Beurel, 2022). In line with this, we found (3 studies out of 4) increased levels of S100B in depressed BD participants as well as manic participants compared to euthymic states. Our findings may suggest that BBB permeability is involved in the pathophysiology of acute episodes but may also represent a transient phenomenon. The association between mood states in BD and BBB dysfunction remains a highly promising focus of future research.

The emerging literature of BD has suggested mood-state-specific increases in various inflammatory markers, such as TNF-α, IL-6, and C-reactive protein (Futtrup et al., 2020). Given the mechanistic links between BBB dysfunction and neuroinflammation, it is possible that BBB dysfunction may be a component of the link between neuroinflammation and acute mood states (Dickerson et al., 2013; Leboyer et al., 2012). Five studies found a correlation between the chronicity of BD and measures of BBB
permeability. Serum MMP9, ICAM and S100B levels were related to early or later stages of BD (Karabulut et al. 2019; Reininghaus et al., 2016). Greene et al. (2020) found Claudin-5 levels in the orbitofrontal cortex to be negatively correlated with illness duration. Interestingly, Claudin-5 levels in major depression are also associated with the duration of depressive episodes (Medina-Rodriguez & Beurel, 2022). Extensive BBB leakage was also related with longer and more severe episodes in BD (Kamintsky et al. 2020). This might suggest that BBB permeability increases with, and may be a marker of, chronicity or progression of disease, while also representing a confounding factor for past and future studies. Carefully controlled work comparing people with BD with different degrees of disease progression across multiple BBB biomarkers will be necessary to determine whether BBB dysfunction increases with BD progression.

The heterogeneity of findings and use of indirect markers of BBB dysfunction likely means that no clear conclusion can be drawn from the included post-mortem studies of BBB markers in BD. The results included in this review indicate variation of BBB permeability throughout the brain, rather than a consistently increased permeability in any particular brain area. Cell adhesion molecules, such as ICAM, are expressed in endothelial cells of the vasculature and may be dysregulated by inflammation (Diétrich, 2002). The increased levels reported by the studies included in this review, mirror previous findings in MDD (Medina-Rodriguez & Beurel, 2022), and could indicate an inflammatory response in the brain with upregulation of ICAM in the brain vasculature rather than a breakdown of the BBB. The genotype studies we included also identified a significant association with single-nucleotide polymorphisms (SNPs) in the genes coding for the proteins of the cell adhesion and extracellular matrix pathways in people with BD (O’Dushlaine et al., 2011) which mirrors findings in schizophrenia (Zhang et al., 2015; Rempe, Hartz, & Bauer, 2016). Additionally, MMP9 polymorphisms appear to have a relation with BD and the depressive symptomatology experienced during the disorder, in
line with recent findings pointing to a relationship between MMP9 and depression (Li et al., 2022; Saravanan et al., 2022). However, the manner in which BD is affected by MMP9 is yet unknown.

The effect of medication on levels of markers of BBB dysfunction was investigated by 26 studies included in this review. 18 studies found no effect of medication on markers of BBB integrity, regardless of the type of medication examined. S100B levels were reported to be lower in BD participants who were taking psychotropic medications, however the specific medications used were not indicated (Hidese, 2020; Tsai & Huang., 2017; Schoeter et al., 2002). QA1lb was reported higher in BD participants treated with antipsychotics (Zetterberg et al., 2014). Lithium treatment was positively correlated to increased glial cell levels, while it was associated with decreased serum GFAP mRNA levels (respectively, Pantazolopoulos et al., 2015; Ferensztajn-Rochowiak et al., 2016). Given these findings, there is no conclusive evidence that pharmacotherapy in BD is associated with BBB dysfunction. However, considering previous reports on the effect of some antipsychotic on the BBB permeability, this is an area that requires further investigation (Elmorsy et al., 2014).

Overall, the quality of the included studies in this review was acceptable. Case-control studies assessed with the Newcastle-Ottawa scale found that all studies, except for two (O'Dushaine et al., 2011; Rybakowski et al., 2009), reached high scores, indicating a low probability of bias. Meanwhile, cross-sectional studies assessed using the Joanna Briggs Institute Checklist for Analytical Cross-Sectional Studies found that more than half of the studies had a high-quality marking, while 12 were moderate and only one ranked as low quality (Janković & Djordijević, 1991) (Supplementary tables 2 and 3).
**Limitations**

There are several limitations to the methodologies used in the studies included in this review. Measuring post-mortem levels of BBB related proteins provides at best an indirect measure of BBB integrity. Imaging methods, such as dynamic contrast enhanced techniques and positron emission topography, have been successfully used to assess the integrity of the blood-brain barrier, however there is only one study using these methods to assess BBB integrity in BD (Moyaert et al., 2023). Furthermore, not every CSF/serum marker explored in this review are well established as markers of BBB dysfunction. The two most common markers are CSF/serum albumin ratio and S100B blood/CSF measures (Roh, Cho, Yoon, & So, 2017; Marchi et al., 2004; Koh & Lee, 2014). Albumin ratio can be also affected by decreased CSF production and flow rate; therefore, it can only be indicative of blood-CSF barrier permeability (Bechter, 2020; Bechter et al., 2010). Meanwhile, S100B is also produce in adipose tissue, meaning that their serum levels can be influences by factors such as individuals' Body Mass Index (Steiner et al., 2010). Therefore, how well serum S100B levels indicate BBB permeability is questionable. Importantly, because of the diversity of methodologies used in the studies included in this systematic review, heterogeneity between study protocols was not sufficient to allow a meta-analysis.

Additionally, reporting of BD participant characteristics was poor with some studies failing to report potentially relevant variables such as mood state at time of study, specific BD diagnosis and illness duration. Many studies did not conduct any sub-group analysis within BD groups based on these characteristics; particularly lacking were studies that considered BD progression/illness duration. Therefore, there were a limited number of studies on which to draw conclusions about the specific nature of the association between BD and BBB dysfunction which may limit the validity of the findings in this review. The included age range in the blood/CSF papers was also quite narrow,
particularly excluding older adults which may have affected these results. Many of the post-mortem studies had small sample sizes, which could have affected the study power, potentially causing less robust effects to fall short of statistical significance. Finally, it is worth noting that only studies written in English were included, limiting our ability to consider the results of those studies reported in a different language.

**Future research**

More research is needed to conclusively address the question of whether BBB dysfunction is associated with BD, particularly regarding associations with mood state. This review found significant limitations with the current literature, especially in the heterogeneity between studies. We would suggest that future studies should compare participants with different BD types (I, II, NOS, with and without psychosis) and in different mood states to explore the nature of the potential association of BBB dysfunction and BD. Medication use should also be controlled for and reported. Additionally, studies should classify BD participants by illness duration and aim to clarify whether BBB plays a role in BD progression. We would also suggest that studies with the objective of measuring BBB dysfunction should measure BBB permeability using imaging methods, as these can provide permeability rates and leakage locations (Moyaert et al., 2023; Sun et al., 2021). Our review suggests a great potential for the future use of neuroimaging, CSF and blood BBB biomarkers to better understand disease processes in BD, particularly in relation to chronicity and mood state.

5. **Conclusions**

This review provides tentative evidence towards an association between BBB dysfunction, specifically increased BBB permeability, and bipolar disorders. It is unclear whether this relationship means that markers of BBB dysfunction may be a state or trait marker of bipolar disorders. Markers of BBB dysfunction have been shown to be
correlated with chronicity of bipolar disorder, which could be due to primary processes of the disorder or its treatment, worsening physical health, or other psychosocial factors. As such, it remains to be seen to what extent these pathologies co-occur, and in what proportion of people with bipolar disorders. Further research, ideally longitudinal follow-up studies, is needed to answer these questions.
6. Acknowledgments

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7. Declaration of interests

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8. References

References with an asterisk (*) are those included in the systematic review


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**Figure 1. Blood-brain barrier structure and chain effects of its breakdown.**

A. A single layer of endothelial cells forms the lumen of the blood vessel. Joined by tight junction and cell adhesion proteins. Pericytes sit around the endothelial cells without covering them entirely. A basement membrane covers this whole structure. Astrocytic end-feet project to this whole complex covering the surface. Glial fibrillary acidic protein (GFAP) and S100B calcium-binding protein B sit within astrocytes.

B. 1) Breakdown of tight junction proteins (ICAM, Zonulin and Cadherin-5), these diffuse into the bloodstream. 2) As a result, there is an increased diffusion of toxins from the bloodstream into brain tissue. 3) Increased toxin concentration causes matrix metalloproteinases (MMP) to activate, excessive MMP activation leads to damage to the basal membrane and astrocytes. Increased activity of the MMPs also leads to further breakdown of tight junctions, worsening this effect. 4) S100B and GFAP are now released from astrocytes and can diffuse into the bloodstream. 5) Overall, there is increased permeability of the BBB. A neuroinflammatory response to BBB breakdown starts taking place, which in turn affects other cerebral tissue, such as neurons and their functioning.
Figure 2. PRISMA flowchart summarising literature search and study selection. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71