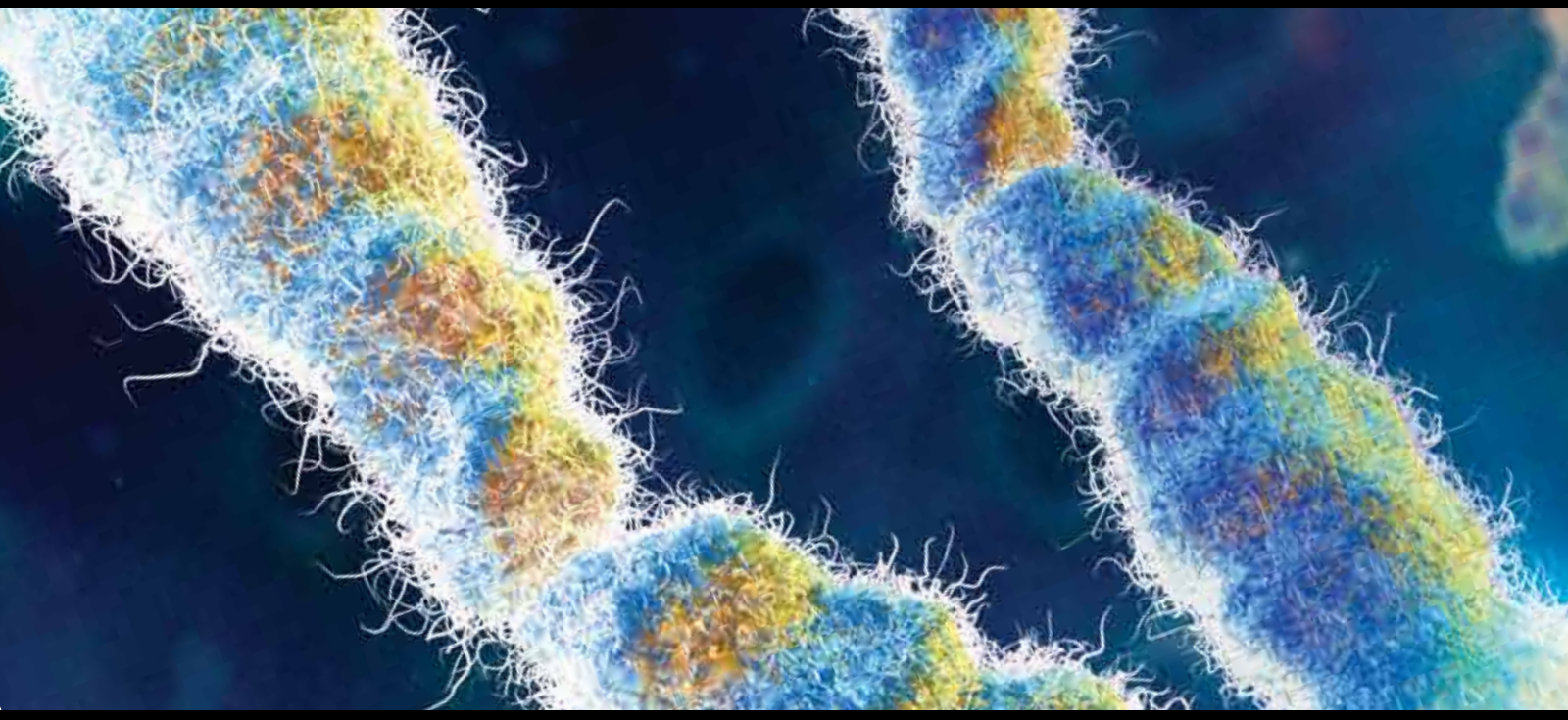
Journal of Aging Research

Cerebrovascular Disorders: Role of Aging

Guest Editors: Aurel Popa-Wagner, Charles L. Rosen, and Emil Toescu



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Editorial Board

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Editorial

Cerebrovascular Disorders: Role of Aging

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Therapeutic development for neurological diseases, whether cerebrovascular disease, vascular dementia, or Alzheimer's disease (AD), has been largely unsuccessful. Despite the apparent success of numerous pharmacologic agents in preclinical animal models, few have translated successfully from bench to bedside. As such, a vast need remains unmet and additional investigation is required.

Speculation concerning the reasons for this failed translation from bench-to-bedside often highlights either the lack of reproducibility or a poor choice of the outcome measure parameters. However, in most of the literature, the lack of clinical relevance of the animal model used often goes unnoticed. The role of age in neurologic disease development is well known, in fact, age is the greatest risk factor for development of AD as well as stroke. In sharp contrast to this clinical reality, the vast majority of preclinical studies utilize young or young-adult animal models of neurologic disease, despite the suggestion of collaborative advisory groups such as the Stroke Therapy Academic and Industry Roundtable (STAIR).

The ageing process affects various biological axes, from the status of the cardiovascular system to the status of the inflammatory responsiveness and finally to the changes in the ability of the neural systems to handle pathological insults and a significant reduction in the recovery capability. At one end of this spectrum of changes in cerebral cardiovascular status are the morphological changes in vasculature, manifested as large increases in the vascular path due to increased tortuosity of arterioles in the deep white matter [1] and an age-associated decrease in capillary number and length, leading to a significant increase (25%) of the intercapillary distance in both the hippocampus and cortex [2] and white matter [3]. Such processes would lead to tissular hypoxia that in young or adult brain would trigger an angiogenesis response, mediated by HIF-1 as a transcription factor for VEGF. However, in the aged brain, there is a reduction in the angiogenesis response, due to decreased responsiveness to HIF-1. Such age-associated changes in the blood vessels architecture are even more relevant to the brain, because of the specifics of vascularization: the system of feeding arteries is situated near the surface of the brain, from where end-arteries are penetrating through the gray and white matter in a network that terminates in a capillary bed but with very few shunts [4].

As a result of such anatomical changes, there is little surprise that in the aged brain there is a significant reduction in the cerebral blood flow (CBF), affecting mainly the cortex, and more sparingly the subcortical regions, as revealed by a variety of imaging techniques, from positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) to high-resolution, contrast-enhanced MRI and arterial-spin labeling (ASL) [5]. However, as it has been pointed out [6], CBF is affected not only by morphological changes, but it is also modulated by a variety of functional parameters such as perivascular innervation [7],

astroglial control of arteriolar constriction [8], and autocrine endothelial signaling in response to rheostatic forces and neural environment [9].

For the clinical category of cerebrovascular diseases, the stroke is, by a distance, the most significant entity, both in terms of prevalence and consequences. The stroke can result from either an occlusion of the vessel (ischemia), which can be either transient (e.g., the transient ischemic attack, TIA) or of longer duration; or from the rupture of a vessel, leading to regional hemorrhage, either within the cortical matter or in the dural cavities. While the biological and medical consequences of a stroke are significant at any age, the incidence and the severity of a stroke are significantly increased with age. The paper from Russo et al. provides a systematic review, looking at the first stroke incident and shows that the trend continues for the very old (older than 80 years old).

Several factors are likely contributors to this increased severity of stroke with age. One such factor is a metabolic decrease in the capacity of neural cells to counteract extreme stressors and/or neurotoxic challenges (decreased metabolic reserve) [10] that appear in the penumbra region of a stroke, in which a combination of hypoxia and resulting decrease in ATP provisions will lead to the generation of a hyperexcitable environment, posing significant metabolic challenges.

Stroke in the very old is a very frequent condition, with an unfavourable outcome, and makes a relevant contribution to the social burden of stroke and may require more efficient, dedicated stroke services (Russo et al.). Aged individuals recover less well from stroke, and rehabilitation aims at improving the physical and cognitive impairments and disabilities of patients with stroke. Therefore, studies on behavioral recuperation after stroke in aged animals are necessary. Various experimental settings have been used to assess the recovery of sensorimotor functions, spontaneous activity, and memory after ischemia in aged rats. Overall, the results indicate that aged rats have the capacity to recover behaviorally after cortical infarcts, albeit to a lesser extent than their young counterparts [11, 12]. It should be kept in mind, however, that before stroke aged rats are already impaired as compared to young animals and show significantly decreased performance in some tests like spontaneous activity [13]. Accelerated glial reactivity to stroke in aged rats correlates with reduced functional recovery [13] including Morris watermaze [14]. Behavioral and histological effects of chronic antipsychotic and antidepressant drug treatment in aged rats with focal ischemic brain injury are discussed in [14].

Another crucial component of the brain tissue response to stroke is the inflammatory response. As reviewed in the article by Shah and Di Napoli (2011) the age-associated changes in the immune function determine that in the aged and individuals, the response to an acute stroke involves a more intense inflammatory reaction in the first phase of acute ischemia, involving cytokine activation and chemokine expression that lead to an early scar formation and fibrosis. In agreement with previous observations [15], the article also points out the fact that the biology of the aged brain is different from that of the young brain, and this has significant implications for the current translational efforts to define effective therapies, since a large majority of interventional studies are performed on young and young-adult animals.

Another important perspective in asserting new potential therapeutical interventions for stroke is to assess if the differences in responsiveness between young adult and old brains are due only to functional changes or if they involve more elaborate changes in protein expression. Recent detailed analyses of the genomics data from adult and aged animals indicate changes in the balance of the various systems and regulatory processes. Using categorized DNA arrays, we found inappropriate gene regulation in response to stroke both in the ipsilateral and the contralateral hemisphere of aged rats. The gene expression profile in the brains of poststroke aged rats is indicative of increased cell death due to DNA damage and apoptosis, especially in the first week after stroke. Similarly, we reported persistent downregulation of genes that are required for neurogenesis after stroke in aged rats [16].

However, the category of cerebrovascular diseases is not restricted to the pathologies involving the large vessels. Cerebral small vessels disease (cSVD) is a group of pathological processes associated with established vascular risk factor such as hypertension, atherosclerosis, diabetes mellitus, and atrial fibrillation. The consequences of small vessel disease on the brain parenchyma are mainly lesions located in the subcortical structures such as lacunar infarcts, white matter lesions, large hemorrhages, and microbleeds. This compendium of tissue lesions has been compiled in the last decade or so by the ever expanding use of more powerful and sensitive brain imaging technologies, rather than by a better understanding or direct assessment of actual small vessels territories. As a result, the cerebral small vessels diseases are now invoked in explaining a variety of clinical syndromes, and van Uden and collaborators discuss in their paper the possibility that such vascular pathology is underlining the clinical manifestations of the late onset depression in the aged people (I. W. van Uden et al. (2011)). Even more daring, Teggi et al. propose that cSVD might also explain some forms of the Meniere disease [17].

Another area of neuropathology in which small vessel disease has a well-established role that gathers ever increasing recognition in both the clinical and basic research world is that of being a leading cause of cognitive decline and functional loss in the elderly. Vascular dementia is a broad concept that has evolved slowly. While general anecdotal references linking putative cerebral vascular events with reductions in cognitive performance have existed for long time, it is the articles of Binswanger and Alzheimer around the turn of the 20th century describing in detail the various types of vascular lesions that could be encountered in human brains that set the field on a strong morphopathological footing, and it took another 70 years until Hachinski suggested that dementia of vascular origin was a consequence of multiple strokes [18]. Subsequently, the concept continued to evolve to include multiple mechanisms related to deficiencies in cerebral blood supply, including large vessel disease, small vessel pathology, consequences of cerebral hypoperfusion and hemorrhage.

While the risk factors for vascular diseases are relatively well established, much less consensus exists on the specific risk factors for vascular dementia, that is, apart, from the acknowledged role of increased age. Ongoing interest in cerebrovascular diseases research has provided data showing that Alzheimer's proteins and other factors may be involved in the pathogenesis of gradual ischemic brain injury. Thus, both focal and global brain ischemia in rodents produce a stereotyped pattern of selective neuronal degeneration, which is just the same as in Alzheimer's type dementia. As hypothesized in the article by Enciu et al., there is an overlap of events between chronic hypoxia and AD on several levels, such as hypoxic-triggered cellular pathways, inflammatory environment, growth factor signaling, and calcium homeostasis. A reduction of CBF and a series of molecular events precede the major ischemic events in vascular cognitive impairment. Based on these subtle changes, intervention at early stages could prevent the full-blown development of dementia, which might represent a "point of no return" for the neurovascular units and neuronal networks with few chances for efficient treatment. In their extensive review, Enciu and her colleagues also discuss some of the molecular mechanisms that are triggered by the cerebrovascular diseases and that lead to overcognitive dysfunction and argue convincingly the case for an early intervention at the point at which the dysfunction of the neurovascular units is still potentially reversible.

However, it is important to keep in mind that the genetic makeup is a hugely important factor, from the subtle alterations in the function of various proteins (e.g., ApoE4) to the overt cerebrovascular diseases with a genetic control, as the cerebral autosomal arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) that involves mutations in Notch-3 protein. Equally important is the discovery that fundamental genetic changes may occur with aging, such as the CLOCK genes, as discussed by Thome et al. (2011). Taken together, aging may result in fundamental changes throughout the body, beginning at a gene level and progressing to protein expression or posttranslational modification level.

It is clear that the quest for improved therapeutics will require increased understanding of disease pathophysiology and, in particular, the changes induced by the aging process. Similarly, neurologic disease research has often maintained a "neurocentric" focus, in which the role of the neuron was emphasized. This is unlikely to result in successful development of therapeutics due to the intimate relationship evident between neurons, surrounding glia, and the neurovascular unit—in both health and disease. The effect of aging on astrocyte and microglial response to injury and how this process can be manipulated successfully for therapeutic development must be considered.

Similarly, preclinical studies of neurologic disease utilizing aged animals must incorporate functional outcome measures in addition to histological measures. Clinically, the primary assessment remains functional outcome and presence/absence of significant disability yet preclinical studies often emphasize histological outcomes such as infarct volume. The difficulty of identifying high fidelity functional studies in aged animals remains a vexing problem.

Acknowledgments

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Clinical Study

Does Ménière's Disease in the Elderly Present Some Peculiar Features?

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Object. Aim of our study was to establish some peculiar features of Ménière's Disease (MD) in a group of elderly MD patients, in which the first vertigo spell happened when over 65 years old. *Material and Methods*. We analyzed a group of 73 younger than 65-years-old and a group of 30 elderly MD patients. All patients underwent a neurotological evaluation, an anamnestic evaluation including a lifetime history of migraine, and blood withdrawal for autoantibody screening. *Results*. Some differences were found between elderly and younger MD patients. Elderly MD patients presented a higher prevalence of Tumarkin attacks and a lower prevalence of lifetime history of migraine; moreover, they presented a faster develop of hearing loss and vertigo spells than a subgroup of 32 younger patients matched for the duration of illness. *Conclusions*. Some clinical features of MD in elderly have been pointed out. Particularly, the lower rate of migrainous history and positivity for autoantibodies often associated with MD, in our opinion, support the hypothesis of a vascular disorder acting as a predisposing factor for MD in elderly.

1. Introduction

Ménière's Disease (MD) is an inner ear disorder, characterized by recurrent episodes of rotational vertigo, coupled with fluctuating hearing loss and tinnitus. According to The American Academy of Otolaryngology-Head and Neck Surgery Foundation (AAO-HNSF), the diagnostic criteria for definite MD are the presence of two or more episodes of vertigo of at least 30 min with hearing loss plus tinnitus and/or aural fullness [1].

The prevalence of MD is 190 cases per 100000, and this value increases in the elderly [2].

The commonly accepted pathogenesis of MD is a raised endolymphatic pressure (hydrops), although a direct relationship between MD and endolymphatic hydrops is unproven [3, 4]. In the last few decades, several studies have analyzed possible etiological mechanisms and correlations with other diseases [5–14]. Epidemiological studies support the possibility of a correlation between MD and migraine. MD patients present a prevalence of migraine between 43% and 56%, which is significantly higher than the estimated 10% in the normal population [10, 11].

Migraine is considered per se a causal factor of recurrent vestibular symptoms, including both true rotational vertigo and subjective vertigo [5–15]; there is clinical evidence that migraine may damage the inner ear causing permanent hearing loss or vestibular deficit [16], and in children a fluctuating hearing loss has been considered as a migraine equivalent [17]. It has been supposed that the migrainous vasospasm may induce damage in the inner ear, acting as a disrupting factor for a secondary MD [9].

Since higher levels of circulating immunocomplexes and elevated autoantibody titers have been found in MD patients, the pathophysiology of MD has been supposed to be linked to an autoimmune disorder [18, 19]. An association with the PTPN*22* T allele, which has been described in other autoimmune diseases, has been reported in bilateral MD; these data support the idea of an inflammatory mechanism common to different autoimmune diseases [20].

Unilateral MD patients have a higher level of antiphospholipid antibodies than the normal population. The antiphospholipid antibodies, anticardiolipin, β_2 glycoprotein 1 autoantibodies, and Lupus-Like anticoagulant are correlated with thrombotic syndrome. The antiphospholipid

antibodies could mediate vascular diseases by a thrombotic mechanism [19].

Since 10% of cases present a familiar distribution, the genetics of MD has been studied [21–23], considering both possibilities of a single gene mutation provoking the syndrome (COCH gene encoding cochlin and chromosome 12p13.3 mutation) [24, 25] and acting as a predisposing factor (HLA B-27, Antiquitin) [26, 27]. Anticipation in familial MD has been described, consisting of an earlier age of onset in successive generations and more severe clinical manifestations. The genetic model may be linked to a trinucleotide repeat disorder; this mechanism is similar to other neurological diseases such as spinocerebellar atrophy 6 and myotonic dystrophy in which a channelopathy is supposed [14].

New studies on Ménière's disease (MD) in elderly patients may help bring about a prolongation of lifespan in the last decades. An earlier study on Japanese MD subjects reported a peak of onset around the fifth decade for men and in the fourth decade for women [28], with a 10-year shift from previously reported data. The neurotological evaluation of elderly MD patients found vestibular clinical features in the elderly similar to young and middle-aged MD patients except for a higher incidence of oculomotor system alterations [28]. A higher prevalence of MD in patients over 65 years has also been reported in a European population, both for a reactivation of MD and for a "de novo" MD. In elderly MD patients, a high incidence in women and of "drop attacks" has been described [29], and Tumarkin episodes may cause sudden falls. The Tumarkin attacks [30] are caused by an acute stimulation of an otolith organ in patients with MD or delayed endolymphatic hydrops; in some cases, differential diagnosis with drop attack is a puzzling dilemma [31].

The aim of our study was to establish some possible peculiar characteristics of MD in a group of elderly MD patients compared with younger MD patients.

2. Materials and Methods

2.1. Subjects. We studied 103 definite MD patients consecutively recruited at the outpatient facilities of the Vestibular Disorders Ambulatory at San Raffaele Hospital in Milan, from January 2006 until April 2011. The diagnosis of definite MD was established according to AAO-HNSF criteria (American Academy of Otolaryngology, 1995).

Patients were divided into two groups in relation to age of onset of MD. A group of 73 younger MD patients: 41 were females and 32 were males, mean age was 50.1 ± 12 years, and age of onset of MD was 39 ± 9.7 years. Two subjects presented bilateral MD. Delayed hydrops were excluded.

Between these patients, a subgroup of 32 subjects presented a lower than 6 years history of vertigo (3.4 ± 1.8 years). Mean age was 39.6 ± 10.3. Nineteen were females.

The second group was composed of 30 elderly MD patients: 13 were females and 17 males and mean age was 72 ± 4.3 years. All of them presented a lower than 6 years history of attacks and presented unilateral MD. Exclusion criterion for this second group was a lifetime history of previous vertigo episodes before 65 years of age. First vertigo attack happened 2.7 ± 2.3 years before the evaluation.

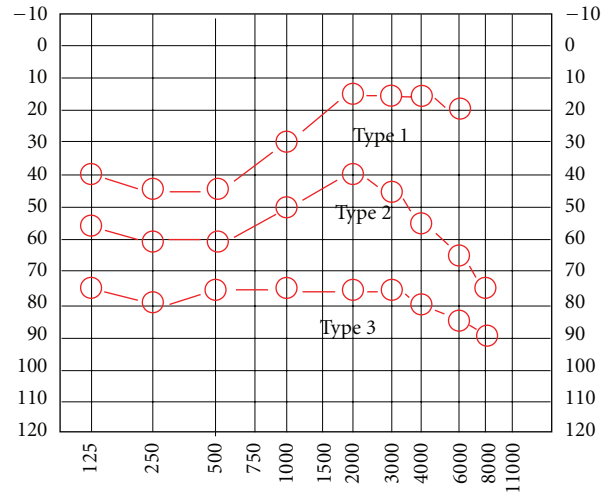

Figure 1: The 3 different types of audiometric exams; on x-axis are the represented frequencies, on y-axis, hearing loss in decibels (db).

All patients underwent a full neurotological examination, including audiometric test at 1 db precision, analysis of spontaneous, and evoked nystagmus with video-oculography (VOG 25, Interacoustics). Anamnesis of Tumarkin attacks and familiar history of vertigo (till grandparents) were assessed by a senior neurotologist. All of them performed a Central Nervous System MRI.

Morfology of audiometric exam was divided in three groups (Figure 1).

(1) Type 1: low frequencies sensorineural hearing loss with normal hearing threshold on high frequencies.

(2) Type 2: reduced hearing threshold at all frequencies, increased at low and high frequencies ("peaked curve").

(3) Type 3: reduced hearing at all frequencies with a threshold below 60 db ("flatcurve").

A lifetime history of migraine was established by a senior neurologist, according to the International Headache Society (IHS) criteria (Headache Classification Sub-Committee of the International Headache Society, 2004) [32].

An autoantibody screening (anti nucleus, mitochondrial, smooth muscle, thyroid, antiphospholipid autoantibodies, and Rheumatoid Factor) was assessed.

The study was approved by our Ethics Committee and patients signed informed consent.

2.2. Statistical Analysis. Continuously distributed variables were described by the mean and standard deviation (SD); categorical variables were described by frequencies and percentages. The significance of any difference between groups was evaluated by t-test for independent samples. A multiple linear regression analysis was performed in order to investigate the correlation between variables.

Table 1: Demographic and clinical data in elderly and younger MD subjects.

	Young MD ($n = 73$)	Elderly MD ($n = 30$)	P value
Age	50.1 ± 12	72 ± 4.3	$P \leq 0.01$
Age at onset of MD	39 ± 9.7	69.3 ± 4.1	$P \leq 0.01$
Migraine	38/73 (51%)	4/30 (20%)	$\chi^2 = 8.93$ $P = 0.03$
Positive autoantibody screening	25/73 (33.8%)	3/30 (20%)	$\chi^2 = 6.31$ $P = 0.02$
Tumarkin	7/73 (9.6%)	11/30 (36-7%)	$\chi^2 = 10.8$ $P \leq 0.01$
Familiar history of vertigo	8/73 (10.9%)	2/30 (6%)	$\chi^2 = 0.5$ $P = 0.5$
Number of spells in the last 6 months	5.5 ± 3.7	10.4 ± 5.4	$P \leq 0.05$

3. Results

Results are presented in Table 1. The elderly MD patients presented a lower rate of lifetime history of migraine ($P = 0.04$) and positive autoantibody screening ($P = 0.04$) than younger MD subjects. Only 1 patient with migraine presented positive autoantibodies.

At the time of examination, 12 of 20 (60%) elderly MD subjects presented a sensorineural hearing loss at all frequencies with a "flat" audiogram, while 8 (40%) had a peak at central frequencies. The elderly MD patients showed a higher prevalence of Tumarkin attacks ($P < 0.01$) and lower for lifetime history of migraine ($P = 0.03$) and positive autoantibodies ($P = 0.02$). Moreover, they presented a higher rate of vertigo spells in the 6 months before evaluation. Ten of 30 (33.3%) elderly MD patients presented microischemic lesions at MRI compared with 10 of 73 (13.7%) in the younger group ($\chi^2 = 5.2$, $P = 0.02$); nine of the 10 subjects in the last group were migraineurs.

Hearing loss (mean value for frequencies between 250 and 2000 Hz) at diagnosis was 64.6 ± 8.8 db in elderly and 53.8 ± 13.8 in younger MD subjects ($P = 0.001$), while the unaffected ear (the two subjects with bilateral MD were not included) was respectively, 18.2 ± 4.5 and 16.3 ± 3.3 ($P = 0.04$).

In the whole group of 103 subjects, a correlation has been established between Tumarkin attacks and lifetime history of migraine ($P = 0.05$) and between positivity of at least one of the autoantibodies and hearing loss ($P = 0.05$).

In order to demonstrate a possible faster evolution of MD in elderly, we compared the results of hearing loss in affected and unaffected ear and the number of vertigo spells in the last 6 months in the group of elderly and in the subgroup of younger MD subjects with a shorter than 6 years history of vertigo attacks. Results and statistics are summarized in the Table 2.

In the group of elderly MD subjects 24, (80%) presented a type 3 audiogram and 6 (20%) a type 2 audiogram; while between the 32 younger MD subjects, 12 (37%) presented a type 3 audiogram, 14 (44%) a type 2, and 6 (19%) a type 1 audiogram ($\chi^2 = 13.1$, $P = 0.001$).

4. Discussion

In our sample of 103 MD subjects, 30 (29%) presented onset of symptoms when over 65 years of age. Our data are in the range of other previous studies [29, 33]. In the sample of elderly MD patients, males were more represented (17 of 30, 56%), the opposite than in the younger sample 32 of 73 (44%), although result has no statistical evidence ($P = 0.2$).

Elderly subjects presented a more "aggressive" evolution of MD; these subjects referred a higher rate of vertigo spells and had a faster evolution toward a "flat" audiometric threshold (with higher values of hearing loss in the affected side) compared with the subgroup of 32 younger MD subjects matched for years since the onset of first symptom. It should be noted that the unaffected ear in elderly presented a lower hearing threshold than younger subjects, but the mean difference was only 2.1 db. As far as we know, no previous data have been published on a possible faster evolution of MD in elderly.

Elderly patients presented a higher rate of Tumarkin attacks, and on the topic our results confirm previously published works; a possible explanation may be linked to a lower compliance of otolithic structures to hydrops in elderly "de novo" MD patients [29, 34]. Alternatively, Tumarkin attacks may be linked to brief periods of ischemia or vasospasm of the anterior vestibular artery, which provides blood to the utricle, vestibular ganglion, and posterior semicircular canal. This branch is supposed to be more fragile than the posterior vestibular artery since the latter probably has a major number of intraosseous collaterals [35]. If so, the pathophysiology of Tumarkin should not differ significantly from that of paroxysmal positional vertigo, whose prevalence is significantly increased in the elderly.

The etiology of MD is at present a puzzling dilemma, and it seems probable that it consists of different pathologic conditions leading to the same cluster of symptoms. All our elderly subjects fulfill the diagnostic criteria for definite MD, but in the two groups, some clinical differences regarding possible "predisposing factors" may be noted, underlining the polymorphic nature of MD.

As previously discussed, epidemiological evidence has been found regarding an association between MD and migraine or autoimmune disorders; moreover, a genetic predisposition may be supposed, since around 10% of MD subjects refer of having at least 1 familiar with recurrent vertigo episodes.

In elderly MD subjects, we found a lower rate of positivity for autoantibodies, lifetime history of migraine and familiar history of recurrent vertigo.

These results and the presence of a higher rate of microischemic lesions are not inconsistent with the hypothesis of a different etiological mechanism, possibly related to vascular disorders, in the genesis of MD in elderly patients, and further studies should assess the question.

Elderly patients commonly present other vascular disorders, and these factors could influence the clinical features

Table 2: Values of hearing loss in affected and unaffected ear (mean value of frequencies between 250 and 2000 hz), number of vertigo spells in the last 6 months, and years since the first attack in the group of 30 elderly subjects and in the subgroup of 32 patients with a lower of 6 years history of vertigo.

	Elderly MD subjects ($n = 30$)	Younger MD subjects ($n = 32$)	P value
Years since first vertigo	2.7 ± 2.3	3.4 ± 1.8	0.08
Hearing loss affected ear	64.7 ± 8.1 db	48.2 ± 10.9 db	0.001
Hearing loss unaffected ear	18.2 ± 4.5 db	16.3 ± 3.3 db	0.04
N° of crises in the last 6 months	6.5 ± 3.7	10.4 ± 5.5	0.002

of MD. The presence of vascular problems in elderly MD patients has been found in other studies, and a higher prevalence of hypertension in MD patients has been reported in a population of 131 subjects [36]. Other studies have focused on a correlation between carotid atheromatosic changes and peripheral vestibular disorders, in some cases mimicking MD [37, 38].

A more recent work reported the presence of microischemic lesions of brain white matter in 31% of MD subjects, while only 25% of BPPV subjects, condition in which a vascular disorder has been demonstrated to act as a predisposing factor [39].

As a final consideration, MD in elderly patients may produce more disrupting results, since most of them present decreased postural and gait control due to a physiological reduction of the motor and sensorial system. Functional balance in elderly subjects with chronic vestibular disorders is worsened when associated with aging, concurrent disorders, use of multiple medications, central vestibular syndromes, mobility, and gait impairments.

5. Conclusions

Hydrops may be more related to a pathophysiological mechanism rather than a specific etiology. Our data support the hypothesis of different, possibly more related to a vascular disorder, predisposing factors for MD in elderly patients compared with MD in younger subjects.

Our data confirm previous works reporting a higher prevalence of Tumarkin attacks in elderly MD subjects; the lower rate of positive autoantibodies may be related to different etiopathological mechanisms of MD in elderly subjects.

Moreover, our data support the hypothesis of a more aggressive pattern in over-65 de novo occurrence MD patients.

Conflict of Interests

The authors declare no conflict of interests.

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Review Article

Neuroinflammation and Cerebrovascular Disease in Old Age: A Translational Medicine Perspective

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The incidence of cerebrovascular disease is highest in the elderly population. However, the pathophysiological mechanisms of brain response to cerebral ischemia in old age are currently poorly understood. Ischemic changes in the commonly used young animal stroke models do not reflect the molecular changes associated with the aged brain. Neuroinflammation and oxidative stress are important pathogenic processes occurring during the acute phase of cerebral ischemia. Free radical generation is also implicated in the aging process, and the combination of these effects in elderly stroke patients could explain the higher risk of morbidity and mortality. A better understanding of stroke pathophysiology in the elderly patient would assist in the development of new therapeutic strategies for this vulnerable age group. With the increasing use of reperfusion therapies, inflammatory pathways and oxidative stress remain attractive therapeutic targets for the development of adjuvant neuroprotective agents. This paper will discuss these molecular aspects of acute stroke and senescence from a bench-to-bedside research perspective.

1. Introduction

Old age is an important risk factor for stroke and is associated with increased patient morbidity and mortality [1, 2]. Many of these patients have associated comorbidities, for example, cardiovascular and respiratory disease. This is further complicated by an increased risk of cognitive and functional decline in elderly stroke patients [3, 4]. Poor functional recovery has also been demonstrated in aged-animal models [5]. The pathophysiological mechanisms of the brains response to an ischemic insult in old age are poorly understood. Most preclinical stroke studies have been performed in young animal models and therefore do not reflect the molecular changes associated with the aged brain [6, 7]. This has been one of the criticisms of preclinical stroke neuroprotection studies and implicated in the resulting failure of clinical stroke neuroprotection trials [8, 9].

Neuroprotective therapies targeting NMDA and AMPA receptors have demonstrated reduced efficacy in aged-animal stroke models [10]. The pharmacokinetic and pharmacodynamic properties of neuroprotective agents may also be different in older patients [8]. This therefore emphasizes the importance of assessing potential neuroprotective therapies in preclinical aged animal stroke models and early clinical studies of elderly patients [6]. A better understanding of stroke pathogenesis in the aged brain would assist in the development of new therapeutic strategies for treatment of this vulnerable age group [5, 11].

Acute ischemic stroke triggers an inflammatory cascade which causes injury to the cerebral tissue, and this process can continue for several days. Cerebral ischemia results in the generation of reactive oxygen species (ROS), which induce the expression of inflammatory cytokines and chemokines. Cytokines upregulate the expression of cell adhesion molecules, which leads to leukocyte infiltration of the cerebral infarct. Cytokines also activate resident microglia, which leads to increased oxidative stress and the release of matrix metalloproteinases. These postischemic molecular changes

lead to dysfunction of the blood-brain barrier (BBB), cerebral edema, and neuronal cell death [12]. The secondary inflammatory response associated with acute stroke has been shown to worsen clinical outcome and results in increased cerebral infarct size [13–15]. Inflammatory mediators and oxidative stress are also implicated in reperfusion injury after thrombolysis and mechanical embolectomy, which can result in further neuronal injury [16, 17]. Furthermore, injury to the brain can make the body more vulnerable to systemic infections. A central nervous system injury-induced immunodepression syndrome has been identified in experimental stroke models leading to spontaneous systemic bacterial infections within 3 days after stroke [18, 19]. This suggests that early administration of potential neuroprotective therapies (within the first 6 hours) would be the optimal time for modifying the neuroinflammatory response.

Therapeutic targeting of the neuroinflammatory pathways has therefore become an important area of translational medicine research in acute stroke [16, 17, 20]. The generation of free radicals and increased oxidative stress is also implicated in the aging process, and the combination of these effects in elderly stroke patients could explain the higher risk of morbidity and mortality [6, 21]. This paper will discuss the neuroinflammatory aspects of acute ischemic stroke and senescence from a translational medicine research perspective.

2. Inflammatory Mediators in Acute Stroke

The cytokines and chemokines are important inflammatory mediators which are upregulated within the cerebral tissue during the acute phase of stroke (Figure 1). As well as being expressed by cells of the immune system, cytokines are also produced endogenously by the resident brain cells (microglia and neurons). Cytokines possess both pro- and anti-inflammatory properties, which play an important role in the progression of the cerebral infarct [22–24]. However, the spatial and temporal upregulation of cytokines and their receptors depends on the ischemic model used [25]. The main cytokines involved in neuroinflammation are the interleukins (IL), IL-1, IL-6, IL-10, and tumor necrosis factor-α (TNF-α). Cytokines are responsible for the initiation and regulation of the inflammatory response and play an important role in leukocyte infiltration into the ischemic regions of the brain [26–30]. The chemokines, monocyte chemoattractant protein-1 (MCP-1), and cytokine-induced neutrophil chemoattractant (CINC) also play an important role in cerebral ischemia and are potent chemoattractant factors [31]. In aged rat models of stroke, it has been demonstrated that the cerebral infarct develops more rapidly after reversible ischemia, with increased microglial response and cytokine expression [5, 32, 33]. This results in accelerated scar tissue formation and is associated with poor functional recovery [5]. Exacerbation of cerebral injury, via increased microglial activation, has also been demonstrated in aged-animal models of intracerebral hemorrhage [34]. The cytokine response has also been demonstrated to increase the risk of neurodegeneration and cognitive decline in aged animal models [35, 36]. Cytokines are also implicated in age-related cerebral atrophy, and an acute-on-chronic cerebral insult is likely to further exacerbate cognitive decline in old age [37].

3. Cytokine Activation in Acute Stroke and Senescence

The interleukins (IL-1, IL-6) and TNF-α have been the best-studied cytokines in the pathogenesis of acute stroke. These inflammatory mediators have also been implicated in the aging process [38–40].

3.1. Interleukin-1. The interleukin-1 (IL-1) family consists of the agonistic isoforms IL-1α and IL-1β, and their endogenous inhibitor, the IL-1 receptor antagonist (IL-1ra) [41]. The expression of IL-1β mRNA is rapidly observed after permanent middle cerebral artery occlusion (MCAo) and remains persistent for several days [42]. The important role of IL-1β in the pathogenesis of cerebral injury after stroke has been demonstrated by treatment with IL-1ra, which decreases neuronal cell death in the penumbral tissue and reduces infarct size after permanent focal cerebral ischemia [43]. The temporal induction profile of IL-1ra after ischemia virtually parallels that of IL-1β which may suggest that the balance between IL-1β and IL-1ra is more important than the levels of IL-1β itself [44].

3.2. IL-1ra in the Treatment of Acute Stroke. The phase II clinical trial of recombinant human IL-1ra (rhIL-1ra) (Anakinra or Kineret) demonstrated that patients with cortical infarcts in the treatment group had a more favorable clinical outcome (Table 1) [45]. The white cell count and inflammatory marker levels were also found to be lower in the treatment group. There is ongoing research into rhIL-1ra, as a potential neuroprotective agent in acute ischemic stroke [46]. A dose-ranging study has been performed in stroke patients to assess if Anakinra can easily cross the BBB and reach effective concentrations when administered intravenously. The results were favorable and showed that IL-1ra can enter the CSF and that the rate of entry can be modulated by altering the administration regime [47]. If the optimal therapeutic window can now be determined in acute stroke patients, this agent might be a promising and effective neuroprotective agent.

3.3. Interleukin-6. Interleukin-6 (IL-6) is a proinflammatory cytokine, which is secreted by monocytes in response to cerebral injury. Elevated levels of IL-6 in acute stroke patients correlate with a larger infarct volume and poorer clinical outcome [15, 58]. Increased IL-6 levels are also associated with senescence and frailty in old age [38]. This may further exacerbate stroke evolution in elderly patients, and this association of IL-6 with senescence requires further investigation [32, 35]. However, the role of IL-6 in acute stroke is far from clear as different regulatory levels have been demonstrated in experimental studies [59]. On one hand, IL-6 regulates synthesis and expression of acute-phase reactants, but it also possesses anti-inflammatory effects, which have been shown to be neuroprotective in both *in vitro* and *in vivo*

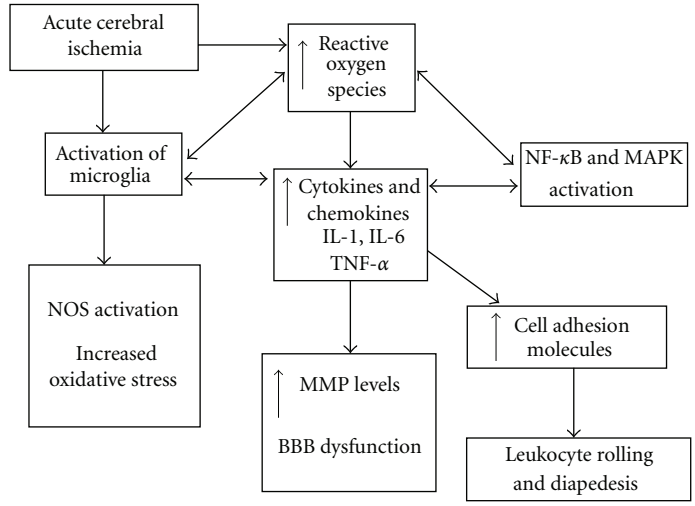

Figure 1: Acute cerebral ischemia and neuroinflammation. Acute stroke triggers an inflammatory cascade via the activation of a number of molecular mediators. The initial phase is associated with the generation of reactive oxygen species (ROS) within the ischaemic cerebral tissue. This is followed by the release of inflammatory cytokines and chemokines, which subsequently results in activation of resident microglia and upregulation of cell adhesion molecules (CAMs). The chemokines are involved in the mobilisation of leukocytes, and these inflammatory cells then interact with the CAMs. This leads to leukocyte infiltration of the ischaemic tissue (diapedesis), which further exacerbates the inflammatory process. Activation of nuclear factor kappa-B (NF-κB) and inducible nitric oxide synthase (iNOS) results in increased oxidative stress and further cytokine activation. Release of matrix metalloproteinases (MMPs) from astrocytes and microglia leads to blood-brain barrier (BBB) dysfunction, cerebral oedema, and neuronal cell death. The aging process further exacerbates these neuroinflammatory pathways, and this has been associated with increased cognitive decline and poor functional outcome in elderly stroke patients. Therapeutic targeting of these molecular pathways is an important area of translational medicine research in cerebrovascular disease.

studies [60, 61]. The effects of IL-6 during the different stages of acute stroke and genetic variation may assist in selective therapeutic targeting of this cytokine [62, 63]. Interestingly, increased cytokine activity has also been demonstrated in the muscles of the paretic limb after-stroke, and this may further hinder recovery [64]. This enhanced inflammatory state in elderly stroke patients may explain the increased risk of morbidity and mortality in this age group [65].

3.4. Tumor Necrosis Factor-α. Increased expression of TNF-α has been demonstrated in experimentally induced stroke models [66]. The initial source of TNF-α within the ischemic tissue appears to be from the microglia and macrophages although it has also been found in ischemic neurons [66, 67]. However, it is important to make a distinction between soluble and membrane-bound TNF-α [68]. Activated microglia and macrophages are major producers of soluble TNF-α within the first 6 hours after cerebral ischemia [69]. [70] TNF-α may show higher production rates in certain regions (e.g., striatum). Transient MCAo animal models and clinical stroke studies have also demonstrated increased peripheral TNF-α levels [22]. Increased levels of TNF-α have also been associated with senescence and neurodegeneration [36]. Intracerebral administration of TNF-α, 24 hours prior to MCAo, significantly enlarges infarct size, and treatment with anti-TNF-α antibodies has shown a reduction in infarct size [66, 71]. Therapeutic targeting of the TNF-α converting enzyme (TACE) is also being explored as a potential method of reducing TNF-α expression in acute stroke [72]. However, as with IL-6, TNF-α has also demonstrated neuroprotective effects in cerebral injury and could be related to the different stages of stroke pathogenesis [73]. Perhaps most importantly, TNF-α activates the NF-κB pathway that is involved in signaling cell death (apoptosis) as well as cell survival. NF-κB will stimulate the production of proinflammatory cytokines [74]. Ultimately, the balance between the two signals will determine the toxic degree of TNF-α [75]. Several hypotheses exist, one suggests that the detrimental effects occur in the early acute phase of the inflammatory response and the more beneficial effects in a later subacute stage.

3.5. TNF-α and Neuroprotective Effects in Ischemic Stroke. TNF-α has demonstrated beneficial effects in ischemic preconditioning when animal models were treated with lipopolysaccharide prior to MCAo [73]. Ischemic preconditioning is a procedure whereby brief episodes of ischemia are protective against a subsequent, more severe insult [76]. One factor that may mediate the neuroprotective effect of ischemic preconditioning is inflammation [77, 78]. Preconditioning with low doses of the proinflammatory agent lipopolysaccharide (LPS) in the rat provides a delayed tolerance and neuroprotection against subsequent challenge via focal ischemia in the brain [79, 80]. Likewise, a mild systemic inflammation elicited prior to stroke in a rat model for periodontitis has a neuroprotective effect by reducing the infarct volume in a rat model for cerebral ischemia [81]. It was hypothesized that the reduction in the infarct volume was due to a reduction in the number of macrophage-like cells that when present cause an enlargement of the infarcted area [81]. Two mechanisms have been put forward to explain the neuroprotective effect

Table 1: Neuroprotective agents targeting neuroinflammation in acute stroke.

Neuroprotective agent	Mode of action	Summary of clinical trials
Recombinant human IL-1 ra (rhIL-1ra)	Interleukin-1 receptor antagonist	In the phase II clinical trial of rhIL-1ra, patients within 6 hours of stroke symptom onset were randomised to either rhIL-1ra or placebo. In the rhIL-1ra-treated group, patients with cortical infarcts had a better clinical outcome [45].
Enlimomab	Anti-ICAM -1 monoclonal antibody	In the phase III clinical trial of enlimomab, patients were randomised to receive either the monoclonal antibody or placebo within 6 hours of acute stroke onset. The modified Rankin scale was worse in patients treated with enlimomab ($P = 0.004$), and treatment was associated with higher mortality. Further development of this drug has been abandoned [28].
UK-279, 276	Neutrophil inhibitory factor	In the Acute Stroke Therapy by Inhibition of Neutrophils (ASTIN) phase II clinical trial, patients were randomised to receive either an infusion of UK-279, 276, or placebo within 6 hours of acute stroke symptom onset. No efficacy was reported on administration of study medication, and the clinical trial was terminated for futility [48].
Cerovive (NXY-059)	Nitrone-based free radical trapping agent	In the phase III clinical trial, Stroke-Acute Ischemic—NXY-059 Treatment II (SAINT II) randomised patients within 6 hours of acute stroke onset to either an infusion of NXY-059 or placebo. There was no significant reduction in stroke-related disability, as assessed by the modified Rankin scale ($P = 0.33$). The Cerebral Hemorrhage And NXY-059 Treatment (CHANT) trial also showed no treatment effect on functional outcome. Further drug development has been abandoned [49, 50].
Edaravone (Radicut)	Free radical scavenger	Lacunar stroke patients treated with edaravone showed significant reduction in infarct size at 1-year followup and early improved neurological outcomes. There was no difference in overall clinical outcomes after 1 year [51, 52].
Uric acid	Antioxidant	The phase II double-blinded study investigated safety and pharmacokinetics of uric acid in acute stroke patients treated with rt-PA. Levels of uric acid increased in the treatment group, with reduction in lipid peroxidation. No safety concerns were reported with uric acid treatment. Further evaluation is ongoing [53, 54].
Acetaminophen (Paracetamol)	Antipyretic effect	In the Paracetamol (Acetaminophen) in Stroke (PAIS) clinical trial, patients presenting within 12 hours of acute stroke onset were randomised to either acetaminophen (6 g daily) or placebo for three days. There was no benefit seen for routine use of acetaminophen in acute stroke but post hoc analysis showed beneficial effects in patients with body temperature between 37 and 39°C [55].
Minocycline	Bacteriostatic antibiotic Anti-inflammatory effects	Stroke patients with NIHSS > 5 and symptom onset between 6 and 24 hours were randomised to either once daily minocycline 200 mg or placebo for 5 days. The NIHSS and modified Rankin scale were significantly lower in the treatment group at 90 days [56]. The Minocycline to Improve Neurologic Outcome in Stroke (MINOS) study was a dose-escalation trial, administering intravenous minocycline within 6 hours of symptom onset. This was shown to be safe and well tolerated up to 10 mg/kg intravenous dosing [57].

of proinflammation; one that depends on inducible NO synthase and peroxynitrite [79, 82], and a second one hypothesizing that LPS preconditioning suppresses neutrophil infiltration into the brain and microglia/macrophage activation in the ischemic hemisphere, which is paralleled by suppressed monocyte activation in the peripheral blood [83]. However, ischemic preconditioning via previous transient ischemic attacks (TIAs) does not seem to have a neuroprotective effect in elderly stroke patients, and these effects require further investigation [84]. Both interleukins and TNF-α are responsible for the activation of inducible nitric oxide synthase (iNOS), which produces nitric oxide (NO) and cyclo-oxygenase 2 (COX-2), a free radical producing enzyme [85]. This increased oxidative stress further worsens neuronal injury and is also related to the aging process [86]. Selective therapeutic targeting of these cytokines during the acute phase of stroke may potentially improve functional recovery. In conclusion, there is no consensus on the effect of TNF-α after ischemic stroke. Neurotoxic or neuroprotective effects will depend on several factors such as the extent of microglial activation in specific brain regions, timing, and threshold of TNF-α expression and of its receptors, and on the conditions that stimulate TNF-α signaling [87, 88]. It is also important to know which form of TNF-α is induced, in which cells, and on which receptor it will exert its effect.

3.6. Interleukin-10. Interleukin-10 (IL-10) is an anti-inflammatory cytokine which inhibits both IL-1β and TNF-α [89]. It has been demonstrated to reduce cerebrovascular risk in clinical studies of ischemic stroke [90]. IL-10 regulates

a variety of signaling pathways and promotes neuronal and glial cell survival by blocking the effects of proapoptotic cytokines, as well as promoting expression of cell-survival signals [89]. As IL-10 has been shown to be an anti-inflammatory cytokine, exogenous administration of this cytokine could be a possible therapeutic strategy to reduce cerebral injury after stroke.

3.7. Transforming Growth Factor-β. Transforming growth factor-β (TGF-β) is another anti-inflammatory cytokine and is present within microglia [91]. Animal models of stroke have demonstrated neuroprotective effects of TGF-β in cerebral ischemia [92]. It is mainly expressed during the recovery phase of stroke and may contribute to cerebral remodeling via fibrosis and scar formation. More specifically after stroke, TGF-β reduces glial activation, decreases the expression and efficacy of other cytokines, and suppresses the release of harmful oxygen and nitrogen-derived products. TGF-β has been shown to reduce infarct volume by attenuating chemokine expression in the ischemic brain of animal models [93]. However, as TGF-β can inhibit apoptosis of neurons, but not necrosis, its possible protective influence is consequently limited to the penumbra. TGF-β could therefore be neuroprotective by blocking apoptotic pathways in the ischemic penumbra and aiding recovery of reversible ischemic brain tissue [94]. On the other hand, TGF-β stimulates glial scar formation and production of beta amyloid precursor, which can lead to a higher risk of cognitive deficit.

3.8. Granulocyte-Colony Stimulating Factor (G-CSF). The cytokine growth factor, granulocyte-colony stimulating factor (G-CSF) has also shown some beneficial effects in aged animal stroke models, possibly via neurogenesis [95]. G-CSF promotes leptomeningeal collateral growth after common carotid artery occlusion and increases circulating blood monocytes and Mac-2-positive cells suggesting mechanisms coupled to monocyte upregulation [96]. Moreover, G-CSF stimulates neuronal differentiation of adult neural stem cells in the brain and improves long-term recovery in more chronic stroke models. G-CSF is being further evaluated in clinical stroke studies [97, 98].

These anti-inflammatory cytokines could therefore play an important role in potential neurorestorative and neuroregenerative therapies [99]. However, in aged animal models, there is accelerated gliosis and scar formation after-stroke, which could reduce the efficacy of these new therapeutic approaches [5, 100]. TGF-β signaling has been shown to increase in aged animal models of stroke via increased activity of microglia and astrocytes [101]. The effects of aging and anti-inflammatory cytokine expression therefore require further research in stroke pathogenesis and investigation of potential new treatments in aged animal models [6, 11].

4. Chemokine Expression in Acute Stroke and Effects of Aging

The chemokines are chemotactic cytokines, which mediate both leukocyte migration and microglial activation. There are 40 different known chemokines thus far, which all share a common structural pattern with 4 cysteine residues, which leads to their classification into four subfamilies of which two have an important role in stroke pathogenesis: the C-X-C and C-C family [102]. The C-X-C family attracts neutrophils and the C-C family monocytes/macrophages [103]. These are extensively expressed after cerebral ischemia [103, 104]. IL-6 and TNF-α both regulate the expression of MCP-1 and CINC within the cerebral tissue [104, 105]. Animal and cell culture studies have also demonstrated that both MCP-1 and CINC play an important role in ischemia-induced inflammatory response and cerebral tissue damage [103–105]. These studies indicate that CINC release precedes neutrophil accumulation and that MCP-1 plays a significant role in the migration of macrophages into the penumbral zone during cerebral ischemia. Increased MCP-1 levels have also been associated with aging and increased risk of neurodegeneration in animal studies [106].

The maximal expression of MCP-1 has been observed within 48 hours after-cerebral ischemia [31]. The different temporal production of MCP-1 and CINC contributes to the regulation of infiltrated leukocytes and the inhibition of MCP-1 and CINC signalling [103]. These chemokines are also implicated in BBB dysfunction, which has been demonstrated to occur earlier in aged rat stroke models [107, 108]. This is associated with accelerated gliosis and scar tissue formation in aged animals [5, 100]. Modification of this response may improve functional recovery and efficacy of potential new neuroregenerative therapies.

4.1. Interleukin-8. IL-8 is also classed as a chemokine (CXCL8) and is thought to contribute to tissue damage by activating neutrophil infiltration [109, 110]. Anti-IL-8 antibody was shown to significantly reduce brain oedema and infarct size [110]. IL-8 has also been shown to be associated with cerebral atrophy in the aging brain [37]. These chemokines could therefore be attractive targets for potential neuroprotective treatments in acute ischemic stroke [110, 111]. Therapy with a broad spectrum pan-chemokine inhibitor, given at the onset of reperfusion, can reduce infarct volume by 50% after a 1-hour MCAo in rats [112]. These animals showed less macrophage accumulation in the peri-infarct area, but not in the core of the insult. This supports the hypothesis that inflammatory cells contribute to extended damage within the ischemic penumbra.

5. Early Gene Expression in Acute Stroke

Cerebral ischemia and the resulting increased oxidative stress activate early gene expression. This plays an important role in the neuroinflammatory response after cerebral injury and results in the production of cytokines, acute phase proteins, and other inflammatory mediators [113]. Microarray studies have demonstrated that over 400 genes could be activated during cerebral ischemia [113, 114]. One of the important transcription factors implicated in the inflammatory cascade is nuclear factor kappa-B (NF-κB). This is a major mediator in the brain's response to ischemia and reperfusion and in the pathogenesis of acute stroke [30, 115]. NF-κB activation

has also been associated with age-related neurodegeneration [116]. NF-κB is activated by a number of factors that are present during cerebral ischemia, which include activated glutamate receptors, reactive oxygen species (ROS), TNF-α, and IL-1β [115, 117]. NF-κB is an important regulator of the inflammatory cascade, and many inflammatory mediators such as inflammatory cytokines, cell adhesion molecules (CAMs), and iNOS have NF-κB binding sequences in their promoter regions [118]. It has several different targets and effects in various cell types and tissues, which can appear paradoxical [119, 120]. In some studies, preventing NF-κB activation was shown to be protective, whereas in other studies, activation of NF-κB enhanced neuronal survival [119–121]. These conflicting results may be due to the fact that NF-κB can upregulate both proinflammatory and prosurvival factors that act in different ways depending on cell subtype [121].

Therapeutic targeting of the NF-κB pathway has therefore become an attractive treatment option, as a central target of the neuroinflammatory cascade. Proteasome inhibitors have shown promising results in animal models of acute stroke [122]. NF-κB, in its inactive form, is normally complexed to the inhibitory protein, inhibitory κB (IκB). Phosphorylation of IκB by IκB kinase (IKK) leads to activation of NF-κB. Phosphorylated IκB is then ubiquitinated and subsequently undergoes degradation by the proteasome. Proteasome inhibition has become an attractive target for drug discovery research in an attempt to reduce NF-κB activation by preventing IκB degradation, thus resulting in a dampening of the inflammatory response [123]. Hypothermia has been also shown to attenuate NF-κB transcriptional activity in aged rat models of stroke, and the use of hypothermia in acute stroke is currently being explored in clinical trials [124, 125]. However, NF-κB activity may be beneficial during the recovery phase of stroke and involved in cerebral remodelling [121]. The effects of cerebral ischemia and senescence can also result in proteasomal dysfunction, which can progress to cell death [126, 127]. Therefore, careful evaluation of potential drugs targeting the ubiquitin-proteasome system and NF-κB is required.

6. Sirtuins and Telomerase Effects on Aging and Neuroprotection

Sirtuin proteins have been associated with longevity and influence aging by regulation of transcription and apoptotic factors [128]. Humans possess seven sirtuin proteins with sirtuin1 (Sirt1) being the best studied. Sirt1 is a deacetylase enzyme and influences inflammatory pathways via regulation of NF-κB activity [129]. The flavonol compound, icariin, has demonstrated antioxidant activity and neuroprotection via upregulation of Sirt1 [130]. Resveratrol, a chemical found in red wine, has been shown to increase activity of Sirt1 and attenuate inflammatory response in animal models of stroke [131]. It has also shown neuroprotective effects via ischemic preconditioning associated with Sirt1 activation [132]. Resveratrol is being further evaluated in clinical studies [133].

Telomeres are repetitive sequences of DNA, located at the end of chromosomes and have a protective role in preventing chromosomal damage. Telomere attrition is associated with senescence and exacerbated by oxidative stress [134]. This has been implicated in the pathogenesis of atherosclerosis and vascular disease [135]. The telomerase enzyme and its catalytic subunit telomerase reverse transcriptase (TERT) maintain telomere length during cell replication. TERT knockout animal models of stroke have demonstrated enhanced neuroinflammatory response and increased infarct volume [136]. Sirt1 has been shown to have a telomere protective effect by reducing oxidative stress and maintaining telomerase activity [137]. The effects of telomerase and Sirt1 are exciting new areas of translational stroke and aging research.

7. Effect of Heat Shock Proteins in Acute Stroke and Senescence

Heat shock proteins (HSPs) are molecular chaperones, which play an important role in cellular metabolism. They also have an important role in controlling the neuroinflammatory pathways [138]. HSP70 is the major HSP and is constitutively expressed in neuronal tissue [138]. Neuronal injury and increased oxidative stress induce HSP70 expression. Animal HSP70 knockout studies have demonstrated increased cerebral infarct size [139]. The HSPs also reduce NF-κB activation in animal models of cerebral ischemia by interfering with I-κB phosphorylation by IKK [138]. This attenuates the neuroinflammatory process, and the neuroprotective effects of HSPs are being further investigated for potential treatment in acute stroke [140]. The HSPs have also demonstrated protective effects from aging and associated with human longevity [141]. Chaperonotherapies could therefore be an attractive therapeutic option for neuroinflammatory disease treatment in old age [142, 143].

8. Cell Adhesion Molecules in Acute Stroke Pathogenesis

The accumulation and infiltration of the cerebral tissue by leukocytes is a complex process that requires the interaction between several cell adhesion molecules (CAMs) and chemokines [144, 145]. The leukocytes roll on the endothelial surface and then adhere to the endothelial cells, which leads to diapedesis (Figure 1). The rolling of leukocytes is mediated by interaction of E- and P-selectin (found on the surface of endothelial cells), and L-selectin (normally found on the surface of leukocytes) with their respective ligands [144]. Inhibiting the activity of P-selectin alone by treatment with monoclonal antibodies (ARP 2–4, RMP-1) after the onset of the insult does not reduce the infarct volume significantly [146–148]. This suggests that the involvement of P-selectin in the inflammatory response after ischemic injury starts early. Firm adhesion and activation of leukocytes is mediated by binding of the CD11/CD18 complex to CAMs, such as intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell

adhesion molecule-1 (PECAM-1), and the mucosal addressin [117, 144, 149]. Increased circulating levels of CAMs have also been associated with aging, which could exacerbate leukocyte infiltration [150]. IL-6 and TNF-α also regulate the expression of CAMs on the endothelial cells and induce infiltration of the ischemic penumbra by leukocytes at the site of inflammation [144]. There is ample evidence from animal models of MCAo that expression of CAMs is associated with increased cerebral infarct size [144]. When MCAo in experimental stroke was followed by reperfusion, administration of anti-CAM antibodies decreased infarct size [151]. However, anti-CAM treatment has not been successful in clinical studies of acute ischemic stroke. The enlimomab study used a monoclonal antibody against ICAM-1, which was administered within 6 hours of acute ischemic stroke onset (Table 1). The three-month outcome mortality data and adverse events were worse in the enlimomab group, and it appears that there may have been a proinflammatory response [28]. Surprisingly, for PECAM-1, there is suggestion of possible neuroprotective properties aside from the neurotoxic effects. PECAM-1 knock-out mice show facilitated leukocyte transendothelial migration after histamine treatment which contradicts the current hypothesis that PECAM-1 stimulates migration [149]. Further research into therapeutic targeting of CAM is ongoing [152, 153].

9. Inflammatory Cells in Acute Stroke

Neuroinflammation is characterized by an accumulation of inflammatory cells and chemical mediators within the cerebral infarct (Figure 1). Neutrophil infiltration in acute stroke has been demonstrated by single photon emission computer tomography (SPECT) studies [154]. This is associated with increased cerebral infarct size [26]. A significant inflammatory response has also been demonstrated in aged animal models of stroke [33, 155]. Clinical studies have shown that peripheral inflammatory cells also play an important role in the pathogenesis of cerebral ischemia [26]. This has also been demonstrated in numerous animal models of acute stroke [92]. MRI animal studies have shown neutrophil infiltration into the infarct zone within a few hours of ischemia, and this process peaks at 24 hours [156]. Blood-derived macrophages and activated microglial cells contribute to the postischemic brain damage by expressing iNOS and production of cytotoxic agents and ROS [30]. Macrophage activation has been shown to exacerbate cerebral injury in aged animals [34]. How microglia become activated after ischemic stroke is still not clear. A possible mechanism is rupture of necrotic neurons in the core of the insult, leading to release of their contents into the extracellular space and scavenging of these contents by microglia [30]. Microglia/macrophages surrounding the ischemic tissue will migrate toward the ischemic lesion and engage in close contact with neurons ("capping"). As these neurons die later on, this capping ensures early recognition and fast phagocytic removal of dying/dead neurons [157]. In an activated state, microglia will produce inflammatory and cytotoxic mediators contributing to cell damage and cell death. On the other hand, microglia are a major producer of TGF-β1 which supports the hypothesis that microglial activation is also neuroprotective [25]. Finally, resident macrophages scavenge and remove necrotic debris and harmful components. Indeed, these data suggest that early activation is detrimental and later activation beneficial. Different subsets of microglia may have different roles after ischemic stroke and thus improve or reduce the chances of survival of ischemic neurons [158, 159]. Perhaps an ideal therapy should modulate the microglial response in order to stimulate neurogenesis [160]. Transgenic mice in which microglial proliferation can be inhibited (eliminated) show increased infarct volume (by 13%) after a 1-hour occlusion, which suggests that proliferating resident microglial cells exert a neuroprotective role after ischemia [161]. Microglial activation has also been associated with stimulation of the toll-like receptor 4 (TLR4). Permanent MCAo models of TLR4-deficient mice were shown to have reduced infarct size [162]. TLR4 plays an important role in the initiation of the inflammatory response during cerebral ischemia and has become another important target for neuroprotective therapy. However, TLR activity seems to decline with aging, and further investigation into its role in cerebrovascular disease is required [163].

Inhibition of leukocyte activation and infiltration into the ischemic cerebral tissue have therefore been an important area of neuroprotection drug research [152, 164]. The neutrophil inhibitory factor, UK-279, 276, a recombinant protein inhibitor of the CD11/CD18 receptor, demonstrated reduced infarct size in animal models of stroke. However, the Acute Stroke Therapy by Inhibition of Neutrophil (ASTIN) study did not show any patient benefit and was terminated for futility (Table 1) [48]. Neuroimaging studies have demonstrated inflammatory cell infiltration during acute stroke and identification of salvageable ischemic penumbra [165]. These imaging techniques can be used as important surrogate markers for future anti-inflammatory drug studies. The use of both neuroradiological markers and biomarkers as surrogate measures of drug treatment in clinical stroke trials is an important Stroke Treatment Academic Industry Roundtable (STAIR) recommendation [8].

10. Free Radicals and Increased Oxidative Stress

Increased free radical production and oxidative stress play an important role in the pathogenesis of both acute stroke and senescence [21, 166]. Activated microglia/macrophages further increase free radical generation and exacerbate cerebral injury in aged-animal models of stroke [34]. This is associated with mitochondrial dysfunction and results in neuronal cell death [167]. Nitric oxide (NO) is one of the important reactive oxygen species (ROS). It possesses both neuroprotective and neurotoxic properties in cerebral ischemia. This is related to the activation of the three different isoforms of NO synthase (NOS) at different stages of the ischemic process. The three isoforms of NOS are endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). NOS catalyses the chemical conversion of L-Arginine to NO and citrulline. The constitutive isoforms (eNOS and nNOS) are activated by increased levels of intracellular calcium, during

the acute phase of cerebral ischemia. Neuronal NOS has a much higher capacity for NO generation than eNOS, and this is responsible for neuronal damage during the early stages of ischemic stroke. Inducible NOS activation occurs later, usually 12–48 hrs after the initial ischemic insult [85]. This is associated with a much higher production of NO, and for a longer period, compared to its two isoforms.

The effect of eNOS is well known for its vasodilatory properties, via the action of cyclic GMP. However, the activity of eNOS has been shown to diminish in aged-animal models [168]. Studies using eNOS knock-out mice have demonstrated increased infarct size following transient MCAo [169]. The upregulation of eNOS via the action of statins has provided an additional neuroprotective property to this class of lipid-lowering drugs [99]. On the contrary, nNOS knock-out mice have been shown to develop smaller infarct volumes in MCAo [170]. There is a strong association between the activation of NMDA receptors and calcium-dependent increase in nNOS activity. The toxic ROS, peroxynitrite ($ONOO^-$) produced from NO reactions has been associated with neuronal cell death, via lipid peroxidation and DNA damage [30, 171]. Increased expression of proapoptotic proteins in aged animal stroke models, via constitutive NOS activation, has also been associated with neuronal cell death [172]. Inducible NOS upregulation results in further NO generation during the later stages of cerebral ischemia [85]. Leucocytes and endothelial and glial cells are the main sources of iNOS expression. Selective inhibitors of iNOS have been shown to display neuroprotection for up to 5 days after-ischemic insult [85]. Again, peroxynitrite is the main ROS involved in neuronal cell death. Interactions between iNOS and cyclo-oxygenase 2 (COX-2) have been linked to penumbral cell death in late cerebral ischemia [85]. Due to this late and prolonged activation of iNOS, it remains an important therapeutic target for potential antioxidant and spin-trap agent therapy [173].

Another important source of ROS is NADPH oxidase. This enzyme predominantly produces the superoxide anion, which reacts with NO to generate peroxynitrite [166]. Recently, there has been increasing interest in the role hydrogen sulfide (H_2S) in the pathogenesis of cerebral ischemia [174]. H_2S is formed from the amino acid cysteine, via the action of the enzyme cystathionine beta-synthase. However, H_2S has demonstrated both beneficial and detrimental effects during acute cerebral ischemia [175, 176]. Clinical studies have demonstrated worse neurological outcome in stroke patients with higher plasma cysteine levels, reflecting increased H_2S activity [175]. However, H_2S has also demonstrated antioxidant properties via free radical scavenging and inhibition of iNOS [176, 177]. This dual effect of H_2S could be related to the different stages of cerebral ischemia, and more research is required to investigate any potential therapeutic benefit of targeting this molecular pathway [174].

Due to the destructive nature of ROS in cerebral ischemia, therapeutic interventions have been an important area of translational stroke research. The increased oxidative stress associated with aging may further exacerbate cerebral ischemia and result in poor functional recovery in elderly stroke patients [21, 167]. However, clinical studies using natural antioxidants (vitamin C and E) and B vitamins to lower homocysteine have not shown any benefit in reducing vascular risk [178, 179]. These vitamins also seem to have little effect on the aging process, possibly because of low antioxidant activity at the smaller recommended doses [180]. The SAINT II [Stroke-Acute Ischemic—NXY-059 (Cerovive) Treatment] study investigated the effect of the nitrone spin-trap agent, NXY-059, in patients presenting within 6 hours of acute stroke symptom onset [8]. This nitrone-derived free radical trapping agent was shown to be an effective neuroprotective agent in animal models of stroke, with a large therapeutic window for treatment [181]. Unfortunately, results from the phase III studies were negative, for both ischemic and hemorrhagic stroke, and further development of the drug has been abandoned (Table 1) [49]. However, lessons from the SAINT studies have allowed further revision of the STAIR criteria and will lead to more rigorous translational research studies in the future [9, 181]. Research is ongoing into antioxidant spin trap agents in the treatment of acute stroke and senescence [21, 173].

Recent clinical studies of antioxidant agents have been more encouraging and shown positive results in early clinical stroke trials [51, 53] (Table 1). Edaravone (Radicut), a free radical scavenger, is currently being investigated as an antioxidant agent in the treatment of acute stroke [182]. Edaravone was shown to reduce activation of NF-κB and MMP-9 in animal models of rt-PA-related hemorrhage [183]. This drug was also shown to reduce oxidative neuronal damage [184]. Clinical studies have shown some benefits of edaravone in lacunar stroke patients with reduction in infarct size and early neurological recovery [51] (Table 1). Uric acid has also been shown to have antioxidant activity and neuroprotective effects in animal models of ischemic stroke [185]. Clinical studies have demonstrated reduced levels of uric acid during the acute phase of stroke, which may exacerbate oxidative stress. The use of adjuvant uric acid treatment with rt-PA was shown to reduce lipid peroxidation and may have beneficial effects in patient outcome [53] (Table 1). Further clinical trials of uric acid in acute stroke are being planned [54].

A viable alternative to conventional drug-based neuroprotective therapies is brain/body cooling, or hypothermia. This provides neuroprotection by reducing oxidative stress, DNA damage, and neuronal apoptosis [186, 187]. In animal studies of focal ischemia, short-term hypothermia consistently reduces infarct size. Nevertheless, efficient neuroprotection requires long-term regulated lowering of whole body temperature. Exposing poststroke-aged rats to a mixture of air and a mild inhibitor of oxidative phosphorylation, H_2S, for 2 days, resulted in sustained, deep hypothermia ($30.8 \pm 0.7^\circ C$). Long-term hypothermia led to a 50% reduction in infarct size with a concomitant reduction in the number of phagocytic cells. At the transcription level, hypothermia caused a reduction in the mRNA coding for caspase 12, NF-κB, and grp78 in the peri-infarcted region, suggesting an overall decrease in the transcriptional activity related to inflammation and apoptosis. Behaviorally, hypothermia was associated with better performance on tests that require

complex sensorimotor skills, in the absence of obvious neurological deficits or physiological side effects, in aged rats [188].

Mild-to-moderate intraischemic hypothermia reduces ATP depletion, anoxic depolarization, glutamate release, and apoptosis, maintains BBB integrity, inhibits white matter injury, and blocks necrosis (if started during ischemia itself) [189]. The effects of hypothermia on inflammation show a more modulating response. However, hypothermia has been suggested to only delay neuronal damage rather than to provide permanent protection. Furthermore, a distinction has to be made between short and long cooling periods. It has been suggested that the disadvantages of delayed cooling could be overcome by performing a prolonged hypothermic protocol [190]. However, the modulating influence of hypothermia on neuroinflammation could also differ depending on these parameters and requires further investigation [191]. Depth and duration of cooling both influence outcomes in experimental and clinical settings and may make translation to the clinic difficult [192].

11. Blood-Brain Barrier Dysfunction

Cerebral ischemia is associated with the release of matrix metalloproteinases (MMPs), as part of the neuroinflammatory response. These proteases are involved in the breakdown of the microvascular basal lamina, which results in the disruption of the BBB [193]. These changes are most prominent in the core infarct, where neuronal damage is maximal. The gelatinases (MMP-2 and MMP-9) are the main MMPs involved in destruction of the basal lamina. MMP-2 is expressed constitutively in the CNS and is normally present within cerebral tissue. MMP-9 is normally absent, and this is the major MMP associated with neuroinflammation [194]. These enzymes are released from endothelium, glia, and infiltrating leukocytes. They target laminin, collagen IV, and fibronectin proteins, which are the major constituents of the basal lamina. This is associated with BBB dysfunction and leads to cerebral edema [12]. Reduced infarct size has been shown in rat models of stroke treated with MMP inhibitors, and also in MMP-9 knock-out mice studies [195, 196].

MMP-9 levels play an important role in the development of cerebral edema and hemorrhagic transformation of infarcted cerebral tissue [197]. Clinical studies have demonstrated a strong correlation between elevated plasma MMP-9 levels and risk of hemorrhagic transformation in the acute phase of ischemic stroke [197]. Elevated MMP-9 concentrations have also been shown to be a predictor of thrombolysis-related intracerebral hemorrhage in patients treated with tissue plasminogen activator (t-PA) for acute ischemic stroke [198]. The combination of MMP inhibitors with t-PA could be a future treatment option in reducing bleeding complications associated with thrombolytic therapy [173]. However, recent animal studies have demonstrated that MMP inhibitors could worsen longer-term neurological outcome, and further evaluation of these drugs in acute stroke is required [199]. Broad-spectrum inhibitors of MMPs, such as BB-94 and KB-R7785, administered after stroke onset, reduce damage after permanent MCAo in mice by 26% [200, 201]. Such treatments, however, cause serious side effects due to their low specificity and explain why these inhibitors have not been used in clinical practice [202]. On that account, more selective inhibitors or knock-out mice for MMP-2 or -9 have been explored. MMP-2 knock-out mice subjected to 2-hour occlusion show massive upregulation of MMP-9, and obviously no improvement could be observed [203]. A selective inhibitor for MMP-2 and -9 (SB-3CT) in mice subjected to a 2-hour MCAo reduces lesion size up to 6 hours after ischemia onset, and this inhibitor is well tolerated in animals [202].

BBB dysfunction has been identified as a major cause of cerebral injury in aged animal stroke models [52, 107]. After ischemia, subtle dynamical changes in BBB permeability occur and can be transient or permanent depending on the severity of the insult. The latter is characterized by endothelial swelling, astrocyte detachment, and blood vessel rupture in the ischemic area, while transient BBB disruption shows endothelial hyperpermeability to macromolecules in the peri-infarct area [204]. Transient BBB disruption shows a biphasic pattern with an initial opening 2-3 hours after the onset of the insult, while 24–48 hours after reperfusion a second opening occurs, leading to vasogenic edema and increased intracranial pressure [204]. Furthermore, production of proinflammatory cytokines and adhesion molecules will be stimulated. Such a disruption results in rapid but significant changes in the molecular relationship between astrocytes and the microvascular extracellular matrix, which has a feedback effect on the neurons they supply and protect [205]. BBB dysfunction in old age has been shown to be closely related to white matter lesions and lacunar infarction [206, 207]. Cerebral amyloid angiopathy is also associated with BBB disruption in old age and subsequent increased risk of intracerebral hemorrhage [208].

BBB permeability studies have demonstrated minimal disruption within the first 6 hours after-MCAo but increased permeability by 21 hrs [209]. However, this BBB dysfunction occurs much earlier in aged rat models [107]. This was examined by comparing young (3 months) and aged (18 months) MCAo rat models. BBB disruption was assessed at 20 min and 24 hrs after-MCAo, with t-PA-induced reperfusion at 120 min. The results showed that BBB disruption in aged rats occurred earlier and increased nearly twofold at both 20 min and 24 hr time points compared to the younger animals (Figure 2) [107]. Neuronal damage in aged rats was also much worse compared to young rats at 24 hr, with aged rats suffering larger infarct size and reduced functional recovery. These findings suggest that early BBB disruption in aged stroke patients could contribute to a greater degree of neuronal injury. There have been concerns that t-PA treatment in elderly stroke patients may increase the risk of hemorrhagic complications, but recent clinical studies have shown no increased risk [210]. BBB dysfunction in old age is therefore an important area of translational stroke research [20, 210, 211]. The development of new MRI techniques for investigating BBB dysfunction in stroke will be an important investigational tool in future translational research [212].

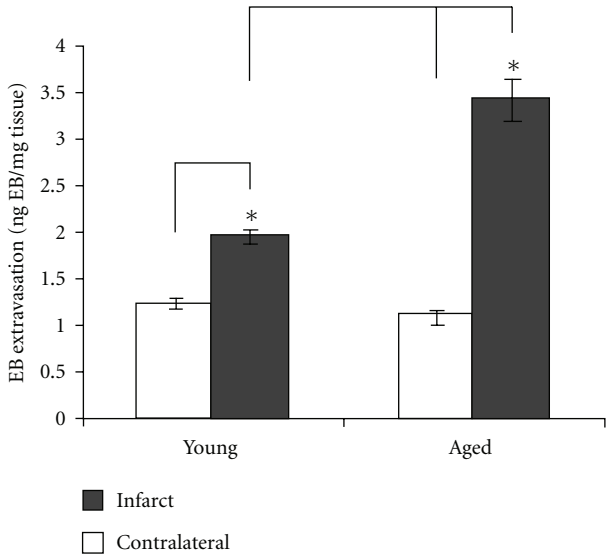

Figure 2: Extravasation of albumin across the BBB 20 min after-MCAO. The aged animals exhibited greater BBB permeability in relation to the corresponding young rats in the infarcted hemisphere ($P < 0.001$). (Copyright: DiNapoli et al. [107]).

12. Clinical Aspects of Neuroinflammation in Acute Stroke

In addition to the development of the localized inflammatory response in the brain, acute stroke also evokes an immune response at the systemic level [15, 213]. This is characterized by the release of proinflammatory mediators into the systemic circulation [14, 15]. The clinical manifestation is called the systemic inflammatory response syndrome (SIRS; Table 2). SIRS is evident in both ischemic and hemorrhagic stroke [214–216]. The degree of the inflammatory response has also been shown to be related to the size of infarct volume [13]. The inflammatory response is also associated with the development of hyperthermia during the acute phase of stroke [217]. This is related to stroke severity and associated with poor patient outcome [217, 218]. Animal stroke models have also demonstrated increased infarct size in hyperthermic conditions [219]. The neuroprotective effects of hypothermia in animal stroke models have demonstrated reduced activation of NF-κB and inflammatory pathways [124]. Clinical research studies have also been investigating the neuroprotective effects of hypothermia, and further trials are planned [125, 220]. The effects of antipyretic treatment in hyperthermic acute stroke patients, as part of the Paracetamol (Acetaminophen) in Stroke (PAIS) trial, did not show any benefit in stroke patients, but post hoc analysis of temperature between 37 and 39°C did show improved patient outcomes with acetaminophen treatment [55] (Table 1). The role of prophylactic antibiotic use in acute stroke patients, in an attempt to treat associated infections and reduce inflammatory complications, is another area of ongoing research [221]. Treatment with minocycline, a bacteriostatic antibiotic with possible anti-inflammatory effects, has shown beneficial effects in a pilot study of acute stroke patients

Table 2: The systemic inflammatory response syndrome (SIRS).

SIRS-diagnostic criteria
SIRS diagnosed if 2 or more of following criteria are present:
Temperature >38°C or <36°C
Respiratory rate >20 breaths/min
Heart rate >90 bpm
White cell count >12,000 mm^3 or <4,000 mm^3 or >10% immature neutrophils

(Table 1), and further evaluation of this drug is ongoing [56, 57].

The neuroinflammatory response has also been associated with cognitive decline and delirium, which is frequently seen in elderly stroke patients [222, 223]. This seems to compromise the reduced functional reserve in the aged brain and is related to underlying cerebral small vessel disease [4]. An increased cerebral inflammatory response has also been demonstrated in aged animal studies with associated increased cognitive deficits [35]. Increased CRP levels have been associated with poststroke cognitive impairment in elderly patients [224]. The reduction of this systemic inflammatory response could potentially improve cognitive and functional outcomes in elderly stroke patients, and further research of these effects in aged stroke animal models is therefore important [181, 225]. With the increased risk of stroke in postmenopausal females, hormonal effects in stroke pathogenesis are also an important area of research. Many preclinical stroke studies have demonstrated neuroprotective and anti-inflammatory effects of estrogen [226]. However, hormonal replacement therapy in female patients has been associated with increased vascular risk in clinical studies [226]. Male sex is an important risk factor for stroke, but little is known about the effects of androgenic hormones in stroke outcome. Some studies have shown that high testosterone levels during the acute phase of stroke are associated with worse clinical outcome but may have a neuroprotective effect during the recovery phase [227]. However, clinical studies show increased vascular risk associated with testosterone supplementation in older men, but this may have some benefit in male patients with low testosterone levels [228]. These contradictory results in preclinical and clinical effects of sex hormone treatment therefore require further translational research.

C-reactive protein (CRP) is an indicator of underlying systemic inflammation. It is an acute-phase reactant and has a pronounced rise in concentration after tissue injury or inflammation. It does not seem to have a significant role in the aging process *per se* but more related to disease pathogenesis [229, 230]. CRP has a long plasma half-life and could also be a potential mediator as well as a marker of cerebrovascular disease [231]. The association of increased levels of CRP with ischemic stroke has been reported in several clinical studies [15, 232]. It has been shown that increased levels of CRP are associated with a worse outcome in patients with ischemic stroke [232, 233]. Increased levels of CRP are also associated with an increased risk of future stroke in elderly patients

[90, 234]. However, the role of CRP in the pathogenesis of ischemic stroke is not completely understood. It is unclear whether CRP is just a marker of systemic inflammatory processes or directly involved in pathogenesis of cerebral tissue damage [231]. Further research to investigate any potential therapeutic benefits of inhibiting CRP in vascular disease is ongoing [235].

13. Conclusion

The incidence of stroke is highest in the elderly population, and the underlying pathogenic changes in combination with senescence have been associated with increased cerebral injury [11]. Acute cerebral ischemia results in a complex inflammatory cascade resulting in the activation of a variety of inflammatory cells and molecular mediators. Aged animal models have demonstrated a more intense inflammatory response during the acute phase of ischemia, followed by early scar formation and fibrosis. There is also earlier BBB dysfunction in older animals with increased permeability and neuronal injury. The neuroinflammatory response involves several molecular pathways, which are interconnected with the aging process and cognitive dysfunction. These complex molecular interactions in old age make it difficult to draw firm conclusions from research observations made in young animal models of stroke and translate these findings into clinical studies. The importance of conducting preclinical stroke studies in aged animal models will therefore be an important part of translational stroke research (STAIR criteria) in the future [6, 8].

Numerous animal models of stroke have demonstrated reduced infarct size on modification of the inflammatory response although these inflammatory mediators also have beneficial effects during the recovery phase. Clinical studies have suggested that cerebral infarct size and patient outcome are affected by the inflammatory response. Unfortunately, clinical neuroprotective drug trials targeting the inflammatory pathways in acute ischemic stroke have thus far been disappointing. Most of the preclinical stroke studies have been performed in young animal models and therefore do not reflect the molecular changes associated with the aged brain. This has been one of the criticisms of preclinical stroke neuroprotection studies and implicated in the failure of clinical trials. A more rigorous bench-to-bedside research approach into investigating neuroprotective agents in a target elderly stroke population may allow a more successful transition into clinical trials and hopefully clinical practice [8, 181]. A multitarget approach together with reperfusion therapies may be the best therapeutic approach in the future.

There is sufficient data to support that hypothermia acts at multiple levels of the ischemic cascade and of the neuroinflammatory response. Hypothermia attenuates the expression of several inflammatory mediators at certain time points and appears to be an attractive therapeutic option, but more research is required to discern which positive or negative effects contribute to neuroprotection [236]. The ability of hypothermia to modulate many aspects of the inflammatory response may render translation to the clinic feasible [125]. Another advantage of hypothermia could be the creation of a larger therapeutic time window to administer other neuroprotective agents and thus improve outcome after transient focal cerebral ischemia. With the success of thrombolysis in the treatment of acute ischemic stroke and ongoing clinical trials of interventional reperfusion therapies, together with better MR imaging techniques, adjuvant neuroprotective therapies remain an attractive option in the future [27, 29].

Conflict of Interests

The authors report that they have no conflict of interests.

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Review Article

CLOCK Genes and Circadian Rhythmicity in Alzheimer Disease

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Disturbed circadian rhythms with sleep problems and disrupted diurnal activity are often seen in patients suffering from Alzheimer disease (AD). Both endogenous CLOCK genes and external Zeitgeber are responsible for the maintenance of circadian rhythmicity in humans. Therefore, modifications of the internal CLOCK system and its interactions with exogenous factors might constitute the neurobiological basis for clinically observed disruptions in rhythmicity, which often have grave consequences for the quality of life of patients and their caregivers. Presently, more and more data are emerging demonstrating how alterations of the CLOCK gene system might contribute to the pathophysiology of AD and other forms of dementia. At the same time, the impact of neuropsychiatric medication on CLOCK gene expression is under investigation.

1. Introduction

Alzheimer disease (AD) is the most frequent form of dementia and one of the most devastating psychiatric disorders, with an estimated 33.9 million people worldwide affected [1]. With increasing life expectancy, AD prevalence will further rise dramatically within the next decades. While the typical neuropathological features have been well known since their first description in 1906 [2], the complex pathophysiology of this condition is still not fully understood. Some progress has been made in elucidating possible risk genes [3, 4] as well as the role of advanced glycation end products [5, 6], amyloid plaques [7], neurofibrillary tangles [8], and other pathological factors which probably interact within the so-called "pathogenic cascade" [9]. Further, presently available treatment strategies are not sufficient and not satisfying as they, at best, slow down the progression of the symptoms but do not provide an option to reverse them. Intervention may frequently occur at advanced stages of neurodegeneration which are no longer possible to alleviate [10]. Additionally, treatments are often associated with a plethora of side effects.

From a clinical point of view, patients, their families, carers, and health professionals are confronted with severe challenges in managing the condition. One such typical challenge is the disturbed diurnal-nocturnal rhythm of many Alzheimer patients [11–13]. Interestingly, circadian CLOCK gene polymorphisms might be linked to sleep-wake disturbances in Alzheimer patients, although the authors of a recent paper focusing on this hypothesis reported largely negative findings for the 122 circadian-related polymorphisms included [14]. The molecular and cellular basis of circadian rhythmicity consists of a complex interaction between various transcription factors, commonly described as CLOCK genes, which represent an internal timekeeping system that interacts with external synchronising factors: the so-called Zeitgebers [15, 16]. Via this interaction, humans and many animals maintain a recurring 24-hour (circadian) system for a plethora of biological and behavioural processes. Desynchronisation can be caused by various factors such as shiftwork, airtravelling through time zones, substances and medication, and neuropsychiatric disorders [17, 18].

Severe desynchronisation is regularly observed in patients suffering from AD and, therefore, the elucidation of the role of CLOCK genes in this condition might contribute to a significantly improved understanding of its underlying pathomechanisms.

2. Sleep and Circadian Rhythmicity in Alzheimer Patients

Nocturnal sleep disturbance is often reported by caregivers of Alzheimer patients and seems to be associated with disease severity [19]. Additionally, sleep quality is often very poor [20] and AD patients suffering from apathy seem to exhibit less consolidated nocturnal sleep [21]. Furthermore, disturbed sleep patterns in patients can have adverse consequences for the health of caregivers [22].

Interestingly, a delayed endogenous circadian phase of core body temperature was found in patients with probable AD by Harper and coworkers [13]. The same group also described that the deviation from normal circadian rhythmicity increases with the severity of Alzheimer pathology [23] and that there might be distinct differences in the pattern of desynchronisation depending on the type of neurodegeneration [12]. These findings can be interpreted as support for the hypothesis that disturbed circadian rhythmicity in patients with AD is mediated by pathological CNS (central nervous system) changes.

Hatfield et al. [23] monitored rhythmicity via actigraphy and cortisol determination in a group of mildly and moderately demented Alzheimer patients over the course of a year and report that as disease progresses, so does the activity rhythm, but the cortisol rhythm is relatively spared. These findings suggest that in the earlier stages of the disease process, circadian deterioration may not be a global phenomenon, but may affect specific domains. A recent study by Hu et al. [24] shows that AD attenuates the scale invariance of the motor rhythm, perhaps reflecting pathological alterations in the SCN (suprachiasmatic nucleus). Such pathological changes in the SCN have been noted in postmortem analysis of the brains from severely demented patients, with neuronal loss and tangles noted, but rarely plaques in the SCN [25]. There are also decreases in specific neurochemical phenotypes in the SCN that are lost [25, 26], with such neurochemicals (VIP (vasoactive intestinal peptide), AVP (arginine-vasopressin)) being crucially implicated in SCN function. Such disturbances in SCN function and the resultant circadian abnormalities are postulated to in part underpin "sundowning" in Alzheimer patients, in which behavioural agitation arises in the late afternoon/evening [27]. In this context, the results of an animal model experiment are interesting: the study involved the implantation of genetically modified, beta/A4-expressing cells into the SCN of adult rats. It was possible to demonstrate that such overexpression of beta/A4 amyloid led to significant alterations in the activity pattern of the animals and disruption of circadian rhythmicity [28]. The 3xTg (triple transgenic) mouse models of AD, which exhibits both Abeta and tau neuropathology, show deteriorated circadian organisation of locomotor behaviour, although there was no weakening of the ability of the system to respond appropriately to light stimulus, whilst the SCN of these animals also showed a decrease of VIP and AVP cells [29]. Bero et al. [30] demonstrated, again in a murine model, that the concentration of interstitial-fluid Abeta and, thus, the Abeta aggregation depend on endogenous neuronal activity, implying that disturbed sleep could contribute to amyloid deposition. Craig et al. [31] suggested that the common and distressing sleep disruption in Alzheimer patients is associated with genetic factors, specifically with a variation of the MAO-A (monoamine oxidase A) gene. Other obvious candidate genes are CLOCK genes. While this gene family has been implicated in other neuropsychiatric disorders [32], a recent study failed to reveal possible associations between CLOCK gene single-nucleotide polymorphisms (SNPs) and sleep-wake disturbance in AD [14]. Nevertheless, even if CLOCK gene variants have not yet been identified as possible risk factors, it is possible that the expression of these genes is altered in Alzheimer patients and that a changed expression pattern of these genes contributes to disturbed circadian rhythms [33].

3. The Role of CLOCK Genes in AD

It has been shown that age, the most important risk factor for AD, influences the expression of molecules involved in immunity as well as in cell signaling and transcription factor regulation, such as transforming growth factor-beta and phosphorylated SMAD3 (Mothers against decapentaplegic homolog 3), in the mouse suprachiasmatic (SCN) and paraventricular nuclei, that is, the neuroanatomical areas crucially involved in the maintenance of circadian rhythmicity [34].

In a recent postmortem study, Cermakian and coworkers found significant differences in the expression pattern of CLOCK genes between Alzheimer patients and controls. The RNA (ribonucleic acid) expression level of the CLOCK gene PER1 (period circadian protein homolog 1), PER2 (period circadian protein homolog 2), and BMAL1 (brain and muscle aryl-hydrocarbon-receptor-nuclear translocator-(ARNT-) like) were quantified via RT-PCR (reverse transcription polymerase chain reaction) for the following brain areas: bed nucleus of the stria terminalis, cingulate cortex, and the pineal gland [33]. A previous study had already shown that in the brain of Alzheimer patients vasopressin gene expression is altered in the SCN [35].

Additionally, a possible noncircadian role of CLOCK genes in psychiatric disorders has been discussed, mainly affecting behavioural phenomena; however, this could also be an epiphenomenon of underlying rhythmicity at the cellular and systemic level [36].

A further important point is also the possibility of using a "resetting" of the internal clock in the treatment of AD. For example, there is evidence that the use of light therapy may delay cognitive decline in AD as well as noncognitive symptoms such as depression [37, 38].

In this context, the possible impact of AD medication on CLOCK gene expression and, thus, indirectly on circadian rhythmicity must also be discussed. To our knowledge, there are no studies published which address this issue specifically for medication used in Alzheimer treatment. However, there is considerable evidence that neuropsychiatric medication can significantly alter the expression of CLOCK genes in various brain areas. In animal models, haloperidol alters the

circadian CLOCK gene expression in the murine CNS [17]. Further, medication with typical neuroleptic haloperidol has been found to alter the rhythmicity of rest-activity cycle and to deteriorate the cognitive state in early-onset Alzheimer patients [39].

4. The Dilemma of How and When to Monitor the CLOCK Genes

There are significant difficulties in monitoring CLOCK genes, either when one monitors the genes themselves or their RNA or protein products. Starting at the gene level, it is important to know exactly when the material was collected during the day from patients with AD. Since the genes are influenced by circadian rhythms, a sample collected during the night will be different from ones collected during the day (or even morning/evening). Finding a matched control may also be difficult. Background substraction and data manipulation to eliminate false positive or false negative results is also important and difficult to address. It is also important to know what is influenced by circadian rhythms: the gene, the RNA, the protein, protein posttranslational modifications, protein-protein interactions, or a combination of more than one? Also, if only CLOCK proteins are responsible for the circadian phase, what phenomenon affects the level of the proteins per se? Is it the increase in RNA translation (and increased gene transcription) leading to increased protein expression or is it just prevention of protein degradation? Is it reduced rate of phosphorylation or increased rate of dephosphorylation? Trying to answer these and other questions as well as finding the right way to investigate these genes and their transcriptional and translational products, as well as posttranslational modifications and protein-protein interactions is challenging and time consuming.

5. Discussion

During recent years, better understanding of the molecular and cellular basis of the clinically frequently observed sleep disturbances and disrupted diurnal and nocturnal rhythms of Alzheimer patients is slowly evolving. CLOCK genes are crucially involved in the maintenance of such rhythmicity, and studies linking this molecule family to brain and body processes in Alzheimer patients can further elucidate the etiopathogenesis of this most frequent dementia. However, to date, the number of such studies is limited and there are only very few reliable and reproducible data available. Further research is urgently needed, especially regarding the exact role CLOCK genes play within the AD pathological cascade and regarding the exact interaction with other pathogenic factors such as amyloid, neurofibrillary tangles, oxidative stress, advanced glycation end products, lipidomic alterations, altered immune modulation, excitotoxicity, and so forth.

Another important feature of CLOCK genes is their close interrelation with external, that is, environmental factors; thus, very few other gene families exhibit such strong gene-environment interaction and interdependency. Therefore, they might be crucial in the integration of exogenous factors and noxae (such as stress) and endogenous vulnerability factors (such as inherited genetic variants), ultimately leading to the clinical phenomenology of AD.

Finally, using and applying this knowledge for the development of improved treatment strategies with better efficacy and tolerability will be an important challenge which, if faced, might lead to significant contributions to the improvement of the quality of life of millions of people suffering from AD.

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Research Article

Depressive Symptoms and Amygdala Volume in Elderly with Cerebral Small Vessel Disease: The RUN DMC Study

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Introduction. Late onset depressive symptoms (LODSs) frequently occur in elderly with cerebral small vessel disease (SVD). SVD cannot fully explain LODS; a contributing factor could be amygdala volume. We investigated the relation between amygdala volume and LODS, independent of SVD in 503 participants with symptomatic cerebral SVD. *Methods*. Patients underwent FLAIR and T1 scanning. Depressive symptoms were assessed with structured questionnaires; amygdala and WML were manually segmented. The relation between amygdala volume and LODS/EODS was investigated and adjusted for age, sex, intracranial volume, and SVD. *Results*. Patients with LODS had a significantly lower left amygdala volume than those without ($P = 0.02$), independent of SVD. Each decrease of total amygdala volume (by mL) was related to an increased risk of LODS (OR = 1.77; 95% CI 1.02–3.08; $P = 0.04$). *Conclusion*. Lower left amygdala volume is associated with LODS, independent of SVD. This may suggest differential mechanisms, in which individuals with a small amygdala might be vulnerable to develop LODS.

1. Introduction

A first depressive episode at older age is defined as late onset depressive symptoms (LODSs). LODSs are usually defined by their occurrence after the age of 60 years, while early onset depressive symptoms (EODSs) appear before. The prevalence of LODS and EODS ranges from 10% to 32% [1–4] in the elderly population. Patients with EODS show a higher rate of family history of depression than patients with LODS; genetic factors appear to be important. While psychological and genetic factors presumably play an important role in EODS [5], there are probably other nongenetic factors that play a role in LODS [5, 6] such as structural changes in the brain [7, 8].

Population-based neuroimaging studies suggest a possible role for frequently occurring white matter lesions (WMLs) in the etiology of LODS [9–12]. Their dominant view is that WMLs disrupt white matter tracts connecting cortical and subcortical structures (e.g., frontostriatal circuits), which result in LODS [13, 14].

As WML are part of the cerebral small vessel disease (SVD) spectrum, also including lacunar infarcts, it could be that the association between WML and LODS is driven by lacunar infarcts as they reflect more severe structural damage than WML. In addition, SVD might not fully explain the presence of depressive symptoms in elderly as there are patients with LODS without SVD.

A functional or structural change in the amygdala may explain the residual depressive symptoms as the amygdala is involved in mood regulation, and structural MRI studies showed a lower amygdala volume in patients with both LODS and EODS than in healthy controls [15–18]. However,

these studies did not adjust for the possible confounding role of SVD. Conversely, none of the population-based studies on SVD and LODS took amygdala volume into account. We therefore wanted to investigate the relation between amygdala volume and the presence of depressive symptoms in patients with SVD, with adjustment for degree of SVD.

2. Patients and Methods

2.1. Study Population. The Radboud University Nijmegen Diffusion tensor and Magnetic resonance imaging Cohort (RUN DMC) study is a prospective cohort study that investigates the risk factors and cognitive, motor, and mood consequences of functional and structural brain changes among elderly with cerebral SVD.

Cerebral SVD is characterised on neuroimaging by either WML or lacunar infarcts. Symptoms include acute symptoms, such as transient ischaemic attacks (TIAs) or lacunar syndromes, or subacute manifestations, such as cognitive, motor (gait) and/or mood disturbances [19]. As the onset of cerebral SVD is often insidious, clinically heterogeneous, and typically with mild symptoms, it has been suggested that the selection of subjects with cerebral SVD in clinical studies should be based on the more consistent brain imaging features [20].

Accordingly, in 2006, consecutive patients who visited the department of neurology, between October 2002 and November 2006, were selected for participation. Inclusion criteria were (a) age between 50 and 85 years; (b) cerebral SVD on neuroimaging (WML and/or lacunar infarcts). Subsequently the above-mentioned acute or subacute clinical symptoms of SVD were assessed by standardized structured assessments. Patients who were eligible because of a lacunar syndrome were included only >6 months after the event to avoid acute effects on the outcomes.

Exclusion criteria were (a) dementia and (b) parkinson(ism) according to the international diagnostic criteria [21–23]; (c) life expectancy of less than six months; (d) intracranial space occupying lesion; (e) (psychiatric) disease interfering with cognitive testing or followup; (f) recent or current use of acetylcholine-esterase inhibitors, neuroleptic agents, L-dopa, or dopa-a(nta)gonists; (g) WML mimics (e.g., multiple sclerosis and irradiation induced gliosis); (h) prominent visual or hearing impairment; (i) language barrier; (j) MRI contraindications or known claustrophobia.

Patients were selected in a three-step approach. After reviewing medical records 1004 individuals were invited by letter, 727 were eligible after contact by phone, and 525 agreed to participate. In 22 subjects exclusion criteria were found during their visit to our research centre (14 with unexpected claustrophobia, 1 died before MRI scanning, 1 was diagnosed with multiple sclerosis, in 1 there was a language barrier, 1 subject fulfilled the criteria for Parkinson's disease, and 4 met the dementia criteria), yielding a response of 71.3% (503/705). From these 503 individuals one group had symptoms of TIA or lacunar syndrome ($n = 219$), and the remaining ($n = 284$) had cognitive disturbances, motor disturbances, depressive symptoms, or a combination thereof. All participants signed an informed consent form. The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study.

2.2. MRI Acquisition. Imaging was performed on a 1,5 Tesla MRI scanner (Magnetom, Sonata; Siemens Medical Solutions, Erlangen, Germany). The protocol included 3D MPRAGE imaging (TR/TE/TI 2250/3.68/850 ms; flip angle15°; voxel size $1.0 \times 1.0 \times 1.0$ mm) and fluid attenuated inversion recovery (FLAIR) sequences (TR/TE/TI 9000/84/2200 ms; voxel size $1.0 \times 1.2 \times 5.0$ mm, with an interslice gap of 1.0 mm). All scans were performed on the same scanner.

2.3. Amygdala Volumetry. One investigator, blinded to the clinical and other imaging data (IvU), performed manual segmentation of the amygdala, using the interactive software program "ITK-SNAP" [24] (http://www.itksnap.org/). Briefly, this program allowed simultaneous viewing of volumes in coronal sagittal and transversal view, thereby permitting a neat handling of anatomical borders while segmenting the regions of interest. Left and right amygdalae were manually segmented in the coronal plane, from posterior to anterior. Next, segmentations were reviewed in the sagittal plane, because then boundaries were better visualized [25–27]. Segmentation was performed according to previously published protocols [27, 28], and the correct segmentation of anatomical boundaries was verified with the aid of neuroanatomical atlases [28, 29]. In short, the first slice of the amygdala, the posterior border, was identified superior to the hippocampus at the point where the white matter first starts to appear superior to the alveus and laterally to the hippocampal head. The anterior border of the amygdala was defined at the level where the amygdala no longer has an ovoid shape. The medial border is marked by the medial margin of the temporal lobe, which borders Cerebral Spinal Fluid (CSF). The lateral/inferior border is the surrounding white matter and the inferior horn of the lateral ventricle. The amygdala and hippocampus were carefully separated on the sagittal view, moving from the medial to the lateral side of the brain.

Segmentations were done in a standardized way by rating the left amygdala first for half of the patients and the right amygdala first for the other half. Volume was calculated for the left and right amygdala separately by summing all segmented areas, multiplied by slice thickness. Intrarater on a random sample yielded an intraclass correlation coefficient for both left and right amygdalae of 0.8.

2.4. WML Volumetry and Lacunar Infarcts. White matter signal hyperintensities on FLAIR scans, which were not, or only faintly hypointense on T1-weighted images, were considered WML, except for gliosis surrounding infarcts. WMLs were manually segmented on transversal FLAIR images, by 2 trained raters, (IvU, LvO), blinded for all clinical data and amygdala volumes. Total WML volume was calculated in the same fashion as for both amydalae. Inter-rater variability for total WML volume was determined

on a random sample of ten percent yielded an intra-class correlation coefficient of 0.99.

Lacunar infarcts were defined as areas with a diameter >2 mm and <15 mm with low signal intensity on FLAIR and T1, ruling out enlarged perivascular spaces and infraputaminal pseudolacunes [30]. Evaluation of infarcts was performed by one person with a good intra-rater variability with a weighted kappa of 0.80. In ten percent of the scans inter-rater variability was calculated with a weighted kappa of 0.88.

2.5. Brain Volumetry. Gray (GM), white matter (WM) tissue, and CSF probability maps were computed using SPM5 routines (Wellcome Department of Cognitive Neurology, University College London, UK). Total GM, WM, and CSF volumes were calculated by summing all voxel volumes that had a $P > 0.5$ for belonging to the tissue class. Intracranial volume (ICV) was taken as the sum of total GM, WM, and CSF.

2.6. Assessment of Depressive Symptoms. Depressive symptoms were assessed with the Center of Epidemiologic Studies Depression Scale (CES-D) [31]. Depressive symptoms were considered present in patients with a CES-D score ≥16 and/or current use of antidepressive medication, taken for depression, irrespective of their actual CES-D score, because depressive symptoms were considered to be the indication for the medication prescription.

In addition, all patients were asked about their history of depressive episodes. If depressive episodes had occurred, the patients were asked for the age of onset and whether the episodes had prompted them to seek medical advice. A history of depression was considered present if depressive episodes in the past had required attention of a general practitioner, psychologist, or psychiatrist [4].

According to the literature we used the age of 60 years as cut-off point to distinguish between LODS and EODS [4]. Patients at the age or older than 60 years with a CES-D ≥ 16 and/or current use of antidepressive medication, taken for depression, without a history of depressive episodes before or at the age of 60, were classified as having LODS. Individuals with a CESD < 16, without a history of depressive symptoms and without the current use of antidepressive medication, formed the reference group. All others fulfilled the criteria for EODS (first depressive episode <60 years).

2.7. Statistical Analysis. We compared the amygdala and WML volumes (overall, left, right) between the EODS and LODS group with the reference using ANCOVA. Adjustments for age, sex, ICV, WML (or amygdala volume with WML being dependent variable) and presence of lacunar infarcts were made.

The risk of LODS and EODS per milliliter increases in amygdala volume, WML volume, and presence (yes/no) of lacunar infarcts was calculated (expressed as the odds ratio (OR) with a 95% confidence interval; 95% CI) by means of age, sex, ICV, adjusted logistic regression analysis with additional adjustment for the appropriate structural MRI measures (WML volume, amygdala volume, or lacunar infarcts). All data were analyzed using SPSS statistical software, version 16.0. P values < 0.05 were considered statistical significant.

3. Results

Of the 503 patients one was excluded because of an automatic segmentation problem that could not be solved manually. Two patients did not complete the CES-D questionnaire and of two patients the history of depression was not known. There were 101 individuals with LODS (20.3%) and 108 with EODS (21.7%); the reference group comprised 289 persons (58.0%).

Demographic and neuroimaging characteristics of 498 patients are shown in Table 1.

Mean age of the population was 65.6 years (SD 8.8), and 56.4% were male. Mean age of the LODS group was 71.4 years and of the EODS group 59.1 years. Mean age of onset of EODS was 45.1 years (SD 10.9).

Total amygdala volume was 3.4 mL (SD 0.5), and mean volume of left amygdala was 1.8 mL (SD 0.3) and differed significantly ($P < 0.001$), from the right amygdala 1.6 mL (SD 0.3). Amygdala volume decreased significantly with age ($\beta = -0.339$; $P < 0.001$), and women had smaller amygdala (3.2 mL; SD 0.4) than men (3.5 mL; SD 0.5; $P < 0.001$).

Table 2 shows that patients with LODS had a higher WML volume (21.8 mL; SD 20.2) than the reference group (14.7 mL; SD 18.0), although not significant ($P = 0.06$). Independent of SVD, patients with LODS had a significant lower left amygdala volume (1.6 mL; SD 0.3; $P = 0.017$) than the reference group (1.8 mL; 0.28); this difference was not found for the right amygdala (1.5 mL; SD 0.3, $P = 0.432$).

Patients with EODS did not differ from the reference group with respect to WML volume, amygdala volume, and the proportion of lacunar infarcts. We found no significant differences between left, right, and total amygdala volume, WML volume, and presence of lacunar infarcts between patients with LODS and EODS.

Table 3 shows the risk of LODS and EODS and per mL decrease in total, left, and right amygdala volume, WML volume (mL), and presence of lacunar infarcts. Each decrease of both total and left amygdala volume (mL) showed a significant increased risk of the presence of LODS (OR = 1.77; 95CI 1.02–3.08; $P = 0.04$, in total amygdala volume and OR 2.92; 95CI 1.22–7.01; $P = 0.02$ in left amygdala volume), independent of SVD. In addition, there was a nearly significant ($P = 0.08$) increased risk for LODS per increase of WML volume. This was not found for EODS and decrease of amygdala volume (OR = 0.95; 95CI 0.56–1.61; $P = 0.86$) per increase of WML volume, or presence of lacunar infarcts.

4. Discussion

In this study of 498 elderly patients with cerebral SVD, left amygdala volume was related to LODS, independent of WML volume and the presence of lacunar infarcts; this was not found for EODS and amygdala volume (left, right nor total).

Table 1: Baseline Characteristics of patients with Late Onset Depressive Symptoms (LODSs), Early Onset Depressive Symptoms (EODSs), and patients without depressive symptoms.

Depressive symptoms	Late onset	Early onset	Reference	Overall
	(n = 101) (20.3%)	(n = 108) (21.7%)	(n = 289) (58.0%)	(n = 498) (100%)
Age yrs	71.4 (6.0)	59.1 (6.2)	65.9 (9.0)	65.6 (8.8)
Sex (male/female)	47/54	60/48	174/115	281/217
MSSE	27.4 (1.7)	28.4 (1.5)	28.3 (1.6)	28.1 (1.6)
Education (>primary school (%))	77.2%	95.4%	93.4%	90.6%
Mean CES-D	21.0 (8.0)	16.9 (11.0)	7.7 (5.0)	12.4 (9.3)
WM volume	438.4 (57.1)	484.7 (62.9)	465.3 (68.8)	464.1 (66.9)
GM volume	597.4 (66.7)	649.8 (60.5)	632.3 (65.8)	629.0 (67.1)
ICV	1660.8 (156.1)	1659.9 (146.5)	1687.9 (159.4)	1676.3 (156.3)
Median WML volume	16.8	4.5	8.0	8.0
Number of lacunar infarcts	40 (39.6%)	30 (27.8%)	101 (34.9%)	171 (34.3%)
Use of antidepressive medication	30 (29.7%)	30 (27.8%)	0 (0%)	60 (12.0%)

Number represent mean (SD) or number.
MMSE: Mini Mental State Examination; CES-D: Center for Epidemiological Studies Depression Scale; WM: white matter; GM: gray matter; ICV: intracranial volume; WML: white matter lesions.

Table 2: Adjusted mean amygdala volume, White Matter Lesions (WMLs) volume, and lacunar infarcts in patients with Late Onset Depressive Symptoms (LODSs), Early Onset Depressive Symptoms (EODSs), and patients without depressive symptoms.

	Patients with LODS (n = 101)	P-value†	Patients with EODS (n = 108)	P-value†	Reference group (n = 289)
Total amygdala volume (mL)*	3.2 (0.5)	0.05	3.5 (0.5)	0.82	3.4 (0.5)
Left amygdala (mL)*	1.6 (0.3)	0.02	1.8 (0.3)	0.71	1.8 (0.28)
Right amygdala (mL)*	1.5 (0.3)	0.43	1.7 (0.3)	0.90	1.6 (0.28)
WML volume (mL)**	21.8 (20.2)	0.06	9.5 (13.8)	0.94	14.7 (18.0)
Lacunar infarcts (%)***	32.9%	0.42	35.8%	0.47	32.0%

Number represent mean (SD) or number.
Data is shown of the comparison of LODS/EODS versus the reference group, *adjusted for age, sex, ICV, WML volume, and presence of lacunar infarcts, **adjusted for age, sex, ICV, presence of lacunar infarcts and amygdala volume, ***adjusted for age, sex, ICV, WML and amygdala volume, †compared with the reference group.

Before conclusions can be drawn, there are some methodological issues that need to be addressed. The proportion of patients with depressive symptoms in our study is relatively high (42%) for LODS and EODS together. In other studies the proportion of patients with depressive symptoms varies between 9.6%–32% [1–4, 32]. A possible explanation for our relatively high proportion of patients with depressive symptoms could be the fact that we purposely included patients on the basis of presence of SVD; consequently the median degree of WML volume in our study is higher than in population-based studies [33]. As these lesions are related to LODS it seems reasonable to expect a concomitant increased presence of depressive symptoms. Our finding of the (borderline significant) association between WML volume and LODS is in line with findings from these population-based studies [1, 4, 34]. Another explanation is that we classified patients as suffering from depressive symptoms once they had had a depressive episode in their medical history or when they used antidepressive drugs at baseline examination (while previous studies usually did not assess detailed information on the use of medication) [35]. The third explanation could be that we included patients with a history of lacunar stroke and transient ischaemic attacks. It is known that in this population the prevalence of depressive symptoms is higher compared to the general population [36].

To elucidate the etiological mechanisms of LODS its current widely used definition suffers from a conceptual problem, due to the fact that age during the first depressive episode determines the classification. It could very well be that recovery after EODS occurs while depressive symptoms develop again after sixty years of age. Despite the fact that these patients still fulfil the definition of EODS because of their history, they may have developed their LODS on the basis of another underlying pathology including SVD. This could have led to overrepresentation of WML among the EODS group.

There is a conceptual problem with LODS and EODS. By definition the EODS sample included many subjects with major depression. All had consulted a doctor or were at one

Table 3: The risk of LODS and EODS per decrease in amygdala volume, white matter lesion (WML), volume and lacunar infarcts.

	Patients with LODS ($n = 101$)		Patients with EODS ($n = 108$)	
	OR	95% CI	OR	95% CI
Total amygdala volume*	1.77	1.02–3.08†	0.95	0.56–1.61
Left amygdala volume*	2.92	1.22–7.01†	0.98	0.41–2.30
Right amygdala volume*	1.53	0.59– 4.03	0.85	0.34–2.14
WML volume**	0.99	0.97–1.00	1.01	0.99–1.03
Lacunar infarcts***	1.32	0.78–2.23	0.80	0.46–1.39

Number represent odds ratio (OR), 95% confidence interval (CI), *adjusted for age, sex, WML, ICV volume, and presence of lacunar infarcts, **adjusted for age, sex, total amygdala volume, ICV, and presence of lacunar infarcts, ***adjusted for age, sex, ICV, WML, and amygdala volume, †$P < 0.05$.

time treated with antidepressants. By contrast, the LODS sample was defined primarily by the CES-D score (and/or current antidepressant use). Some may have had major depression and some minor depression. These potential differences may affect results both ways.

Another limitation is the cross-sectional nature of our study, which prevents us from proving causality. The RUN DMC study has a longitudinal design, and followup is already planned to evaluate the effect of brain changes on depressive symptoms [37].

Strengths of our study include its design of a homogeneous population that covers the whole spectrum of cerebral SVD, its size and high response rate of over 70%, and the use of a single expert who segmented the amygdala, blinded to clinical information. Particularly the definition of the anatomic boundaries of the amygdala is a notorious problem in amygdala segmentation. Our use of one single, experienced rater minimized the effect of differential segmentation between several raters thereby limiting the effect misclassification. Our results are in line with those from meta-analyses on amygdala volume in nonclinical samples that found a mean volume of both left and right amygdala in 39 studies of 1.7 mL, with a range from 1.0 to 3.9 mL [28].

Although the amygdala is critical to the interpretation of emotion [16, 17] and implicated in mood disorders, volumetric studies of the amygdala in patients with mood disorders have provided inconsistent results [18]. Studies of chronic or recurrent depressive patients have found identical [38, 39], smaller [40–42] but also larger [43] amygdala volumes compared to controls. Postmortem studies showed a smaller amygdala in depressed patients compared to controls, probably due to fewer glial cells [15].

Our data showed a significant relationship with LODS and left amygdala volume; we did not find this relation with the volume of the right amygdala. This is concordant with previous neuroimaging and postmortem studies that have reported left lateralized atrophy in the prefrontal cortex and amygdala in mood disorders [15, 41, 44, 45]; however previous results are inconclusive as others also report on an association between a decrease in right amygdala volume and mood [40]. In addition some functional imaging studies have shown lateralization, with activation of the left amygdala, in relation to emotion and emotional information processing [46, 47].

A possible explanation for a smaller amygdala in patients with LODS could be the coexistent SVD, abnormalities in blood flow, metabolism, and neurotransmitter receptors [48, 49] which may, either directly or indirectly, lead to amygdala atrophy. Intact connectivity of the frontostriatal circuits is important in mood regulation [14, 50]. According to the "vascular depression" hypothesis [14] SVD, that tends to have a high prevalence in the frontostriatal regions, disrupts fiber tracts within these circuits, probably leading to depressive symptoms [11, 34].

There are two other studies that investigated the role of amygdala morphometry in LODS patients. One [51] showed that, despite insignificant amygdala volumetric findings, variations of amygdala shape can be detected and localized. They investigated so in 11 healthy elderly individuals and 14 depressed elderly individuals. A population-based cohort study found a relation between a history of depression, particularly early onset, and an increased risk of for Alzheimer's disease; however this risk was not mediated by smaller hippocampal or amygdala volumes at baseline [32]. In contrast to our results they did not find a relation between a history of depression or depressive symptoms at baseline and smaller amygdala volume. As discussed earlier, this could be because of the difference in degree of SVD between this population-based cohort and our study sample in which we included only subjects with some degree of SVD. As amygdala volume is related to the degree of SVD [52], our study sample will probably have smaller amygdalae. In addition there was a difference in WML segmentation, most studies used semiquantitative methods, and in contrast we manually segmented the WML volume. This in combination with the higher proportion of depressive symptoms in our study sample (42% versus 27%) can explain the relation we found between amygdala volume and LODS in contrast to the findings of this population-based cohort. Finally, the relation between LODS and amygdala volume in our cohort was mainly driven by the left amygdala, while this population-based cohort summed both amygdalae in their analysis.

In conclusion, amygdala volume is associated with LODS, independent of SVD and not with EODS. Future research should consist of prospective studies in order to assess whether baseline presence of SVD increases the risk of amygdala atrophy at followup and whether this coincides with LODS. Innovative MRI techniques including diffusion tensor imaging (DTI) could offer promising tools in order to identify white matter tracts between the amygdala and other parts of the brain and the effects of SVD in those tracts with respect to the incidence of LODS.

Conflicts of Interests

The authors declare that there is no conflict of interests.

Authors' Contribution

The authors certify that all coauthors have contributed sufficiently to the writing of the paper. They have read and approved submission of the final version of the paper, and they have taken due care to ensure the integrity of the work.

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Review Article

Neurobiology of Vascular Dementia

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Vascular dementia is, in its current conceptual form, a distinct type of dementia with a spectrum of specific clinical and pathophysiological features. However, in a very large majority of cases, these alterations occur in an already aged brain, characterized by a milieu of cellular and molecular events common for different neurodegenerative diseases. The cell signaling defects and molecular dyshomeostasis might lead to neuronal malfunction prior to the death of neurons and the alteration of neuronal networks. In the present paper, we explore some of the molecular mechanisms underlying brain malfunction triggered by cerebrovascular disease and risk factors. We suggest that, in the age of genetic investigation and molecular diagnosis, the concept of vascular dementia needs a new approach.

1. Vascular Dementia—Historical Considerations

Just how far back in time should one go when searching data of vascular dementia (VaD)? In 1549, Jason Pratensis published *De Cerebri Morbi,* linking dementia to stroke [1], and in 1658, Johann Jakof Wepfer theorized that a broken brain blood vessel may cause apoplexy (stroke) [2]. The correlation between atherosclerotic disease and dementia was clearly identified only at the beginning of the 20th century by two well-known contributors to the field of neurodegeneration: Alois Alzheimer and Otto Binswanger [3]. The modern era of vascular dementia began in the 1960s, under the leadership of the Newcastle College of Medicine [4]. The concept of VaD was ever since under permanent scrutiny and revision, in light of new clinical, pathological, and imagery data (Figure 1). In the early 1970's, multiple infarct dementia was recognized as a major type of dementia, apart from Alzheimer's disease, characterized not by "neuronal atrophy" but by atherosclerotic burden. In 1975, Vladimir Hachinski defined the "ischemic score," later used for the clinical diagnostic of vascular dementias [5]. However, the concept of VaD soon became controversial due to an increased discrepancy between the incidence of cognitive disorders and that of the "strategic stroke." Furthermore, the early prevention of multi-infarct dementia (MID), the aging of the general population, and an arising need to define "normal aging" versus "pathological aging" [6] added to this controversy. The struggle to identify preventable and treatable factors widened the pathogenic spectrum of VaD [7]. Several epidemiological studies reported associations of hypertension, type 2 diabetes, obesity, and inflammation with VAD and, in some cases, AD. These all coincide with those of stroke, which in turn is an established factor for

cognitive decline and VAD [8] and underlines furthermore the need for a new classification of dementia types [9]. During the last two decades, there was a switch of exploration from classical pathology to new imaging techniques at the molecular level. Therefore, new pathogenic pathways were identified, which greatly increased the complexity of mechanisms of neuronal loss due to cerebral vascular injury [10].

In each stage of clinical and imaging research, new attempts were made to define VaD as an individual, self-standing class of dementia. Mayer-Gross et al. presented in the late 60s a set a criteria including dementia with focal signs and symptoms consistent with stroke, a fluctuating course, preservation of intellectual powers, and personality until late in the disease. Importantly, definition included vascular risk factors such as hypertension [11].

The "multi-infarct dementia" (MID), first described by Vladimir Hachinski, was characterized by a number of small ischemic strokes that may not result in focal neurologic deficits, but in time, by cumulative damage, would lead to cognitive decline. Later, the Hachinski ischemic scale, used for MID diagnosis, was modified by Loeb and Gandolfo to include CT scan criteria [12].

In the 1990s, as acknowledgment of overlapping features of various types of dementia, VaD criteria included clinical and imaging features of *probable* and *possible* disease. Criteria for *definite* VaD would require histopathological evidence from biopsy or autopsy [13].

Currently, the most widely used criteria for VaD include the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC), *International Statistical Classification of Diseases* (ICD), and National Institute of Neurological Disorders and Stroke—Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria [14].

2. The Concept of Brain Ageing

The concept of brain ageing stated at first that cell death might be responsible for the progressive deterioration of different physiological functions. Studies on aged animals [15] from over two decades ago reported neuronal loss with aging, with or without cortical thinning (depending on the type of method used for quantification), but with diminution of the total volume of gray matter. By the end of the 1980s, reports of preserved neuronal number, despite cortical thinning in human brain [16], started to challenge the previous data and were followed by confirmatory studies on animals [17–20]. This controversy was solved by modern imaging investigational methods, starting with computer tomographic analysis in the early 1980's [21] and continuing with recent PET and MRI analyses [22, 23]. These techniques demonstrated that brain atrophy does occur with age in the healthy, nondemented elderly, involving both gray and white matter, but the loss is rather of neuronal connections, not of neurons. Furthermore, quantitation of neurons showed that, despite frontal and medial temporal cortical thinning, the number of neurons is preserved in healthy adults. Freeman et al. reported that, in frontal and temporal neocortical regions, the neuronal count remained relatively constant over a 50-year age range, suggesting that the atrophy is a reflection of the 3D neuronal network loosening rather than perikaryal loss [24]. The prefrontal cortical neurons seem to be particularly vulnerable to ageing, as a decrease in dendritic branching has been reported in neocortex of both rat [25] and human brains [26–28]. By contrast, there is no significant change in dendritic length of hippocampal granule cells, nor a reduction in spine density in the dentate gyrus of aged humans [29] or rats [30].

White matter reduction is also a consistent finding in the aged human brain, possibly as an indicator of defective myelination (although oligodendrocyte number seems to increase). White matter loss is strongly correlated with vascular risk factors, particularly hypertension and stroke [31], two pathologies included in the broad spectrum of VaD risk factors. However, the involvement of white matter abnormalities and the presence of lacunae yielded contradictory results in terms of functional integrity and cognitive impairment [32].

At the molecular level, aging is a "decrease in homeostatic reserve" [33] which interferes with neuronal ability to limit and buffer the increase of reactive oxygen species (ROS) production, to sustain a protective response to cytotoxic stimuli or to limit vicious cycles such as inflammatory environments. DNA damage increases with age (some of which is ROS related), somatic mutation in human lymphocytes being nine times more frequent in aged human subjects than in neonates [34], and mitochondrial DNA being even more sensitive than nuclear DNA. Mitochondrial aging brings its share of vulnerability to stress in aged cells, with decreased ATP reserves [35] along with affected cellular calcium removal systems and low buffering capacity [36]. Moreover, one should take into account the fact that, in the brain, these processes affect, at different rates, different cell types that share a homeostatic balance. On the other hand, understanding aging of the nervous tissue, as compared to other tissues, could be a more challenging task due to a more complex regulation, signaling, and intercellular interactions.

3. VaD from a Molecular Perspective

The molecular perspective on VaD is rather limited; the general concept of this type of cognitive impairment has derived from clinical and imaging findings and is correlated, at the cellular level, with neuronal death and the sudden interruption of neuronal networks. The main pathological changes leading to different forms of vascular dementia take place in both large (atherosclerosis and thrombosis) and small (lipohyalinosis and fibrosis) cerebral vessels, secondary to common vascular risk factors, such as hypertension, diabetes mellitus, and dyslipidemia. The reduction in cerebral blood flow (CBF) starts early during vascular disease [37] and, therefore, a major vascular event can be preceded by a variable period of chronic hypoxia. As a result, the brain cellular microenvironment might change and adaptive

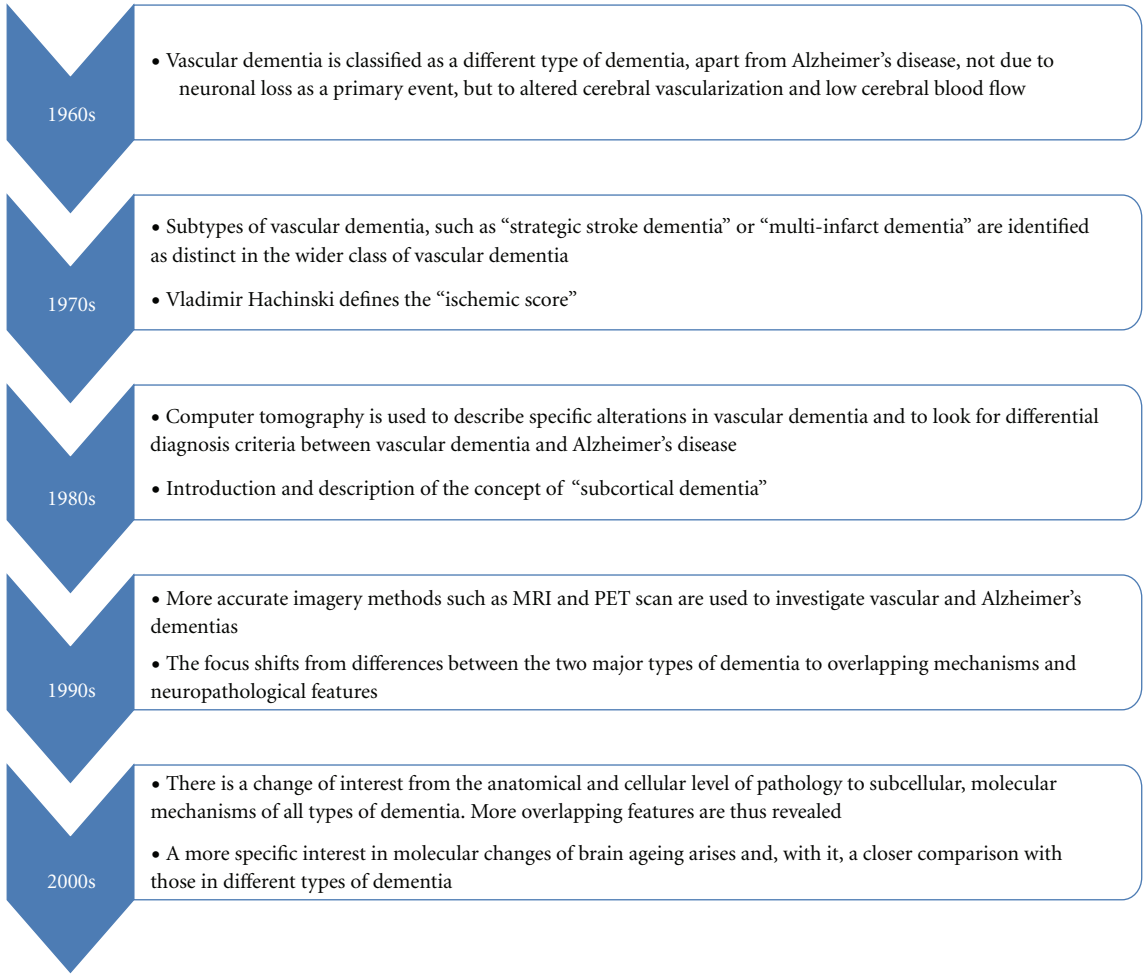

Figure 1: Evolution throughout time of vascular dementia concept.

processes may lead to cellular malfunction, rather than cellular death.

3.1. Cerebral Blood Flow and Ischemia-Triggered Molecular Events. Normal aging is associated with low cerebral flow and velocity at rest [38] and an attenuation of responsivity to hypoxia, hypercapnia, or blood pressure alterations [39]. These modifications may appear due to either histological alterations of the vessel wall (thickening of basement membrane, loss of pericytes, and an overall reduction in cortical vascular bed) or lower metabolic demand. The same changes in blood flow, but at a higher rate, were documented in subcortical ischemic VaD patients by PET studies, with some groups reporting a preferential decrease in frontal lobe regions [40]. In laboratory rats, chronic hypoxia increases the CBF for several days, after which a decrease towards the baseline is noted, probably due to compensatory mechanisms such as increased hematocrit and decreased metabolic needs [41]. Hypoxia inducible factor-1 (HIF-1) was used by Ritz et al. as a marker of hypoxia in the cortex of young (2 months) and old (9 months) spontaneously hypertensive rats (SHR) and stroke-prone SHR, in their study on hypoxic alterations of nonneuronal populations [42]. Interestingly, the increase in HIF1α was documented only in aged animals, along with an imbalance between microvessels and astrocytes at the level of the neurovascular unit. In hypoxic conditions, HIF-1α is upregulated, dimerizes with HIF-1β (the constitutively expressed subunit of HIF-1), translocates into the nucleus, and binds to hypoxia-responsive elements (HREs) of target genes, such as vascular endothelial growth factor (VEGF), glucose transporter-1 (GLUT1), lactate dehydrogenase (LDH), erythropoietin (Epo), and nitric oxide synthase (NOS).

3.2. Inflammatory Cytokines, Adhesion Molecules, and Endothelial Malfunction. Endothelial malfunction is considered to be a first step in the development of atherosclerosis, and may be objectified by overexpression of inflammatory cytokines and adhesion molecules, leading to monocyte recruitment in the nascent atherosclerotic plaque and overproduction of reactive oxygen species (ROS), as a sign of mitochondrial, peroxisomal, and lysosomal alteration.

Measurements of plasma markers in VaD patients showed increased levels of proinflammatory cytokines (IL1, IL6, TNFα) as well as anti-inflammatory cytokines (IL-10)

[43]. IL-6 and TNFα levels increase with aging in animals and humans, and IL-6 transgenic mice also show progressive proliferative cerebellar angiopathy and blood-barrier (BBB) breakdown. These events indicate the endothelium as one of the main targets of proinflammatory cytokine IL-6 [44]. The same transgenic strains indicated for the first time a causative relationship between local production of IL-6 in the brain and the age-related decline in learning and cognitive function, demonstrating dendritic vacuolization, stripping of dendritic spines, decreased synaptic density, and loss of GABA-producing neurons in the hippocampus. In association with neurodegenerative changes, a diffuse nonproliferative gliosis with marked activation of astrocytes and microglia was identified in GFAP-IL6 mice [45]. Furthermore, studies in transgenic mice overexpressing TNF and/or its receptors (p55 and $p75^{NTR}$) demonstrated that IL-6 is a potent microglial activator and, depending on the receptor it activates, (i) an endothelial activator (via $p75^{NTR}$), leading to increased expression of adhesion molecules, BBB disruption, and CNS leukocyte infiltration or (ii) a demyelinating agent and oligodendrocyte apoptosis inducer via p55 [46]. According to Batti and O'Connor, although TNFα has no effect on synaptic transmission or long-term potentation (LTP) under basal conditions, it severely impairs the recovery of postsynaptic transmission after hypoxic exposure [47]. They also showed that the TNFα effect is p38/MAPK mediated, a signaling pathway involved as well in hypoxic neuronal death in the CA1 region of the hippocampus. But, in addition to its neurotoxic nature, TNFα may also exert neuroprotective effects [48, 49] in selected signaling contexts.

Suggested to be another marker of chronic inflammation [50], E-selectin is an endothelial adhesion molecule, that is involved in weak linking of circulating leukocytes. Its expression is upregulated by IL-1 and TNFα. Elevated levels of E-selectin have been previously linked to experimental and clinical brain ischemia [51], and high levels of soluble selectin (sE-selectin) have been correlated with severe cerebrovascular disease [50]. Generating immune tolerance against E selectin by repeated low-dose mucosal administration in lab rats had a protective effect against hemorrhagic strokes in HRS rats and against VCI development in Wistar rats, as shown by Wakita et al. [52].

3.3. Oxidative Stress. The impact of ROS on cognitive function is elegantly demonstrated by studies of superoxide dismutase (SOD) isoenzyme transgenic mice. Overexpression of mitochondrial SOD has a neuroprotective role against drug-induced neurotoxicity, overexpression of cytoplasmic SOD improves age-related impairments in LTP, and overexpression of extracellular SOD is correlated with better spatial memory in laboratory rats [53]. Following cerebral ischemia, the production of free radicals was increased in aged rats and human endothelial cells, mainly by overproduction in the monocyte/macrophage system, especially when stimulated by inflammatory mediators [54].

3.4. Effect of VaD Molecular Alterations on Neuronal and Glial Populations. Hypoxia is associated with increased expression of all NO synthase isoforms, including neuronal (nNOS), astrocyte and microglia-inducible isoform (iNOS), and endothelial isoform (eNOS) [55], which are involved in neuronal death through inhibition of mitochondrial respiration and NMDA/Ca^{2+}-induced exotoxicity [56, 57]. Brain cells are particularly sensitive to ROS aggression due to their high content of polyunsaturated fatty acids, which constitute a substrate for lipid peroxidation. Exposure of brain cells to oxidative stress increases the accumulation of cholesterol in cell membranes [58], leading to decreased fluidity and impaired transmembrane transport.

Hypoxia also upregulates the expression of BDNF—a neurotrophic factor with important roles in neuroplasticity and hippocampus-related learning. This might serve as a protective mechanism against a paucity of hippocampal BDNF mRNA and BDNF plasma levels at older ages [59]. BDNF is further reduced by vascular risk factors such as hypertension and poor glucose metabolism [60]. However, hypoxic upregulation of BDNF is not accompanied by upregulation of its high-affinity receptor Trk-B, but of its low-affinity receptor $p75^{NTR}$, a TNF superfamily receptor. The $p75^{NTR}$ expression is upregulated by hypoxic conditions and is correlated with an increase in caspase-3 activation in cortical and hippocampal neurons, leading to apoptosis [61]. The upregulation of $p75^{NTR}$ is linked to NOS stimulation and to Ca-mediated regulation of expression, suggesting a complex transformation of the pattern of molecular expression in chronic ischemia and VaD.

4. Mixed versus Pure Dementia

"Mixed" dementia is, by the very definition of Vladimir Hachinski himself, "Alzheimer's disease and cerebral infarcts contributing to the dementia" [6], but other coexisting pathologies are also common in dementia such as Parkinson disease (in about 20% of patients with AD) and dementia with Lewy bodies (up to 50%) [62].

Many data suggest that "pure" vascular dementia is rare and is the exception, rather than the rule [63–65]. Vasculopathy as a trigger of AD neuropathological features has been proposed repeatedly before [66–68], and it is very likely that a patient with late-onset AD may already have a vascular burden and shares with VaD vascular risk factors. Moreover, Zhang et al. demonstrated that the low-oxygen dependent increase in HIF1α expression was accompanied by an increase of BACE1 protein levels and a secondary increase in Aβ production [69]. These data suggest that restoration of normal oxygen levels to hypoxic tissues, for example, by the use of small molecules that lower the affinity of oxygen for hemoglobin, could be an interesting issue for research [70, 71].

Activation of inflammation is a consistent finding in AD, as shown in cell culture models [72, 73], animal models [74, 75], and postmortem studies on AD brains [76–78].

Inflammation was related to the onset of cognitive decline and also correlated with disease progression by measurements of serum TNFα and the TNFα/IL1-β ratio. Patients with AD show elevated levels of TGF-β that are

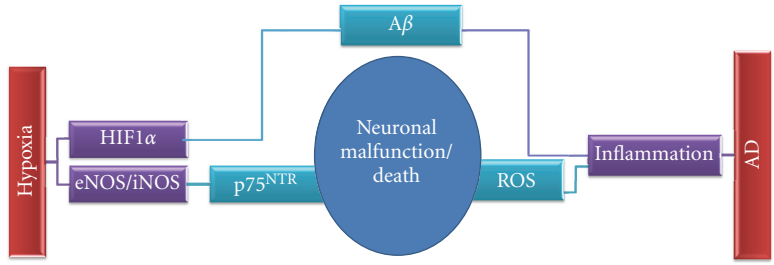

Figure 2: Some mechanisms converging towards neuronal malfunction in two major types of dementia.

correlated with low expression of TGF-R in the affected brain areas, especially around cerebral vessels with CAA [45]. Furthermore, inflammation is associated with ROS production, and oxidative stress has a dual relationship with Aβ peptide: (i) it favors the aggregation of Aβ into a fibrillar form and (ii) it mediates the toxic effect of Aβ on neuronal cells, as shown by the protective effect of antioxidants and free radical scavengers [79]. In turn, some Aβ peptides (such as the 25–35 form) have an intrinsic lipoperoxidizing effect, as established on neocortex-derived synaptosomes [80]. Oxidative stress is demonstrated by the increased amount of 4-hydroxynonenal (HNE), which was shown to interfere with plasmalemmal ATPases and transporters, including Ca^{2+} shifters, further increasing metabolic imbalance in AD.

Downstream Aβ production and accumulation results in secondary endothelial malfunction through: (i) amyloid angiopathy; (ii) NOS inhibition [50]; (iii) atherogenesis correlated with endothelial activation and overexpression of inflammatory cytokines and adhesion molecules, even before Aβ deposition [81]; (iv) lipid peroxidation in the frontal cortex in AD brains [82]; (v) BBB alteration [83].

To conclude, there is an overlap of events between chronic hypoxia and AD on several levels, such as hypoxic-triggered cellular pathways, inflammatory environment, growth factor signalling, and calcium homeostasis (Figure 2). Thus, from the molecular level perspective, the diagnostic criteria for neurodegenerative diseases have become ill defined or insufficient and there is a true need for redefinition.

5. Overlapping of Normal Aging and Neurodegenerative Diseases at Cellular and Molecular Level

Normal aging and various types of neurodegeneration share common molecular events (Table 1), such as alteration of cerebral blood flow, neuroinflammatory environment, and endothelial malfunction.

Aging favors the production of proinflammatory cytokines, mostly through microglial and astrocytic activation [54]. Aging has also been associated, at the cellular level, with increased production of reactive oxygen species (ROS) [109]. Oxidative alteration of enzymes and the subsequent loss of enzymatic activity is a trait of the aging brain, particularly, in the anterior frontal lobe [49]. Oxidative stress leads to the accumulation of free cholesterol [79], along with ceramides, lipid peroxides, and derived aldehydes (such as HNE), that covalently bind to membrane proteins, altering their functions.

Oxidative stress is involved as well in the disruption of Ca^{2+} homeostasis, an effect studied especially in neurons, where Ca^{2+} is a vital mediator of neuronal signaling. It appears that, in aged neurons, several Ca^{2+} homeostatic systems are affected [33] and there is impairment in the maintenance of a nontoxic Ca^{2+} overload [120].

Although it seems that levels of nNOS and eNOS do not change with age, still there is an increase in NOS activity in aged rat cortex. These two NOS isoforms are Ca^{2+} induced, which correlates with the above-mentioned impairment of aged cells to deal with Ca^{2+} overload. Furthermore, consistent with the Ca^{2+}-independent nature of iNOS, there are several reports underlining its absence in the normal aged cortex of lab rats [15, 60, 105].

6. Conclusions

Instead of considering VaD a pure result of neuronal death and the interruption of neuronal networks that support cognitive function, we hypothesize that early brain malfunction is induced by vascular risk factors and chronic hypoxia. A reduction of CBF and a series of molecular events precede the major ischemic events in vascular cognitive impairment. Based on these subtle changes, intervention at early stages could prevent the full-blown development of dementia, which might represent a "point of no return" for the neurovascular units and neuronal networks with few chances for effective treatment.

Abbreviations

Aβ: Amyloid beta peptide
$p75^{NTR}$: Low affinity receptor for tumor necrosis factor α
HIF 1α: Hypoxia Inducible Factor 1α
ROS: Reactive oxygen species
eNOS: Endothelial nitric oxide synthase
iNOS: Inducible nitric oxide synthase.

Table 1: Comparison between normal aging and neurodegenerative diseases from a molecular perspective.

Parameter	Normal aging	Vascular dementia	Alzheimer's disease	Other neurodegenerative disorders
CBF	Diminished with lower velocity, but with preserved dynamic adaptability [84]	Diminished in parietal and frontal lobes, some authors reported also a decrement in superior temporal gyri, thalami, anterior cingulate gyri [85]	Diminished only in parietal cortices and later in advanced disease in frontal lobes [86]	Diminished in preoccipital and occipital regions in PD [87] and LBD [88]
VEGF -A	Low basal levels produced by astrocytes [89]	Upregulation of VEGF and VEGF R2 in astrocytes [90]	Low serum levels and decreased secretion by peripheral immune cells [91]	FTLD—associated with VEGF gene promoter polymorphism in selected populations [92]
Inflammatory cytokines				
IL-6	Increased mRNA compared to young subjects [93]	High blood levels, associated with high CRP may be associated with high risk [94]	Positive immunoreactivity in amyloid plaques and increased concentration in AD brain, compared to age-matched subjects [95]	Increased in cerebral and cerebellar cortex of Huntington patients [96]
TNFα	Increased basal levels in aged laboratory animals with week induction injury response [97]	Modulates neuronal cell loss in cerebral ischemia [98]	Increased expression in AD brain, along with TNF-R1 [99]	Increased in plasma [100], CSF of PD patients and in PD brains, especially in areas with greatest loss of dopaminergic neurons [101]
TGFβ1	Detected at low levels in CSF and produced in CNS at low levels by neuronal cells [102]	Increased in CNS and CSF after stroke [103]	Increased in areas with amyloid burden [104]	CAA—directly related to amyloid vascular deposition [105]
Adhesion molecules	sVCAM increased [106]	sVCAM increased in atherosclerotic disease [107]; sE-selectin increased in severe cerebrovascular disease [108]	sVCAM elevated in late onset AD [50]	sVCAM increased in Down Syndrome [100]
ROS	Increased accumulation with aging [109]	Increased in ischemia animal models and stroke patients [110]	Increased: Aβ-related ROS generation and MAOS [111]	Increased in PD *in vitro* models [112] and animal models [113]
Lipid metabolism	Accumulation of ceramides and free cholesterol in cerebral cortex [114]	Hypercholesterolemia is a known risk factor for VaD	Increased levels of cholesterol, and activation of cholesterol biosynthesis pathway [115]	PD dementia does not correlate with apoE polymorphism or lipid profile [116]

Table 1: Continued.

Parameter	Normal aging	Vascular dementia	Alzheimer's disease	Other neurodegenerative disorders
GLUT 1	Altered structure and function of GLUT-1 [117]	Downregulated in prolonged hypoxia [118]	Low expression in AD hippocampus and double transgenic APP/PS1 animal model Learning increases expression in mouse brain [119]	Insufficiently investigated in neurodegeneration, but involved in "Glut-1 deficiency syndrome"— a treatment-resistant form of epilepsy [120]
BDNF	Decreased mRNA in human plasma and hippocampus [121]	Increased expression following hypoxic stress in cell cultures [122, 123] and lab animals [123]	Decreased expression in hippocampus temporal and frontal cortex [124]	Reduced BDNF expression in the caudate and putamen in HD patients [52] Reduced mRNA BDNF expression [125] and protein [126] in striatal neurons in PD patients
Calcium	Reduced homeostatic reserve [33]	Involved in ischemia-induced excitotoxicity [127]	Aβ disrupts Ca homeostasis in cortical neuronal cell cultures [117]	Excitotoxicity and excessive Ca^{2+}-mediated nitric oxide production are believed to contribute to the death of dopaminergic neurons in PD [118]; Huntingtin transgenic mice express mitochondrial Ca overload upon glutamate stimulation [119]

MAOS: membrane-associated oxidative stress VDCC: voltage dependent calcium channels, FTLD: frontotemporal lobar dementia, LBD: Lewy body dementia, and HD: Huntington disease.

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Review Article

Stroke in the Very Old: A Systematic Review of Studies on Incidence, Outcome, and Resource Use

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Background and Purpose. Stroke incidence increases with age and is likely to increase in the aging populations. We investigated incidence, outcome, and resource use in very old subjects with stroke. *Methods*. We performed a systematic review of available data through electronic search of the literature databases and manual search of reference lists. Data were extracted for the age groups of over 80, 80 to 84 years old, and over 85. Overall incidence rates, expressed as the number of first strokes per 1000 person-years, were estimated using Poisson regression analysis. Odds ratios for the comparisons between subjects over and under 80 were calculated with the Mantel-Haenszel method. *Results*. We found a high incidence of stroke in the very old. The estimated incidence rates were 20.78 (95% CI 19.69 to 21.87) in subjects over 80, 17.23 (95% CI 15.97 to 18.49) for those 80 to 85 years old, and 20.78 (95% CI 16.74 to 23.78) for those over 85. Subjects over 80 contributed 29.95% of strokes; rates were similar among genders. Thirty-day case fatality rate and occurrence of dependency were higher in subjects over 80, although associated with less frequent hospital and stroke unit admission and less diagnostic resource use. *Conclusions*. The contribution of very old subjects to the global burden of stroke is relevant and may require efficient dedicated stroke services.

1. Introduction

In many Western countries, subjects in the oldest age classes, usually referred as the oldest old or very old, represent the fastest to growing segment of the population and make a huge contribution to health care costs [1]. Stroke is one of the leading causes of death and of severe disability in most countries, and its incidence increases steeply with age [2]. Thus, in the forthcoming years, stroke may represent a massive epidemic, causing many disabled patients and deaths in Western countries [2–5]. The availability of data on incidence, classification, and prognosis of stroke in the very old and information on resource use is important to plan health services and to focus treatment strategies. However, studies in the very old are sparse, small, and differing in methodology [4, 6–35].

We performed a systematic review of the available evidence on incidence, outcome, and resource use of very old people with stroke.

2. Materials and Methods

In the present paper, data were identified by search of Medline and from the references of relevant articles published after 1980. Different subsets of studies were potentially eligible for different parts of this paper. The search terms "stroke", "isch(a) emic stroke", "intracerebral", "intraparenchymal", "subarachnoid", "h(a) emorrhage" were firstly used. Then the search was refined by applying any of the following terms: "population-based", "community-based", "community", "epidemiology", "epidemiological", "incidence", "occurrence", "survey", "surveillance", "prognosis", "outcome", "management", and "resource use". Lastly the terms "very old", "oldest old", "very elderly", and "over 80" were applied for the final search refinement. Only papers published in English were reviewed. The reference list of the identified papers were also manually searched. Stroke had to be defined according to the WHO definition, that is, the occurrence of rapidly developing signs of focal or global disturbance of

cerebral function, lasting longer than 24 hours or leading to death, with no apparent cause other than that of vascular origin [36].

Two of the authors reviewed all selected papers reporting data on occurrence, management, and outcome of stroke in subjects over 80 years of age, 80 to 85 years old, or over 85 years. Data on absolute and relative incidence of stroke, stroke type and demographics, outcome, diagnostic procedures, and treatment were assessed. Any repeated reporting of the same study was excluded, so that each data set was considered only once. Population-based studies performed in different period on the same population were considered only once, by using the final data assessment. Stroke type classification was considered only in those studies where CT, MRI, or autopsy findings were available for at least 80% of stroke cases. Strokes were classified into four major types: ischemic stroke (if CT or MRI within 30 days of stroke showed infarct or no relevant lesion and/or autopsy showed ischemic stroke), primary intracerebral haemorrhage (if shown on CT, MRI, or autopsy), subarachnoid haemorrhage (classified by characteristic findings in CSF analysis and/or autopsy, CT, or cerebral angiography), and undetermined stroke (no CT, MRI, autopsy, cerebral angiography, or (for subarachnoid haemorrhage only) CSF examination was done).

The incidence of first ever stroke was calculated per 1,000 person-years. Poisson regression analysis was used to compare incidence rates from different studies. Fitted values were assumed as the best estimate of the true stroke incidence in the very old. Odds ratios with 95% confidence intervals (95% CI) were calculated for mortality, dependency at the modified Rankin scale (mRS), and healthcare resource use with the Mantel-Haenszel method. Sensitivity analysis was performed by excluding those studies that produced a significant deviance change when removed from the model. Since only few studies used the classical cutoff of age 80, results were presented separately for subjects over 80, 80 to 84 years old, and over 85.

3. Results

Sixteen studies reporting data on stroke incidence in the very old were identified including altogether 2406 patients 80 years old or older with a stroke occurring over 114,074 person-years at risk. Incidence rates and confidence intervals are reported in Table 1. Only two studies reported incidence data in subjects over 80 (estimated overall incidence rate of 20.78 per 1,000 person-year; 95% CI 19.69 to 21.87), and three studies reported incidence in people aged 80 to 84 years (overall incidence 17.23/1,000; 95% CI 15.97 to 18.49). In both analyses, there was a significant heterogeneity ($P < 0.0001$). Incidence in people over 85 was reported by 15 studies, with a significant variability among studies ($P < 0.0001$) and rates ranging between 10.34 and 33.48 per 1,000 person-year; the estimated overall incidence rate was 20.78/1000; 95% CI 16.74 to 23.78.

Incidence was almost similar between men and women in the considered age classes of very old people (Table 1). About one-third (29.95%) of strokes occurred in subjects over 80, 15.21% between 80 and 84 years of age, and 16.78% in those over 85.

The distribution of stroke type was reported by 6 studies (Table 2). The estimated overall occurrence rates indicated that the great majority of subjects suffered from an ischemic stroke (88.27%); intracerebral hemorrhage occurred in a proportion of subjects (11.17%) similar to that of all ages subjects (13.43), while subarachnoid hemorrhage was quite rare in the very old (0.55%).

Stroke outcome in oldest subjects was reported by two population-based and three hospital-based studies. Thirty-day case fatality rates and the proportion of dependent subjects (modified Rankin scale > 2) were reported in Figure 1. Mortality was consistently higher among subjects over 80 as compared to subjects under 80 (OR 3.07; 95% CI 2.81 to 3.35). The proportion of dependent subjects was also significantly higher among subjects over 80 in all studies, but in the L'Aquila stroke registry, with an overall OR of 1.77 (95% CI 1.57 to 1.99).

Sparse data are available on resource use by very old subjects with acute stroke (Figure 2). However, in the majority of studies, there was a general tendency to lower use of healthcare resources in subjects over 80, with a tendency to less frequent hospital and stroke unit admission and less frequent neuroimaging Doppler sonographic, echocardiographic, and angiographic studies.

4. Discussion

Data on stroke incidence, clinical and demographic characteristics, and healthcare resource use in the very old are scarce and often inconsistent. The age cut-off of 80 years was close to the average life expectancy in many Western countries and was crucial, considering the sharp drop in the general population after that age [1, 34]. However, only a few studies reported incidence of stroke in patients over 80. On the other hand, there were only moderate differences in the incidence rates among subjects 80 to 84 years old and those over 85. In fact, incidence of stroke was very high in subjects 80 to 84 years old (17.23/1,000) as well as in those over 85 (20.78/1,000).

There is a considerable heterogeneity among incidence rates in different studies. The lowest rate was reported by the study performed in Basle where death certificates were not directly searched [20]. Though lifestyle characteristics of the population and risk factors control might have influenced incidence in the elderly in different studies, rising important clues on the implementation of preventive measures, the play of competing risks in subjects over 85 and of end-cohort effects might also explain most of the differences.

As strongly recommended by several authors, we analyzed incidence of first-ever stroke only, since the inclusion of subsequent strokes, occurring in a highly selected population of stroke survivors, may produce highly biased results [37]. However, in order to estimate the true occurrence of any stroke, either first or recurrent, a 30% of events should be added, producing even more impressing incidence rates in the very old [17].

Table 1: Studies on incidence of stroke in the elderly.

Study	Rate ∗ 1000	95% CI	M/F	% very old
Age over 80 years				
LASR	21.54	20.39–22.69	1.04	30.23
Dijon	10.68	7.71–13.65	0.99	23.90
Overall	*20.78*	19.69–21.87	*1.03*	*29.95*
Heterogeneity $\chi^2 = 24.23$; $P < 0.0001$				
Age 80–84 years				
LASR	17.41	16.11–18.71	1.09	15.46
Dijon	8.87	5.26–12.48	1.32	11.22
ILSA	14.38	9.62–19.15	1.13	14.11
Overall	*17.23*	15.97–18.49	*1.15*	*15.21*
Heterogeneity $\chi^2 = 11.42$; $P < 0.0001$				
Age over 85 years				
LASR	30.00	27.71–32.28	1.21	14.77
Basle	10.34	7.76–12.92	1.22	22.68
Rochester	23.51	19.28–27.74	0.61	23.39
Auckland 1991	19.13	15.91–22.35	0.62	17.34
Dijon	13.03	8.05–18.00	0.74	12.68
Innherred	30.39	30.28–30.51	1.16	21.06
London	18.93	13.63–24.23	1.01	21.33
Perth	23.89	17.87–29.92	1.39	15.95
Belluno	33.48	26.17–40.78	0.68	16.46
Oxfordshire	19.87	15.78–23.95	0.90	13.19
Aosta	32.37	22.37–42.36	1.81	15.35
Frederiksberg	15.99	11.41–20.58	1.36	17.56
Umbria	21.80	15.82–27.77	0.75	17.30
ESPro	21.17	16.19–26.15	1.20	19.21
Arcadia	26.61	22.06–31.16	1.51	23.06
Overall	*20.78*	16.74–23.78	*1.07*	*16.78*
Heterogeneity $\chi^2 = 24.2$; $P < 0.0001$				

Table 2: Distribution of stroke type in the very old.

Study	IS		ICH		SAH	
	%	95% CI	%	95% CI	%	95% CI
LASR	86.89	84.46–89.32	12.70	10.3–15.1	0.41	0.00–.86
Innherred	90.63	83.48–97.77	7.81	1.24–14.39	1.56	0.00–4.6
Belluno	94.03	88.36–99.7	5.97	.3–11.64	0.00	—
Frederiksberg	96.97	91.12–100	3.03	0.00–8.88	0.00	—
ESPro	96.43	91.57–100	3.57	0.00–8.43	0.00	—
Arcadia	86.18	80.08–92.28	12.20	6.41–17.98	1.63	0.00-3.86
Overall	*88.27*	*86.36–90.19*	*11.17*	*9.3–13.05*	*0.55*	*.11–1.00*
Heterogeneity $\chi^2 = 1.06$; $P = 0.9998$						
Overall all ages	*83.75*		*13.43*		*2.82*	

According to our estimates, subjects over 80 years of age contributed almost one third of all strokes pointing out that this small segment of the resident populations makes a relevant contribution to the global burden of stroke. Moreover, although stroke incidence is similar in very old men and women, because of the higher proportions of women in these age classes, women are likely to be responsible for an increasing number of stroke admissions in many countries with a rapidly aging population [1–3, 5, 34].

Besides, despite 30-day case fatality rate was much higher in subjects over 80 than in younger subjects (OR 3.07), surviving patients still showed a higher risk of dependency

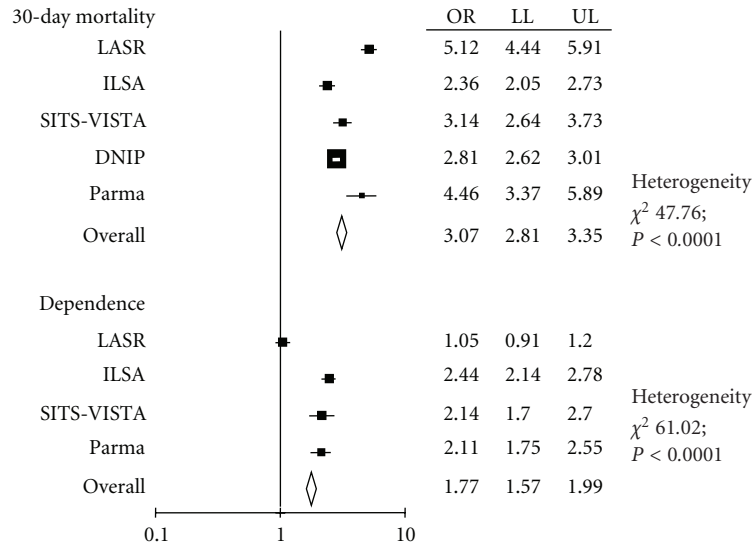

Figure 1: Meta-analysis of studies on stroke outcome in the very old. LASR: L'Aquila stroke registry; ILSA: Italian longitudinal study on aging; SITS-VISTA: International Stroke Thrombolysis Registry and Virtual International Stroke Trials Archive; OR: odds ratio; LL: lower limit; UL: upper limit.

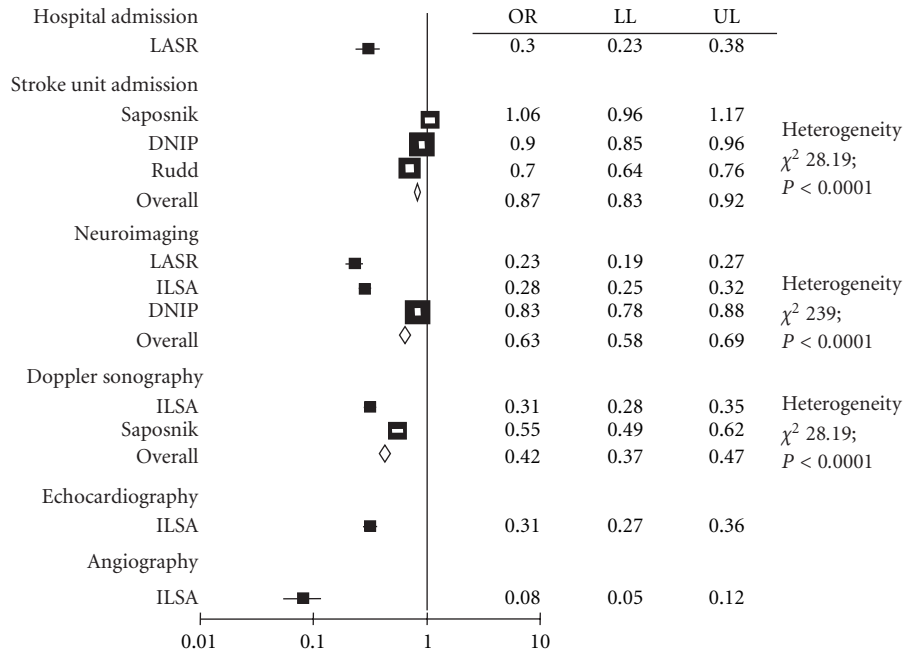

Figure 2: Meta-analysis of studies on healthcare resource use by very old people. LASR: L'Aquila stroke registry; ILSA: Italian longitudinal study on aging; DNIP: Danish National Indicator Project; OR: odds ratio; LL: lower limit; UL: upper limit.

after stroke (OR 1.77). Therefore, very old people also contribute to stroke costs that are likely to increase as general populations get older.

In the available studies, there is a clear tendency to a lower resource use, balancing the tendency to higher costs of stroke in the very old [8, 19, 30, 32, 33]. This tendency may depend on different attitudes and traditions in health care utilization leading to inequalities in the access to health care resources, rather than on fewer requirements and may rise some ethical concern unless adequately prevented. Ad hoc services and dedicated access routes may be useful to reduce inequalities in resource use and to improve health services for the very old.

In conclusion, stroke in the very old is a very frequent condition with an unfavourable outcome, make a relevant contribution to the social burden of stroke, and may require more efficient, dedicated stroke services.

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