Brain microbleeds are small dot-like lesions appearing as hyposignals on gradient echo T\textsubscript{2} magnetic resonance sequences. In Alzheimer’s disease, brain microbleeds are of special interest as they may play a crucial role in the pathophysiology. They may be a missing link between two important theories on the neuropathogenesis of Alzheimer’s disease—the amyloid cascade hypothesis and the vascular hypothesis. Moreover, they may affect the clinical course of the disease and may have therapeutic consequences. The aim of this article is to review available data to understand the meaning of brain microbleeds in clinical terms and underlying pathology in the context of Alzheimer’s disease. We also review the available evidence and highlight the pitfalls of our current knowledge on brain microbleeds in the setting of clinical trials design.

**Keywords:** brain microbleeds; Alzheimer’s disease; dementia

**Abbreviations:** CAA = cerebral amyloid angiopathy; ICH = intracerebral haemorrhage; PiB = \textsuperscript{11}C-Pittsburgh compound B

### Introduction

Brain microbleeds are small dot-like lesions appearing as hyposignals on gradient echo T\textsubscript{2} -weighted magnetic resonance sequences. They have been considered as rare and silent lesions but the development of specific magnetic resonance sequences raised the interest in this radiological construct that is meant to represent a specific underlying microscopic pathology—perivascular collections of haemosiderin deposits. Brain microbleeds may occur in deep or cortico-subcortical locations, potentially related to their pathophysiology. These lesions, which were previously considered as rare and silent, are becoming frequent and clinically meaningful. Literature has focused mainly on community-based samples and specific clinical samples, such as patients with intracerebral haemorrhage (ICH) due to cerebral amyloid angiopathy (CAA). In Alzheimer’s disease, brain microbleeds are of special interest as they may have a crucial role in the pathophysiology of the disease. Moreover, they may affect the clinical course of the disease and may have therapeutic consequences. Some therapeutic trials exclude patients with brain microbleeds because of a putative effect on risk of brain haemorrhage or encephalopathy.
However, the generalization of results from studies in specific populations to Alzheimer’s disease is questionable. To date, no recommendations are available to guide clinicians and researchers for the management of patients with Alzheimer’s disease with brain microbleeds. The aim of this article is to review available data to understand the meaning of brain microbleeds in clinical terms and underlying pathology in the context of Alzheimer’s disease. We will also review the available evidence and highlight the pitfalls of our current knowledge on brain microbleeds in the setting of clinical trials design.

Basis for review and identification of references

References for this article were identified through searches of the medline database (http://www.ncbi.nlm.nih.gov/pubmed/) with the following search terms: ‘Alzheimer’s disease’, ‘dementia’, ‘cerebral amyloid angiopathy’, ‘MRI’, ‘microbleed(s)’, ‘microhaemorrhage(s)’, ‘petechial haemorrhage(s)’ between January 1966 and July 2010. The references from identified articles and the authors’ own files were also searched for relevant publications. Only articles published in English or with available translations of relevant data were reviewed. The final reference list was chosen on the basis of relevance to the topics covered in this article.

How frequent are brain microbleeds in Alzheimer’s disease?

An overview of the five studies in which prevalence of brain microbleeds in Alzheimer’s disease was studied is presented in Table 1 (Nakata et al., 2002; Hanyu et al., 2003; Cordonnier et al., 2006; Nakata-Kudo et al., 2006; Pettersen et al., 2008). StatsDirect software was used to estimate the pooled prevalence of brain microbleeds in Alzheimer’s disease. As two studies described overlapping samples (Nakata et al., 2002; Nakata-Kudo et al., 2006), we included the study with the largest sample size in the meta-analysis. Based on considerable heterogeneity of the individual studies, a random effects model was chosen resulting in a pooled proportion of 23% (95% confidence interval 17–31%) (Fig. 1). An overall qualitative assessment of the number of brain microbleeds in Alzheimer’s disease suggests that, among patients with brain microbleeds, approximately half have only one brain microbleed and the other half exhibit two or more brain microbleeds. In rare cases, dozens of lesions can be seen. Prevalence of brain microbleeds in different disease settings, as compared with Alzheimer’s disease, is represented in Fig. 2.

Table 1 List of publications reporting prevalence of brain microbleeds in Alzheimer’s disease populations

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of patients</th>
<th>Number of patients with microbleeds</th>
<th>Prevalence of microbleeds (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nakata et al. (2002)</td>
<td>38</td>
<td>7</td>
<td>18 (9–33)</td>
</tr>
<tr>
<td>Hanyu et al. (2003)</td>
<td>59</td>
<td>19</td>
<td>32 (22–45)</td>
</tr>
<tr>
<td>Cordonnier et al. (2006)</td>
<td>223</td>
<td>41</td>
<td>18 (14–24)</td>
</tr>
<tr>
<td>Nakata-Kudo et al. (2006)</td>
<td>50</td>
<td>8</td>
<td>16 (8–29)</td>
</tr>
<tr>
<td>Pettersen et al. (2008)</td>
<td>80</td>
<td>23</td>
<td>29 (20–39)</td>
</tr>
</tbody>
</table>

a: Overlapping samples, the largest sample was used for the meta-analysis of prevalence.

Significance of brain microbleeds in Alzheimer’s disease neuropathology

To demonstrate the neuropathological correlates of the radiological construct of brain microbleeds, post-mortem imaging studies...
combined with histology are warranted. However, there are only a limited number of pathological studies regarding brain microbleeds; three studies gathering three controls, 11 patients with ICH and eight patients with Alzheimer’s disease have been published (Fazekas et al., 1999; Tatsumi et al., 2008; Schrag et al., 2010). Fazekas et al. (1999) were the first to demonstrate—in a sample of patients who died from ICH—that histologically, brain microbleeds represent focal leakage of haemosiderin from abnormal small blood vessels (Fazekas et al., 1999). Due to the nature of the samples (mostly hypertensive patients who died from ICH) in this study, brain microbleeds were strongly associated with hypertensive vasculopathy. This association was also reported in a single case study of an elderly hypertensive woman (Tatsumi et al., 2008). In Alzheimer’s disease, only eight brains have been reported to date (Schrag et al., 2010). The majority of the lesions appeared in cortico-subcortical location and amyloid beta deposition was present in the vessel walls adjacent to the bleeds suggesting that cortico-subcortical brain microbleeds are a consequence of underlying CAA. Some argue that all amyloid plaques arise from bleeds, based on the co-location of brain microbleeds and amyloid deposition in the brain, but this finding remains to be replicated (Cullen et al., 2006; Stone, 2008). Available data suggest that different mechanisms can lead to brain microbleeds. This dichotomization is particularly relevant in Alzheimer’s disease for the following two reasons: (i) Alzheimer’s disease and hypertensive vasculopathy—patients with Alzheimer’s disease often have a past history of arterial hypertension even if at the time of diagnosis they are no longer hypertensive (Skoog et al., 1996) and (ii) Alzheimer’s disease and CAA—the accumulation of amyloid proteins within the walls of blood vessels and surrounding them termed CAA, occurs in 78–98% of patients with Alzheimer’s disease and is frequently considered to be a major pathological feature of Alzheimer’s disease (Kalaria and Ballard, 1999). Not only is CAA associated with Alzheimer’s disease, but CAA also contributes to the cognitive decline in Alzheimer’s disease (Pfeifer et al., 2002a). It seems that disentangling the differential or synergistic effects of CAA and/or arterial hypertension on brain microbleeds in Alzheimer’s disease is related not only to the presence but also to the anatomical distribution of brain microbleeds. Typically, deep and infratentorial brain microbleeds are presumed to result from hypertensive vasculopathy, while cortico-subcortical brain microbleeds seem to be closely related to CAA.

**Microbleeds: a missing link between the amyloid cascade hypothesis and the vascular hypothesis?**

Specifically in Alzheimer’s disease, it is conceivable that brain microbleeds can develop through both pathways, as neurodegenerative (including amyloid angiopathy) and cerebrovascular pathology both play a role in the pathophysiology of the disease.

**Amyloid cascade hypothesis**

The most widely accepted theory regarding the pathogenesis of Alzheimer’s disease is the amyloid cascade hypothesis (Hardy and Selkoe, 2002). In short, the amyloid cascade hypothesis states that the development of Alzheimer’s disease is initiated by abnormal cleavage of the amyloid precursor protein, resulting in an imbalance of production and clearance of amyloid β. As a consequence, amyloid β accumulates in the brain in the form of senile plaques. Despite extensive research, the amyloid hypothesis has not provided definitive answers. The first problem with this hypothesis is that amyloid deposition does not correlate with dementia severity or extent of neurodegeneration; and secondly, the amyloid cascade hypothesis does not account for the role of tau in the pathophysiology of Alzheimer’s disease. Still, it seems that abnormal processing of the amyloid β-protein may be one of the initiating events in the development of Alzheimer’s disease, with other processes, including neuronal dysfunction and neurodegeneration, becoming the key pathological processes later on in the disease process (Jack et al., 2010).

**Vascular hypothesis**

Over the last decade, the vascular hypothesis, stating that vascular pathology plays an important role in the pathogenesis of Alzheimer’s disease, has emerged as an alternative (Snowdon et al., 1997; Breteler, 2000; de la Torre, 2004). On MRI, expressions of cerebrovascular disease, such as white matter hyperintensities and lacunes, are more often observed in patients with Alzheimer’s disease than in controls (Schneider et al., 2004; DeCarli, 2006). Still, despite much research, the vascular hypothesis has not led to a breakthrough either, as correlations of cerebrovascular disease with cognitive impairment are only modest (Prins et al., 2005; Schmidt et al., 2005; van der Flier et al., 2005).

There is debate between those who perceive Alzheimer’s disease to be a ‘pure disease’, and those who recognize overlap between cerebrovascular and neurodegenerative processes. Brain microbleeds, being an expression of vascular damage and at the same time being closely related to amyloid deposition, may serve as an additional argument for the notion that amyloid and cerebrovascular pathology act in synergy to cause Alzheimer’s disease.

**Brain microbleeds: a missing link?**

Brain microbleeds might be considered a reflection of an important underlying process in the pathogenesis of Alzheimer’s disease, as they may be a candidate to bridge the amyloid cascade and vascular hypotheses. Figure 3 represents a hypothetical model illustrating the potentially central role of brain microbleeds in the pathogenesis of Alzheimer’s disease. On one hand, brain microbleeds are strongly related to hypertensive vasculopathy (Fazekas et al., 1999; Greenberg et al., 2009). At the same time, they are seemingly a marker of CAA (Cullen et al., 2006; Greenberg et al., 2009; Schrag et al., 2010). The specific underlying pathology of brain microbleeds may differ by brain location. Brain microbleeds in the deep grey matter and infratentorial regions may be the result of microangiopathy, such as ischaemia and arteriolosclerosis.
(vascular route). In contrast, brain microbleeds in cortico-subcortical regions may be the result of deposition of amyloid proteins within the walls of blood vessels (amyloid route). Brain microbleeds can then be seen as the common downstream product of two separate pathways that act as a catalyst towards subsequent degeneration. This hypothetical model integrates knowledge from epidemiological studies on brain microbleeds in healthy populations and in specific patient groups such as ICH populations in which CAA and hypertensive vasculopathy both play a role in the pathophysiology of the haemorrhage and of the dementia frequently associated (Cordonnier et al., 2010).

Limitations

Although CAA and Alzheimer lesions co-occur in up to 90% of patients with Alzheimer’s disease at autopsy (Kalaria and Ballard, 1999; Attems, 2005), only 23% of patients with Alzheimer’s disease exhibit brain microbleeds. Brain microbleeds presumably are only the tip of the iceberg, being a macroscopic reflection of severe CAA, while modest CAA may not necessarily lead to the occurrence of brain microbleeds on MRI. Other artefactual reasons explaining this discrepancy are that MRI data often come from patients with mild disease, while autopsy data by definition concern patients with end-stage disease. Furthermore, to date, no data are available on the combination of post-mortem MRI and autopsy in Alzheimer’s disease cohorts regarding brain microbleeds and CAA lesions. Finally, MRI parameters may also influence the detection rate of brain microbleeds, as with improved magnetic resonance techniques, the prevalence of brain microbleeds may rise not only in the general population as shown in the Rotterdam study (Vernooij et al., 2008), but even more so in patients with Alzheimer’s disease. Alternatively, it is also conceivable that patients with Alzheimer’s disease with brain microbleeds represent a specific subgroup of patients with a slightly different disease process and a different clinical course, who might therefore benefit from a different management strategy. If this is true, then the proposed model may not be applicable to the neuropathogenesis of Alzheimer’s disease in general but rather to this subgroup only. Even then, there could be much value in such a model, which attempts to specify and differentiate the neuropathogenesis of Alzheimer’s disease. The model describes how different routes may result in one common downstream product commonly regarded as ‘Alzheimer’s disease’.

The proposed model clearly does not capture the full breadth of disease processes essential to develop the clinical syndrome of Alzheimer’s disease. For example, the tau protein has not been incorporated in the model, while this protein undoubtedly plays a role of major importance in the neuropathogenesis of Alzheimer’s disease. Likewise, the loss of synapses is an early event in the development of Alzheimer’s disease, but has not been incorporated in the model. In a recently coined hypothetical model combining evidence from MRI, positron emission tomography
and CSF biomarker studies, it has been suggested that the cascade of pathological events starts with abnormal processing of amyloid β, leading to the deposition of plaques in the brain. Later on in the disease process, neuronal dysfunction and neurodegeneration (evidenced by increased tau, atrophy and synaptic loss) become the dominant disease processes (Jack et al., 2010). The model described here adds a possible mechanism for interaction with vascular factors early on in the disease process, and suggests that the synergy between amyloid deposition and cerebrovascular damage may trigger further neuronal dysfunction and neurodegeneration. The latter are not further elaborated on in the current model, but are clearly crucial in the development of Alzheimer’s disease.

Anatomical distribution of brain microbleeds in Alzheimer’s disease

Most studies on brain microbleeds in Alzheimer’s disease demonstrated a cortico-subcortical predominance (Figs 4 and 5) but with some patients exhibiting brain microbleeds both in deep and cortico-subcortical locations. One study specifically focused on the anatomical distribution of brain microbleeds and revealed that the topography of brain microbleeds had cortico-subcortical predominance in 92% of patients with Alzheimer’s disease, with occipital lobes accounting for 57% of these brain microbleeds (Pettersen et al., 2008). Interestingly, the cortico-subcortical pattern of distribution was similar to the CAA related ICH distribution, indirectly suggesting that brain microbleeds in Alzheimer’s disease in cortico-subcortical location are linked to CAA (Rosand et al., 2005).

Associated factors

Arterial hypertension

Brain microbleeds are strongly related with hypertensive vasculopathy. Brain microbleeds are more frequent in hypertensive people, both in general (odds ratio 3.9; 95% CI 2.4–6.4) and in cerebrovascular populations (odds ratio 2.3; 95% CI 1.7–3.0) (Cordonnier et al., 2007). Although disentangling the differential or synergistic effects of CAA and arterial hypertension on brain microbleeds in Alzheimer’s disease is highly relevant, in Alzheimer’s disease cohort data on arterial hypertension in relation to brain microbleeds are available from only one small study (n = 50) (Nakata-Kudo et al., 2006). The odds ratio associated with arterial hypertension was 1.10 (95% CI 0.24–4.99). This odds ratio suggests that the association between arterial hypertension and brain microbleeds is weaker among patients with Alzheimer’s disease than among cerebrovascular disease populations or healthy people. Possible explanations include: (i) arterial hypertension was under-diagnosed at the time of the study since patients with Alzheimer’s disease were no longer hypertensive but used to be (Skoog et al., 1996) or (ii) brain microbleeds in Alzheimer’s disease are more likely to be associated with CAA than with hypertensive vasculopathy.
Magnetic resonance imaging measures of small vessel disease

Vascular pathology is important in Alzheimer’s disease (Schneider et al., 2004; DeCarli, 2006). Associations between brain microbleeds and white matter hyperintensities have often been found (Cordonnier et al., 2006; Pettersen et al., 2008; Vernooij et al., 2008; Goos et al., 2009). Likewise, brain microbleeds have been shown to be associated with lacunes (Roob et al., 1999; Horita et al., 2003; Wardlaw et al., 2006). This provides evidence for microangiopathy as an underlying mechanism for brain microbleeds. In Alzheimer’s disease, some studies reported a correlation between the severity of white matter hyperintensities and the existence of brain microbleeds (Hanyu et al., 2003; Nakata-Kudo et al., 2006; Pettersen et al., 2008; Goos et al., 2009). Unfortunately, they all used different scales precluding any meta-analysis. These associations suggest that brain microbleeds can be interpreted as a third expression of small vessel disease on MRI next to white matter hyperintensities and lacunes.

The synergistic effects of CAA and ischaemic small vessel disease on brain microbleeds in Alzheimer’s disease may be explained not only by the presence, but also by the anatomical distribution of brain microbleeds. In a healthy population, a relationship between severity of white matter hyperintensities and deep, but not cortico-subcortical, brain microbleeds was shown, suggesting that especially deep brain microbleeds are an expression of small vessel disease (Vernooij et al., 2008). In the studies focusing on Alzheimer’s disease, however, relationships with white matter hyperintensities and lacunes were reported despite a clear predominance of cortico-subcortical brain microbleeds, precluding any firm association with brain microbleed location.

Apolipoprotein E genotype

In an analysis of ApoE genotype in healthy elderly subjects subdivided according to brain microbleeds distribution, the Rotterdam Scan study found an association with the ApoE e4 allele pertaining only to the subgroup with isolated cortico-subcortical brain microbleeds and not the subgroup with deep hemispheric or infratentorial brain microbleeds (Vernooij et al., 2008). Given the relationship between ApoE e4 and CAA (Greenberg et al., 1995), these results provide further evidence that isolated cortico-subcortical brain microbleeds often reflect the presence of advanced CAA. No association with ApoE genotype was found in two studies in Alzheimer’s disease (n = 139) (Hanyu et al., 2003; Pettersen et al., 2008). Sample sizes were not designed to disclose any significant results. In a study comparing patients with Alzheimer’s disease with many brain microbleeds to patients with Alzheimer’s disease without any brain microbleeds, patients with many brain microbleeds were more likely to be homozygous for ApoE e4 (Goos et al., 2009).

Cerebrospinal fluid biomarkers

CSF levels of the protein amyloid β 1–42 are reduced in patients with Alzheimer’s disease, probably reflecting the presence of senile plaques, one of the neuropathological hallmarks of Alzheimer’s disease (Blennow and Hampel, 2003; Mattsson et al., 2009; Mulder et al., 2010). In a preliminary study, patients with Alzheimer’s disease with multiple brain microbleeds were shown to have even more severely reduced levels of CSF amyloid β 1–42 than patients with Alzheimer’s disease without any brain microbleeds (Goos et al., 2009). Notably, the brain microbleeds in this study almost invariably had a cortico-subcortical location, suggesting a link with CAA. Congruent with this finding, it was recently shown that patients with CAA (diagnosed according to the Boston criteria (Knudsen et al., 2001)) had more strongly reduced levels of CSF amyloid β 1–42 than patients with Alzheimer’s disease (Verbeek et al., 2009). In the latter study, amyloid β 1–40 was also found to be reduced in patients with CAA, while such an effect was not found in patients with Alzheimer’s disease. The described associations with CSF biomarkers provide support for the notion that brain microbleeds, especially those with cortico-subcortical location, are in some way directly linked to (vascular) amyloid, one of the major pathological hallmarks of Alzheimer’s disease.

Positron emission tomography

Using 11C-Pittsburgh compound B (PiB) positron emission tomography, it is possible to visualize in vivo and quantify amyloid deposition both in the brain parenchyma and in the vessels (Klunk et al., 2004; Tolboom et al., 2009). Some studies suggest that in patients with CAA, an increased PiB retention in occipital lobes is observed (Johnson et al., 2007; Ly et al., 2010). Comparison of patients with CAA, healthy controls and patients with Alzheimer’s disease, suggest a different pattern of PiB retention both in terms of intensity and anatomical distribution according to the underlying disease. Unfortunately, no data on brain microbleeds were available. These preliminary data suggest, however, that patients with Alzheimer’s disease, according to the number and location of their brain microbleeds, could show a specific distribution of PiB retention.

Superficial siderosis

Superficial siderosis is a radiological or pathological diagnosis of curvilinear haemosiderin deposits in the subpial layers of the central nervous system. Superficial siderosis can be seen on T2 weighted images as low intensities that hug the surface of the brain. It is thought to represent remnants of prominent leakage of blood from a leptomeningeal vessel into adjacent subarachnoid space or peri-adventitial compartment (Linn et al., 2010). Furthermore, it has been described as closely linked with CAA and even as a potential in vivo diagnostic marker for CAA (Linn et al., 2010). A direct association between superficial siderosis and CAA was described on autopsy in two patients with Alzheimer’s disease (Feldman et al., 2008). Because brain microbleeds and CAA are associated, one may speculate that in Alzheimer’s disease, a subset of patients with brain microbleeds will also exhibit features of superficial siderosis. Unfortunately, no large study focusing on this association has been specifically conducted in an Alzheimer’s disease cohort. The only available data come from the Rotterdam population-based registry—among 1062 healthy subjects, seven (0.7%) exhibited...
superficial siderosis, all of whom had brain microbleeds in lobar locations (Vernooij et al., 2009b).

Clinical significance of brain microbleeds

Studies on the clinical significance of brain microbleeds are slowly emerging, but mostly in populations other than Alzheimer’s disease.

Cognitive dysfunction

In stroke and non-demented patients, brain microbleeds have been associated with cognitive decline (Greenberg et al., 2004; Werring et al., 2004; Yakushiji et al., 2008). There is only one study investigating relationships between brain microbleeds and neuropsychological performance in a relatively large number of patients with Alzheimer’s disease (n = 80) (Pettersen et al., 2008). The authors were not able to find any association with neuropsychological performance. Previous studies, focusing on Mini-Mental State Examination score only, failed to demonstrate the impact of brain microbleeds on cognition (Nakata et al., 2002; Nakata-Kudo et al., 2006; Pettersen et al., 2008). Potentially, those studies have been hampered by their relatively small sample size (n = 168), low number of brain microbleeds and the crude nature of Mini-Mental State Examination. To overcome the problem of low numbers of brain microbleeds, a proof of principle approach was taken in a study comparing patients with Alzheimer’s disease with multiple brain microbleeds with patients with Alzheimer’s disease without any brain microbleeds (Goos et al., 2009). Patients with multiple brain microbleeds had more severe cognitive impairment that could not be explained by disease duration, degree of atrophy or white matter hyperintensities.

Predicting dementia in mild cognitive impairment

Mild cognitive impairment is characterized by isolated memory impairment, not sufficient for a diagnosis of dementia (Petersen et al., 2001). Patients with mild cognitive impairment are at an increased risk to develop dementia, mostly Alzheimer’s disease, with a rate of 12–15% per year (compared with 1–2% in the normal population). A study on the predictive value of brain microbleeds in patients with mild cognitive impairment showed that the observation of at least one brain microbleed on MRI yielded a more than 2-fold increase, though non-significant risk of non-Alzheimer dementia (Staekenborg et al., 2009). Another study showed that occurrence of brain microbleeds on susceptibility weighted MRI in patients with mild cognitive impairment predicted subsequent cognitive decline (Kirsch et al., 2009). It is conceivable that brain microbleeds in healthy populations, especially those in cortico-subcortical locations, are predictive of future development of Alzheimer’s disease, while brain microbleeds in deep locations might be predictive of vascular dementia. To date, however, this has not been investigated in studies with a sufficient duration of follow-up.

Mortality

Only one study investigated the association of brain microbleeds with subsequent mortality in memory clinic patients (Henneman et al., 2009). Brain microbleeds on presenting MRI were associated with a more than 2-fold increased risk of mortality, which could not be explained by other expressions of small vessel disease on MRI (i.e. white matter hyperintensities) or vascular comorbidity. There was a clear dose–effect relationship, with a higher number of brain microbleeds being related with an increased risk, while one or a few brain microbleeds did not have much impact. It is tempting to assume that the increased risk of mortality is due to increased occurrence of haemorrhage, but at present, this remains speculative.

Do brain microbleeds in Alzheimer’s disease infer an increased risk of intracerebral haemorrhage?

There are no data on the natural history of ICH in patients with Alzheimer’s disease, nor are there any data on brain microbleeds in relation to ICH in patients with Alzheimer’s disease.

Are brain microbleeds related to a distinct clinical phenotype?

From the above, a picture arises where patients with Alzheimer’s disease with multiple brain microbleeds may have a distinct clinical phenotype, although this should be confirmed in further studies. These patients have a worse cognitive outcome, higher risk of mortality, and a higher risk of new brain microbleeds. They tend to be ApoE ε4 positive more often and seem to have more abnormal levels of CSF amyloid β than patients without any brain microbleeds, suggesting a link with underlying CAA. It is tempting to think that patients with Alzheimer’s disease with many cortico-subcortical brain microbleeds have this phenotype, while patients with especially deep or infratentorial brain microbleeds might present with a different phenotype. Literature to support such a conclusion is not yet available however. Likewise, it is not clear at this point if patients with Alzheimer’s disease with one or a few brain microbleeds should also be regarded as having a distinct clinical phenotype. This question touches on the subject of whether patients with Alzheimer’s disease with brain microbleeds are a different subgroup, or whether brain microbleeds are a visible expression of an underlying disease process that (almost) all patients with Alzheimer’s disease have. Knowing that almost all patients with Alzheimer’s disease have CAA to some extent, and that brain microbleeds can be regarded as a macroscopic expression of severe CAA, we feel that patients with one or two brain microbleeds are representative of Alzheimer’s disease in general, without a specific clinical phenotype. This is also supported by the available literature on clinical relevance of brain microbleeds reviewed above: associations with any clinical outcome are hard to find, probably due to the large number of patients with only one brain microbleed.
Microbleeds and treatments

To date, there are no evidence-based data to guide the clinician for treatment decision in a patient with brain microbleeds (Cordonnier, 2010). No specific data are available for patients with Alzheimer’s disease. In a population-based setting, brain microbleeds were more prevalent among users of antiplatelet agents (odds ratio 1.7; 95% CI 1.2–2.4) while anticoagulants failed to show a significant effect (odds ratio 1.5; 95% CI 0.8–2.7) (Vernooij et al., 2009a). Because of the cross-sectional design of this study, the causal relationship remains unclear. Anti-thrombotic use may give rise to the development of brain microbleeds. Alternatively, patients with vascular disease, requiring anti-thrombotic treatment may have more brain microbleeds to start with, as a result of their vascular disease. Given the current lack of evidence, the benefits of anti-thrombotic agents seem to outweigh the potential risks in secondary prevention of vascular diseases.

Microbleeds in clinical trials

With studies on the relevance of brain microbleeds in Alzheimer’s disease emerging, brain microbleeds have become a factor of interest in the design of clinical trials, especially with regard to the new generation of immunization therapies. Still, very little literature is available to make recommendations on how to handle brain microbleeds in the context of clinical trials.

Microbleeds and immunization therapy

An early trial with immunization therapy was terminated when patients in the intervention arm developed encephalitis (Check, 2002; Bayer et al., 2005). Autopsy of one of the 15 patients who developed encephalitis convincingly showed that immunization had succeeded in removing amyloid β plaques, while at the same time there was no change in tangles, neuropil threads or vascular amyloid (CAA) (Nicoll et al., 2003). Furthermore, amyloid β immunoreactivity was strongly associated with activated microglia. It has been suggested that the immunization therapy may have triggered an inflammatory response not only against amyloid β plaques, but also against vascular amyloid, resulting in subsequent cognitive decline (Greenberg et al., 2003). These observations suggest that patients with advanced CAA, as evidenced by brain microbleeds on MRI, might be less eligible for amyloid β immunization therapy. Confirmatory evidence for this line of reasoning comes from animal studies, which have similarly shown a reduction in diffuse, but not vascular, amyloid in reaction to passive amyloid β immunization (Pfeifer et al., 2002b). Furthermore, immunized mice were at a 2-fold increased risk of CAA-related cerebral haemorrhage. However, haemorrhagic strokes were not a prominent feature in the Elan human vaccine study (Bayer et al., 2005).

Recently, results of the bapinezuamab trial were reported (Salloway et al., 2009). Overall, the study was negative on the primary efficacy analysis, but exploratory analyses showed a potential beneficial effect of the immunization in ApoE ε4 non-carriers, as opposed to the ApoE ε4 carriers, in whom no such effect was observed. Furthermore, the adverse event of vasogenic oedema was detected on brain MRI in 10% of the treated patients versus none in the control group. The vast majority of patients with vasogenic oedema were ApoE ε4 carriers. The cause of vasogenic oedema is unknown, but may be related to the presence of CAA, which is in turn known to be related to ApoE ε4. Vasogenic oedema can occur spontaneously in CAA and with agents that alter vascular permeability. As such, it may be related to removal of amyloid β from the cerebral blood vessels, or other mechanisms related to amyloid clearance.

The results of the aforementioned study suggest that ApoE ε4 carrier status may influence both efficacy and adverse events of immunization therapy. Taking into account the possible underlying mechanism, it is conceivable that advanced CAA, rather than ApoE ε4 status per se, is the causative feature. Unfortunately, nobody really knows how to take into account the presence of CAA in living patients with Alzheimer’s disease. Prevalence and number of brain microbleeds are the most likely candidates.

Nowadays, the question is no longer whether to exclude or include brain microbleeds in any trial influencing the amyloid pathway, but rather how many brain microbleeds can we include safely and at which number should we decide to stop therapy. Unfortunately, there is no literature available to guide trial designers in this respect, especially since the prevalence and number of brain microbleeds also strongly depends on the specific MRI parameters. In this context, it is of importance to note that in a naturalistic cohort of 254 memory clinic patients (including 74 patients with Alzheimer’s disease) who underwent repeated MRI after an average period of two years, one or more new brain microbleeds were observed in 12% (Goos et al., 2010). This finding implies that with a natural disease course, new brain microbleeds should be expected in 6% of patients on a yearly basis. Presence and number of baseline brain microbleeds were the strongest predictors of incident brain microbleeds, together with other MRI expressions of small vessel disease and ApoE ε2 genotype. Based on the studies on clinical impact of brain microbleeds, the effect of brain microbleeds on, example, mortality or cognitive outcome seems to have a strong dose–effect relationship. An educated guess could therefore be to allow one or a few brain microbleeds, but exclude patients with many brain microbleeds. Furthermore, location of brain microbleeds may be of importance, with cortico-subcortical brain microbleeds being more strongly related to CAA than brain microbleeds in deep or infratentorial locations. One could therefore imagine using a stricter limit for cortico-subcortical locations than for deep or infratentorial locations.

Microbleeds and anti-platelet agents

Another relevant category of trials in relation to brain microbleeds in Alzheimer’s disease, are trials on antiplatelet agents as primary prevention. Preliminary evidence suggests that antiplatelet agents may infer an increased risk of ICH in Alzheimer’s disease. Among 156 patients with Alzheimer’s disease receiving acetylsalicylic acid, four suffered from an ICH compared with none among the
Brain microbleeds and Alzheimer’s disease

154 Alzheimer’s disease receiving placebo (Bentham et al., 2008). The same tendency was found in a recent trial where three patients with Alzheimer’s disease suffered from an ICH among 65 patients with Alzheimer’s disease receiving aspirin compared with none in the cohort of 58 Alzheimer’s disease receiving placebo (Richard et al., 2009). Although the small numbers of patients preclude a definitive conclusion, the finding might suggest that a subgroup of patients with Alzheimer’s disease who use acetylsalicylic acid might be at greater risk of ICH. Unfortunately, the presence of brain microbleeds was not taken into account in these studies. It would be of great importance to stratify these types of analyses by presence of brain microbleeds at baseline, to see if brain microbleeds predispose for the development of ICH in the context of using antiplatelet agents as primary prevention. Conversely, it would also be important to investigate whether patients without brain microbleeds are highly unlikely to develop ICH after aspirin, rendering them safe to use aspirin as primary prevention (Fiala, 2010).

Trials that try to rigorously regulate vascular risk factors including arterial hypertension to prevent the development of Alzheimer’s disease should also take into account the presence of brain microbleeds. It is conceivable that patients with deep brain microbleeds, who clearly show evidence of hypertensive vasculopathy, benefit most from this kind of therapy. Stratification by presence of brain microbleeds might increase the power of these studies.

In summary, guidelines on how to handle brain microbleeds in the context of clinical trials are currently lacking, but it is becoming increasingly clear that the observation of brain microbleeds can be highly relevant. Future studies should take the presence of brain microbleeds into account as a potentially modifying factor of the treatment effect. Furthermore, when thinking about the role of brain microbleeds in the design of clinical trials, one should also take into account that brain microbleeds may develop along two routes (the route of hypertensive vasculopathy and the route of CAA, Fig. 3), potentially related to their preferred location of occurrence. For immunization therapy, it may be the cortico-subcortical brain microbleeds that should be considered, while for trials focused on the treatment of vascular risk factors, deep brain microbleeds may be more relevant.

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References


