Atherosclerosis in the circle of Willis: Spatial differences in composition and in distribution of plaques

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ABSTRACT

Background and aims: Intracranial atherosclerosis is one of the main causes of ischemic stroke. However, the characteristics of intracranial arteries and atherosclerosis have rarely been studied. Therefore, we systematically investigated atherosclerotic changes in all arteries of the Circle of Willis (CoW).

Methods: Sixty-seven CoWs obtained at autopsy from randomly chosen hospital patients (mean age, 67.3 ± 12.5 years), of which a total of 1220 segments were collected from 22 sites. Atherosclerotic plaques were classified according to the revised American Heart Association classification and were related to local vessel characteristics, such as the presence of an external and internal elastic lamina and the elastic fibre density of the media.

Results: 181 out of the 1220 segments had advanced plaques (15%), which were mainly observed in large arteries such as the internal carotid, middle cerebral, basilar and vertebral artery. Only 11 out of 1220 segments (1%) showed complicated plaques (p < 0.001). Six of these were intraplaque hemorrhages (IPH) and observed only in patients who had cardiovascular-related events (p = 0.015). The frequency of characteristics such as the external elastic lamina and a high elastin fibre density in the media was most often associated with the vertebral artery. Only 3% (n = 33) of the CoW arteries contained calcification (p < 0.001), which were mostly observed in the vertebral artery (n = 13, 12%).

Conclusions: Advanced atherosclerotic plaques in the CoW are relatively scarce and mainly located in the 4 large arteries, and mostly characterized by an early and stable phenotype, a low calcific burden, and a low frequency of IPH.

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1. Introduction

Intracranial atherosclerosis is one of the leading causes of ischemic stroke and possesses an increased risk for stroke recurrence. Moreover, it is an important contributor to the development of vascular cognitive impairment and dementia. Apart from atherothromboembolic complications that arise from the carotid arteries, also intracranial atherosclerotic plaques appear to be involved in the onset of cerebrovascular pathology [1]. Unlike the extracranial atherosclerosis prone “high risk” vascular territories such as coronary arteries and aorta, there is a relative lack of knowledge on the histopathology of intracranial atherosclerosis.

Only few large descriptive autopsy studies have been published, which date from the 1960s and 1970s [2–4]. However, pathology reports in which the currently available classification system for atherosclerotic plaques have been applied are limited, they do not include the entire circle of Willis (CoW) [5–7] and smaller arteries were not investigated [8,9]. As the pulse pressure (PP) increases during aging due to stiffening of the walls of the larger arteries, systolic blood pressure (BP) and pulse wave velocity (PWV) increase, which is damaging for small arteries [10,11] and facilitates atherosclerosis [12,13]. We therefore included small arteries of the circle of Willis in our analysis.

Our own prior research did apply the current classification of atherosclerotic plaques to evaluate the phenotype of intracranial plaques yet only included large arteries of the CoW and not the smaller arteries, and showed that advanced intracranial atherosclerosis is rare in large arteries [13]. Another recent study did evaluate the phenotype of intracranial plaques in relation to age.
gender and degree of stenosis, but also this study only included large arteries of the CoW [14].

The present study was set up to systematically investigate the extent, and composition of atherosclerotic plaques, including plaque compositions, in 22 different sites of the entire CoW, at autopsy, and to correlate the findings with atherosclerotic risk factors. We also included features of the underlying vessel wall, such as the presence of an internal (IEL) and external elastic lamina (EEL) and the density of elastin fibers.

2. Material and methods

2.1. Material selection and tissue processing

Sixty-seven CoWs were retrieved from the autopsy population of Academic Medical Center, Amsterdam. Written permission to obtain the brains for research purposes was granted by the families of the patients and the study was conducted in accordance with the Helsinki Declaration. Data about gender, age, clinical risk factors and the cause of death were obtained from hospital electronic patient records. The clinical endpoints were: hypertension – 140/90 mmHg or higher; diabetes mellitus – blood sugar level above 126 mg/dL; obesity – body mass above 30 kg/m² [2]; cardiovascular death – death due to cardiovascular disease [15–17]. The collected brains were fixed in buffered formalin (4%) with 10% NaCl up to 6 weeks before carefully dissecting the CoWs. Twenty-two sites were selected for analysis at the left and right side of the CoW (5 mm thick segments), and grouped into 11 artery types (see Fig. 1a). All segments were decalcified in 12.5% EDTA for 4 days. Segments with macroscopically visible atherosclerosis were selected. If macroscopical indications of atherosclerosis were absent, a representative artery segment was selected (see Fig. 1A). Based on their internal diameter the arteries were grouped into large, medium and small arteries. Large arteries included the middle cerebral (MCA), internal carotid (ICA), vertebral (VA) and basilar artery (BA); medium arteries entailed the anterior cerebral (ACA), superior cerebellar (SCA) and posterior cerebral artery (PCA). Small arteries included the anterior (ACoA) and posterior communicating artery (PCoA) and the posterior (PICA) and anterior inferior cerebellar artery (AICA). After processing and embedding in paraffin, a total of 1220 artery segments were analysed, which included an average of 18 segments per patient.

2.2. Classification of atherosclerotic plaques

Four μm-thick sections were stained with Haematoxylin-Eosin (HE) or Elastic von Gieson (EvG). Atherosclerotic plaques were classified according to a revised American Heart Association Classification [5]. Segments were categorized into three groups: (1) non-diseased, without intimal thickening or inflammatory infiltrates; (2) early plaques showing the accumulation of smooth muscle cells (intimal thickening), foamy macrophages (fatty streak), or smooth-muscle cells and proteoglycans (pathological intimal thickening); and (3) advanced plaques mainly showing collagen rich (fibrous), calcified (fibrocalcified) or lipid (fibrolipid) rich tissue with a fibrous cap overlying an area of which >40% was a lipid core or calcified tissue; (4) complicated advanced plaques showing a cap rupture, intraplaque haemorrhage (IPH), a chronic total occlusion (CTO) or a superficial erosion.

2.3. Artery characteristics

Artery characteristics were evaluated on HE or EvG stained sections. Calcification deposits were grouped as stippling, morula or large deposits [18]. The EEL was classified as absent, continuous or fragmented and elastin fibres as absent, low, moderate or high (see Fig. 3). Furthermore, the internal elastic lamina was classified as absent, fragmented, or coiled, and plaque morphology was defined as eccentric or concentric.

![Fig. 1. Atherosclerotic stages in the circle of Willis.](image-url)

(A) Circle of Willis of a 69-year old patient. Selected sampling sites are indicated with white bars. Segments are selected from the following arteries: the middle cerebral (MCA, N = 118), anterior cerebral (ACA, N = 226), internal carotid (ICA, N = 115), anterior communicating (ACoA, N = 32) and posterior communicating artery (PCoA, N = 104), the posterior inferior cerebellar (PICA, N = 38), anterior inferior cerebellar (AICA, N = 85), superior cerebellar (SCA, N = 110), posterior cerebral (PCA, N = 121), vertebral artery (VA, N = 111) and basilar artery (BA, N = 183). The red arrows indicate a representative examples of intracranial atherosclerosis displayed in the PCA and ICA. (B) Graph showing atherosclerotic stages per artery type, and the increase of advanced plaques as the size of the arteries increases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
2.4. Statistical analysis

The data were statistically analysed using the SPSS software package (Version 20, SPSS IBM, Inc. Chicago, IL, USA). Continuous data were expressed as mean ± SD, and categorical data were expressed as percentages (atherosclerotic stage, elastin fibres in the media, internal and external elastic lamina, calcification and plaque shape). Moreover, independent association analysis of risk factors or cause of death with the atherosclerotic stage was evaluated with the Chi-square test and Fisher’s exact test or by binary logistic regression analysis. Results were considered to be statistically significant at a p-value of ≤0.05.

3. Results

3.1. Patient characteristics

Except for age and gender no clinical information was available of 10 (15%) patients. Thirty (45%) patients died from cardiovascular-related events, 14 (21%) from sepsis, 7 (10%) from neurological events and 6 (9%) from cancer, 10 were (15%) defined as other (Table 1). The sex distribution was almost even: 31 women (72.9 ± 11.1 SD years) and 36 men (62.4 ± 11.5 SD years). The median age was 68 years with a range of 37–90 years. Cardiovascular-related medication included statins (20/67, 30%), angiotensin-converting-enzyme (ACE) inhibitors (16/67, 24%) and beta-blockers related-medication included 808 (66%) had early plaques and 181 (15%) had complicated plaques (see Table 1 and Fig. 1B). Seventy-nine percent of non-diseased, 80% (66%) had early plaques and 181 (15%) had advanced plaques. Of these, 231 (19%) were concentric (34/181). Furthermore, 2% (4/181) was found in the age categories 61–70 years, 1% (2/181) was found in the age category 71–80 years and 49 advanced plaques. In the large arteries (497/1220, 41%) there were from patients with diabetes mellitus (46%, p = 0.037; Table 1).

3.2. Atherosclerotic stage classification and association analysis with patient characteristics

A total of 1220 segments of the CoW were evaluated. The mean number of segments per patient was 18. Of these, 231 (19%) were non-diseased, 808 (66%) had early plaques and 181 (15%) had advanced plaques (see Table 1 and Fig. 1B). Seventy-nine percent of the segments with advanced plaques (143/181) were eccentric and 19% were concentric (34/181). Furthermore, 2% (4/181) was completely occluded. Complicated plaques (p < 0.001) were present in 11 segments, which were derived from 8 patients. These included, 4 chronic total occlusions in the VA, BA, PCA and ACoA, 1 superficial erosion in the VA and 6 intraplaque haemorrhages in the VA, BA and MCA. Three of the intraplaque haemorrhages were associated with rupture of the fibrous cap of the plaque, of which two in VA and one PCA. Plaque complications occurred more often in patients who had died of a cardiovascular related-event (n = 9, 82%, p = 0.015). There was a substantial variation in the topography of atherosclerotic plaques throughout the arteries of the CoW. Small arteries (260/1220, 21%) displayed 64 segments with no plaques, 185 with early plaques (intimal thickening, fatty streaks and pathological intimal thickening) and 11 with advanced plaques (fibrous, fibrofibrillar fibrocalsific). In medium sized arteries (463/1220, 38%), 132 segments had no plaques, 282 showed early plaques and 49 advanced plaques. In the large arteries (497/1220, 41%) 35 had no plaques, 341 showed early plaques, and 121 showed advanced plaques.

Only 11/1220 (1%) plaques were complicated plaques. 5 of these were from patients with diabetes mellitus (46%, p = 0.037; Table 1). Age (p = 0.001), smoking (434/1220, 36%, p = 0.022) and diabetes mellitus (226/1220, 19%, p = 0.022) were all significantly associated with intracranial atherosclerosis (data not shown).

3.3. Calcification

Calcification was present in 33 of the 1220 segments (3%, p < 0.001). These 33 segments were sampled from 5 mainly large arteries, the VA, ICA, BA, MCA and PCA (see Fig. 2 A–D). Sixteen out of the 33 segments showed a stippling calcification pattern (30%), 8 a morula pattern (25%) and 9 had large calcium deposits (23%) (Fig. 2 D).

3.4. External elastic lamina (EEL)

Fig. 3 illustrates features of the EEL, which was present in 41% of the segments either as a continuous (183/1219, 15%) or fragmented layer (317/1219, 26%). The EEL was most frequently observed in the VA (103/111, 93%, see Fig. 3 D), while it was almost absent in the smallest arteries, such as the SCA (9/110, 8%) and AICA (11/85, 13%). Only in the age category 51–60 years a significant association was found between EEL absence and hypertension (p = 0.014). For both hypertension and diabetes mellitus significant associations were found in the age categories 61–80 years (p = 0.047 and p = 0.027) and >80 years (p = 0.001 and p = 0.013) (data not shown).

3.5. Elastin fibres in the tunica media

Elastin fibres in the tunica media were present in different densities (Fig. 3 E–H) ranging from high (280/1219, 23%), moderate (326/1219, 27%), low (478/1219, 39%) to absent (135/1219, 11%). The highest elastin fibre density was found in the largest arteries, such as the VA (110/111, 99%), MCA (117/118, 99%), ICA (112/115, 97%) and...
the BA (146/153, 95%), while the lowest densities were found in the tunica media of smaller arteries such as the AICA (51/85, 60%) and ACoA (23/32, 72%). Age and hypertension or diabetes mellitus were significantly associated with elastin density loss for the age groups 61–80 years (p = 0.037 and p = 0.017) and >80 years (p = 0.034 and p = 0.004) (data not shown).

3.6. Internal elastic lamina (IEL)

IEL characteristics (Fig. 3 I–L) were only assessed in advanced plaques because their presence affects the appearance of the IEL. Coiling of the IEL, a characteristic of the IEL which occurs at the site of an atherosclerotic plaque when the atherosclerosis progresses towards an advanced stage [19], was present in 96/181 (53%) segments, absent in 21/181 (12%) and fragmented in 64/181 (35%). An absent (p = 0.001, OR: 7.47; CI: 2.24–25.0, Table 2) and fragmented (p = 0.02, OR: 2.29; CI: 1.14–4.59, Table 2) IEL was significantly associated with fibrolipid plaques. No significant association was found between the phenotype of the plaque, plaque shape and IEL, however, unlike calcified plaques, the results from fibrolipid plaques did show that they were skewed towards an eccentric plaque shape.

4. Discussion

The main results of this detailed analysis of the extent, the histomorphology and spatial distribution of atherosclerotic plaques throughout the arteries of the CoW show that plaque formation occurs throughout the entire CoW. Early and advanced lesions occurred preferentially in the larger diameter vessels. However, the frequency of advanced plaques was only 15% and complicated plaques (rupture or erosion) were present in 1% of the plaques. Atherosclerotic plaques were associated with age, smoking or diabetes mellitus.

4.1. Topographic distribution and morphology of intracranial atherosclerosis

Previous autopsy studies have shown that atherosclerotic plaque formation was mainly associated with the large arteries of the CoW, such as the ICA and MCA [1,2,4,20–23]. However, in most of these studies plaque formation was screened in only one artery, and smaller diameter vessels were not included. A recent study by Guiterrez also included only large diameter vessels and reported a low frequency of advanced plaques, which is in line with our findings of 15% advanced plaques in the large vessels. We also included smaller arteries (0.5–3 mm internal diameter), such as AICA, PICA, PCoA, SCA and PCA, and showed that the frequency of advanced plaques in these vessels is only 1% (11/1220). The relative lack of advanced plaques in smaller arteries might be explained by a marked fall in blood pressure along the large arteries of the CoW as large intracranial and extracranial arteries, such as the VA and carotid arteries, are assumed to significantly contribute to the vascular resistance in the arterial cerebral circulation [12]. This early pressure drop may thus provide small CoW arteries an intrinsic mechanism to protect themselves from the development of atherosclerotic plaques.

Plaque complications were very rare: about 1% of the samples contained complicated plaques, and IPHs were only present in 6 segments. This is much lower compared to other vascular beds such as the carotid (81%), femoral (63%) and the coronary (61%) arteries [24,25]. The prevalence of intraplaque haemorrhage, in particular in the MCA, is more common among symptomatic than asymptomatic patients [26]. Yet, lower compared to extracranial atherosclerosis [27].

Another important finding was that nearly all advanced plaques appeared to be eccentric rather than concentric plaques. The role of eccentricity has been studied extensively extracranially [28] and showed that eccentricity characterizes arteries that are still able to maintain their vasospastic potential, whereas concentricity associates with loss of that ability. This can be explained by the presence of an arc of normal vessel wall which is seen as the dominate determinant in eccentric plaques for maintaining a vasospastic potential [19].

4.2. Intracranial atherosclerosis and clinical risk factors

In the current study, the clinical background of the patients was...
Fig. 3. Elastin characteristics of the circle of Willis. External elastic lamina: EvG stained sections showing the EEL as (A) continuous and (B) fragmented in the VA or (C) absent in the PCoA. Arrows point towards the EEL. (D) Graph of EEL characteristics per artery type in the CoW. Densities of elastin fibres in the tunica media: EvG stained sections showing elastin fibre densities in the tunica media with (E) high and (F) moderate densities in the VA, and (G) low density in the AICA. Arrows point towards elastin fibres. (H) Graph showing elastin fibres densities in the tunica media per artery in the CoW. Internal elastic lamina characteristics: EvG staining showing examples of the IEL as (I) coiled in the MCA, (J) fragmented in the VA, and (K) absent in the BA. The letters A, M and I indicate the tunica adventitia, media and intima in the images. (L) Graph showing the IEL characteristics of the four largest artery types in the CoW. Images A–C, E–G and I–K were all taken at a 100× magnification, and a scale bar 100 μm. Inserts of the images A–C and E–G were taken at 400× magnification, scale bar 10 μm, and insert I–K at 200× magnification, scale bar 200 μm. Asterisks indicate the luminal area. See Fig. 1 for abbreviations used in the graph.
The association of age and diabetes mellitus with the extent of intracranial atherosclerosis is in concordance with previous studies, which have shown that age, hypertension and diabetes mellitus were associated with the development of intracranial atherosclerosis [29,30]. Our results do not identify hypertension as a contributing factor to intracranial atherosclerosis. This may be due to the relative low number of patients included, and/or to the fact that the clinical data were collected retrospectively. Many papers associate hypertension and the development of mild cognitive impairment or vascular dementia (VD) and show that they are reduced by antihypertensive drugs [31–35]. Furthermore, recent data from the Rotterdam population-based study suggest that a substantial portion of dementia cases could be prevented if modifiable risk factors, such as hypertension, would be eliminated [36]. Yet, there is no evidence from large randomised controlled trials showing the positive effect of antihypertensive treatment on VD and vascular cognitive impairment [35].

Although, there was no association between hypertension and intracranial atherosclerosis in our study we did find significant associations between increasing age, hypertension and decrease of elastin fibre densities in intracranial arteries. This is in line with previous studies that focused on arterial aging in which treated hypertensive and normotensive subjects were followed over a 6-year period to evaluate the progression of aortic stiffness [37].

4.3. Atherosclerosis and the elasticity of intracranial arteries

Elastin morphologic characteristic differences of the arterial wall of the CoW have been reported previously [9], and were not related to the extent and type of plaques. In our study, the IEL displayed multiple specific features, such as coiling and fragmentation, which was associated with the plaque morphology (Table 2). These characteristics only applied to the large diameter vessels. In the small diameter vessels, the IEL appeared to be continuous. CoW arteries and especially the 4 largest arteries, namely VA, MCA, BA and ICA, contained a high elastin fibre density [13] in the tunica media, yet their quantity remains lower than in extracranial arteries, suggesting that CoWs are not elastic arteries, even though, they possess elastin fibres and that the large intracranial arteries are in transition from elastic to muscular intracranial arteries [38,39].

Our interest in including the smaller arteries in our analysis was based on data that for an artery or arteriole of any given diameter, the microvascular pressure is lower in the brain than in the heart, which fits with the above mentioned statement that larger cerebral arteries indeed contribute to the cerebral vascular resistance. Moreover as mentioned earlier, several studies have documented that stiffening of larger arteries during aging increases the BP and PWV, which may damage small arteries and facilitate atherosclerosis [10–12]. However, those studies have either been functional studies, did focus on human study subjects and histological data on the extent of atherosclerosis in small cerebral arteries are not available.

There are several reasons that may explain why we found that atherosclerosis was mainly associated with the largest arteries and not with the small arteries of the CoW. First, atherosclerosis in small intracerebral arteries may only be present in those patients that have advanced atherosclerosis in larger vessels, which was not the case in our study population. This lack of advanced atherosclerosis may be due to the relative young age (mean age 67 years) of our study population. Given the long lag time for developing intracranial atherosclerosis, which only starts after the fourth decade (Resch et al. 1964; Ritz et al. 2014), it is not surprising that we only found earlier stage of atherosclerosis [2,13]. A second reason could be that small intracerebral arteries do have a protective mechanism against atherosclerosis such as elevated levels of antioxidant activity or a specific composition of the glycocalix, inhibiting trapping of chylomicrons and very-low density protein, which results in a reduced deposition of apolipoproteins in the intima of intracranial arteries [40–42]. Of note is that both protective mechanisms have been shown in intracerebral arteries, yet without discriminating larger and smaller vessels. An additional physiological protection may be provided by an increase in arteriolar resistance which may occur concomitantly with the increase in resistance of the larger arteries, thereby preventing an increase in blood pressure in the smaller intracerebral arteries as was suggested by Faraci [12].

Another characteristic that was associated with the large arteries of the CoW was the (dis)continuity of the EEL. Moossy et al. 1966 reported its absence from intracranial arteries. Moreover, Masuoka et al. 2010 showed that the EEL was present in the ICA, however, its presence was highly dependent on the sampled location [8,43]. Our results are in agreement with these studies as we also show that a continuous EEL is mainly associated with the large and more elastic arteries of the CoW.

Altogether, our data demonstrates that early intracranial atherosclerosis is predominant in this cross sectional analysis of our hospital population and that advanced atherosclerosis is exceptional. Even more advanced intracranial plaques show a low calcific burden and low frequency of complications, and is really a feature of the largest arteries of the CoW and not of the smaller arteries.

Table 2

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a Reference category is fibrous.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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