Do gender-specific carotid plaque characteristics and stroke risk prediction suggest different biomarkers for men and women?

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Abstract. B-mode high-resolution ultrasound is a noninvasive technique that provides one of the best methods for the detection of early stages of atherosclerotic disease. Many studies have successfully applied carotid intima-media complex thickness as a technique to monitor arterial wall alterations based upon its association with cardiovascular risk factors, the incident cardiovascular disease, and the outcome. This review paper addresses key issues regarding gender-specific clinical applications of imaging assessment in the context of cardiovascular risk prediction. This will lead to improved management of the single patient, more effective than the application of one-size-fits-all management strategy.

Keywords: carotid imaging, atherosclerosis, plaque biomarker, sex-based differences.

1 Introduction

Atherosclerosis is an inflammatory disease and endothelial dysfunction plays a fundamental role in the initiation and progression within the vessel wall of medium-sized and large arteries [1-3]. The diagnosis of atherosclerosis is crucial for the prevention of cardiovascular events, like myocardial infarction and stroke [4-6]. The primary examination to make this diagnosis is an ultrasound imaging of the carotid artery a safe procedure for the patient, requiring cheap technology. B-scans are easily interpreted by medical doctors and recent 3D ultrasound systems offer a powerful analysis and can reveal pathologies that could be missed in a single 2D image. Carotid intima-media thickness (CIMT) is defined as the distance between the lumen-intima and the media-adventitia interfaces seen on ultrasound images of the carotid artery wall [7-9]. Common carotid artery (CCA) intima-media thickness can be measured by ultrasound near to or below the carotid bulb. For risk assessment CIMT measurements made closer to the bulb might detect early plaque formation. Another important feature that has been reported to be associated with stroke and other cerebrovascular events is carotid plaque detection [10-12]. Plaque echogenicity as assessed by B-mode ultrasound has been found to reliably predict the content of soft tissue and the amount of calcification. Plaques that appear echolucent on B-mode ultrasound are lipid-rich, whereas echogenic plaques have a higher content of fibrous tissue and calcification. The relative risk for cerebrovascular events is higher in subjects with echolucent (low echogenicity) plaques [13-14].

The structure and composition of arterial walls can differ between genders [15-16]. There is convincing evidence for sex-based differences although the relation between sex and dysfunction characteristics needs further insight accessing individual patient data to perform these analyses [17-19]. At every age, most women are of shorter stature and smaller size than most men [20]. Height is related directly to arterial caliber and length as well as cardiac output through effects on stroke volume and heart rate.

Statistical comparisons for gender suggest that females appeared more vulnerable to the progression of arterial stiffening [21-23]. Carotid plaque measurements using maximum wall thickness as 1D size, wall area as

2D size, and wall volume as 3D size are larger in men as opposed to women [24]. Nevertheless, the normalized wall index, which accounts for the total vessel size, shows no significant difference between males and females. Regarding the number of calcifications, men have higher calcification volumes than women and accordingly intraplaque hemorrhage is more common in men than in women [25-26]. Sex differences in stroke incidence, and complication rate have been reported [27-29].

This article is based on previously conducted studies, addressing recent publications on gender-specific differences regarding carotid inflammatory wall, plaque characteristics and stroke risk aiming different biomarkers for men and women what may benefit clinical management and specific treatment [30-31]. Comprehensive parameters on single individuals are the aim of precision medicine that allows for tailored diagnostics and treatment.

2 Atherosclerotic vessel wall characteristics

The vessel wall is a complex dynamic structure that is involved in the regulation of blood flow both in physiologic and pathologic states. There are multiple complex cellular and molecular processes driven by the endothelium that influence the condition of the remaining vessel wall elements. Early atherosclerotic processes result in a relatively predictable asymptomatic progression of disease, followed later by unpredictable manifestations that can result in downstream ischemic sequelae. Ultrasound is currently the first-line imaging method for investigating carotid intima media thickness, carotid stenosis, and carotid plaque features. High-resolution ultrasound techniques allow imaging of a double-layer carotid wall structure (intima-media and adventitia) as presented in Figure 1.

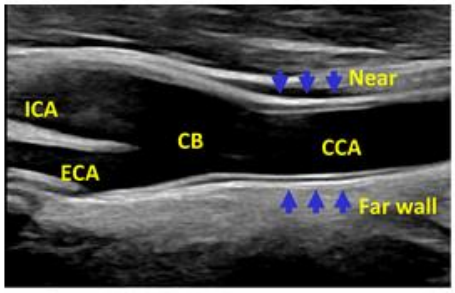

Figure 1. High-resolution ultrasound imaging of a carotid artery.

CIMT is observed as a double line pattern visualized between the intimal-luminal and the medial-adventitial interfaces of the carotid wall in a longitudinal view by B-mode ultrasound. There is a well-documented association between CIMT and cerebrovascular events. CIMT provides a reliable marker in young people, in whom plaque formation or calcification is not established. However, the usefulness of CIMT measurement alone in the improvement of risk cardiovascular models is still controversial [5, 25].

Most studies of vessel wall disease have been based on patients with known carotid artery disease. Approximately 20% of patients with either stroke or a transient ischemic attack have an ipsilateral carotid stenosis of >50%, a group considered, by definition, of advanced disease. The designation of mild or no disease is likely to be an underestimation resulting from comparative imaging using Doppler ultrasound (US), which commonly designates any stenosis measurement <50% as nonsignificant or disease-free [6, 14, 32]. More advanced pathologic features that are straightforward to detect including a thicker vessel wall that provides an easier imaging target.

Advanced imaging has significantly evolved in the last 20 years such that several newer features of the highly dynamic and vulnerable carotid artery plaque can be explored with finer detail using magnetic resonance imaging, computed tomography and ultrasound. Different features of vulnerability identify luminal morphology and ulcerations, intraplaque hemorrhage, fissured layer of connective tissue, lipid heterogeneous material, inflammation and plaque thickness and volume [6,13, 25].

The carotid plaque features assessed using B-mode ultrasound, including echogenicity, surface morphology,

and plaque size. Plaque echogenicity in the carotid vessels can be divided into four types. As shown in Figure 2, plaque type I - predominantly hemorrhage, lipid, cholesterol, and proteinaceous material (homogeneous with predominantly hypoechoic with thin echogenic rim), type II - dense fibrous connective tissue with >50% volume of hemorrhage, lipid, cholesterol, and proteinaceous material (heterogeneous with echogenic plaque with >50% hypoechoic areas), type III - dense fibrous connective tissue with <50% volume of hemorrhage, lipid, cholesterol, and proteinaceous material (heterogeneous with echogenic plaque with <50% hypoechoic areas) and type IV - dense fibrous connective tissue (homogeneous with uniformly echogenic plaque).

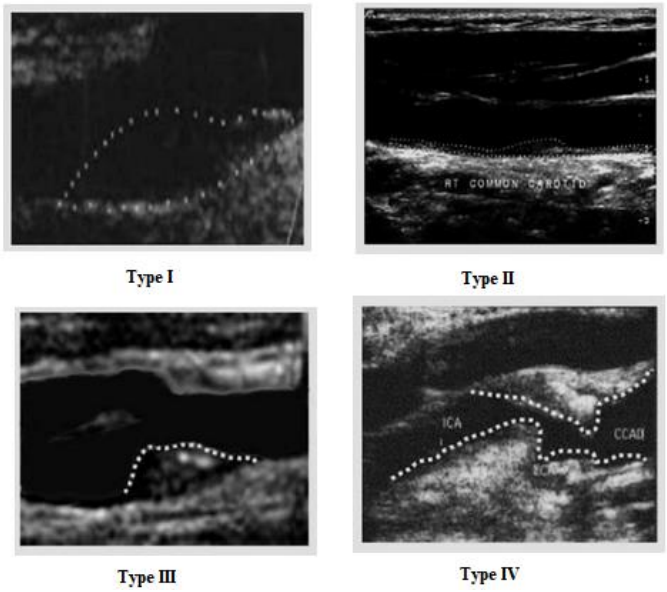

Figure 2. Plaque echogenicity: type I - hypoechoic, type II -heterogeneous with echogenic >50% hypoechoic areas, type III - heterogeneous with echogenic <50% hypoechoic areas and type IV - uniformly echogenic.

Plaque surface morphology is related to severity of stenosis, cardiovascular risk factors, and type of ischemic cerebrovascular symptoms. Surface morphology of the carotid plaque can be classified as smooth, irregular, or ulcerated. Plaques are classified as ulcerated if extension of material is present beyond the vascular lumen into the surrounding plaque [33, 34]. Plaques are classified as irregular if pre- or post-stenotic dilatation is present and/or if the plaque surface morphology shows irregularities without any sign of ulceration. If the plaque is not ulcerated or irregular, it is classified as smooth. The majority of ulcerations are located proximally to the maximum stenosis and ulcerated and irregular plaques are more frequently encountered with a higher degree of stenosis. Complicated plaque is more common in the symptomatic artery of patients with cerebrovascular symptomatology than in the asymptomatic artery; however, this can be ascribed to the significantly higher stenosis degree present in symptomatic arteries compared with asymptomatic arteries.

3 Biomarkers for stroke risk

Severe carotid artery stenosis is a well-established risk factor and accounts for approximately 20% of all ischemic strokes. CIMT and carotid plaque are measures of atherosclerosis and it seems reasonable to combine established traditional risk scores with carotid imaging.

It is well known that cardiovascular diseases present differently among men and women: ischemic heart disease in women develops after 7–10 years compared to men, in fact men are three times more likely to develop acute coronary syndromes under the age of 60, while this tendency decreases with age and the likelihood among sexes equalizes over 75 years. With regards to cerebrovascular events, women over the age of 85 have a higher risk of developing stroke than men, leading to greater disability, mortality and case fatality in this group. Additionally, stroke is more likely to be the first manifestation of cardiovascular disease in women, while ischemic heart disease tends to be the first in men [24,26, 30].

In epidemiological studies in asymptomatic individuals, increased carotid intima-media complex thickness (CIMT) values indicate higher risk of stroke, myocardial infarction, or cardiovascular mortality. Carotid plaque presence, its number and size, volume, surface, echogenicity, or vascularization are all possible measures of adverse cardiac and cerebral events. CIMT measurements and carotid plaque assessment introduction is closely related to the development of high-resolution ultrasound techniques that allowed for the imaging of a double-layer carotid wall structure (intima-media and adventitia). CIMT values are age and sex specific, and males have higher CIMT on average compared to females. A CIMT value over the 75th percentile should be considered abnormal elevating a person with multiple risk factors at higher risk category [35, 36].

The incorporation of additional imaging parameters based on the actual plaque pathophysiology can no longer be ignored for a more accurate analysis of the risk of an ischemic stroke. Non-invasive imaging techniques can provide a useful tool to analyze plaque features among men and women. Imaging biomarkers of vulnerability exist being associated with stroke risk: intraplaque hemorrhage, lipid-rich necrotic core, thin-rupture fibrous cap, carotid plaque thickness, surface morphology and volume. These imaging features of vulnerability present differently among men and women. Indeed, in a population with carotid wall thickening, intraplaque hemorrhage and lipid-rich necrotic core, indicating plaque vulnerability, are highly frequent and more prevalent in men than women. Men tend to have larger plaque volume, more vulnerable plaque composition (intraplaque hemorrhage, lipid-rich necrotic core, thin-rupture fibrous cap) and more frequent coexistence of vulnerable features [37, 38].

Further improvement in risk estimation may be gained by considering not only the largest identified plaque, but also the total plaque burden, plaque area, plaque score (a sum of all plaques heights), or a number of segments containing plaque in both carotid arteries. According to some authors, the average of all the CIMT observed in each carotid segment, is the variable that best describes the total plaque profile, and which has the best predictive power. Additionally, carotid plaque burden measured by 3D ultrasound is highly correlated with coronary artery calcium scores and predictive of major adverse cardiac and cerebral events.

CIMT and carotid plaque reflects atherosclerosis burden in the whole arterial tree. Incidence and severity of cardiovascular risk factors (both traditional and non-traditional) have an impact on CIMT thickness and plaque burden, and more importantly, they are responsible for the rate of carotid atherosclerosis progression. CIMT and carotid plaque may play an additive role in scoring systems evaluating cardiovascular risk.

Most carotid plaque quantification techniques use manual planimetry by repeatedly slicing the 3D US image transverse to the vessel axis with a predefined inter-slice distance. In each transverse slice, the plaque boundaries are traced and the area is measured. The plaque volume is calculated by summing the areas and multiplying them by the inter-slice distance. Quantification of carotid plaque progression or regression requires accurate and reproducible techniques to measure volume and morphology.

The wide array of features of vulnerability offered by MRI, CT and US need not only to be implemented in routine diagnostic algorithms but also to feed them into deep learning algorithms and computer-based decision modeling that will offer optimal strategies for the implementation of these data in an efficient diagnostic process. Carotid stiffness is also significantly associated with ischemic stroke. Carotid stiffness adds value to the existing risk particularly individuals at intermediate cardiovascular risk. During the last decade, automated techniques for sophisticated analysis of vascular mechanics have evolved, such as speckle tracking, and new methods based on deep learning have been proposed with promising outcomes.

Epidemiology tells us that men have higher lifetime risk of stroke than women, but if women suffer from a stroke it is usually more severe, leading to greater disability. These imaging features of vulnerability present differently among men and women. Indeed, men tend to have vulnerable plaques more frequently than women, as demonstrated in a population with carotid wall thickening. Plaque characteristics in asymptomatic patients present more frequently larger percent volume of lipid core as compared to women. With this features, atherosclerotic plaque is more prone to rupture, leading to subsequent ischemic events, consistent with epidemiological data, explaining why men age < 75 years have higher incidence of stroke than women.

4 Discussion

Evidence on possible explanations why men have more advanced plaques is emerging. Both sex and gender may contribute to a worse risk factor profile in men. Higher prevalence of modifiable risk factors as smoking, hypertension, and diabetes in men explains only the tip of the iceberg [39,40]. Differences in carotid anatomy,

affecting hemodynamic parameters, women tend to have larger outflow/inflow ratios. This means they have relatively larger outflow areas (internal and external carotid arteries) compared to the inflow area (common carotid artery), which affects the formation and distribution of carotid plaques. Low ratios, which are more seen in men, result in loss of flow energy, increasing local stress, and endothelial damage. More insight in these underlying mechanisms could contribute to the discovery of sex-specific therapeutic targets for atherosclerosis. These stresses, once again, the importance of including equal populations of male and female patients in studies and investigation of sex as an important component from animal models through clinical trials.

Gender can influence health by reflecting the economic resources and healthcare access of the population, and despite the impossibility to define sex and gender in a binary way, the literature offers very few examples of non-binary trials or gender-related studies. Sex-based plaque differences may not be related just to larger plaque burden in men: in fact, after adjusting the findings for total plaque volume, the prevalence of intraplaque hemorrhage and lipid-rich necrotic core remained higher in men than in women, while the difference in intraplaque hemorrhage and lipid-rich necrotic core volume disappeared, indicating that plaque burden does not fully explain the sex differences in carotid atherosclerosis and that sex plays an important role in the development of a vulnerable plaque rather than in the size of its components. This knowledge may lead to a sex specific management of stroke and transient ischemic attack, with men benefitting from a carotid endarterectomy more than women.

Cardiovascular risk-factors, attention should be paid also to the modern role of women. Nowadays working patterns and activities are similar among sexes, but with women often having family responsibilities on top of time- and energy-consuming working roles. This joint social burden led to increased psychosocial stressors which further increase cardiovascular risk. Thus, modern female lifestyle and under-recognized risk factors, such as anxiety, depression, physical and psychological abuse, socioeconomic status and health literacy should be taken into consideration when adopting cardiovascular risk prevention strategies.

Summarizing, men have more often vulnerable plaques than women. There is a need to investigate the effect of plaque characteristics on stroke per sex separately. This has implications for the interpretation of carotid atherosclerosis in men and women, it is more likely that in men the plaques are more advanced including components like intraplaque hemorrhage. Currently, ultrasonography and computed tomography angiography are the most used modalities for carotid evaluation but cannot reliably identify the presence of intraplaque hemorrhage. Using magnetic resonance imaging in the diagnostic is feasible in clinical practice since only one sequence is needed for the detection of intraplaque hemorrhage. It is important to realize that although the exact mechanisms of sex differences in carotid atherosclerosis are still unclear, we are already able to act on these differences. This knowledge can be used in clinical practice, allowing sex-specific risk scores in order to improve clinical decisions.

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References

[1] M. Scheffle, A. Pellaton, J. Boto, I. Barnaure, B.M. Delattre, J. Remuinan, R. Sztajzel, K.O. Lovblad and M.I. Vargas, "Hemorrhagic plaques in mild carotid stenosis: the risk of stroke", *Canadian Journal of Neurological Sciences*, vol.48, pp. 218–225, 2021.

[2] O. Catalano, G. Bendotti, A. Mori, M. De Salvo, M. Falconi, T.L. Aloi, V. Tibollo, R. Bellazzi, A.F. Bardile, S. Montagna, et al., "Evolving determinants of carotid atherosclerosis vulnerability in asymptomatic patients from the MAGNETIC observational study", *Scientific Reports – Nature*, vol. 11, pp. 23-27, 2021.

[3] A. Gupta, H. Baradaran, H. Kamel, A. Mangla, A. Pandya, V. Fodera, A. Dunning and P.C. Sanelli, "Intraplaque high-intensity signal on 3D time-of-flight MR angiography is strongly associated with symptomatic carotid artery stenosis", *American Journal of Neuroradiology*. vol. 35, pp. 557–561, 2014.

[4] A.S. Larson, W. Brinjikji, L.E. Savastano, I. J. Huston and J.C. Benson. "Carotid intraplaque hemorrhage is associated with cardiovascular risk factors", *Cerebrovascular Diseases*, vol. 49, pp. 355–360, 2020.

[5] M.S. McLaughlin, P.J. Hinckley, S.M. Treiman, S.E. Kim, G.J. Stoddard, D.L. Parker, G.S. Treiman and J.S. McNally, "Optimal prediction of carotid intraplaque hemorrhage using clinical and lumen imaging markers", *American Journal of Neuroradiology*, vol. 36, pp.2360–2366, 2015.
[6] S. Voigt, H. van Os, M. van Walderveen, I.C. van der Schaaf, L.J. Kappelle, A. Broersen, B.K. Velthuis, P.A. de Jong, R. Kockelkoren, N.D. Kruyt, et al., "DUST study group. Sex differences in intracranial and extracranial atherosclerosis in patients with acute ischemic stroke". *International Journal of Stroke*, vol. 16, pp. 385–391, 2021.
[7] L. Saba, A.R. Moody, T. Saam, M.E. Kooi, B.A. Wasserman, D. Staub, A. van der Lugt, J.K. DeMarco, D. Saloner, M. Wintermark, et al., "Vessel wall-imaging biomarkers of carotid plaque vulnerability in stroke prevention trials: a viewpoint from the Carotid Imaging Consensus Group", *JACC: Cardiovascular Imaging*, vol.3, pp. 2445–2456, 2020.
[8] D. Bos, B. Arshi, Q.J.A. van den Bouwhuijsen, M.K. Ikram, M. Selwaness, M.W. Vernooij, M. Kavousi and A. van der Lugt, "Atherosclerotic carotid plaque composition and incident stroke and coronary events", *Journal of the American College of Cardiology*, vol. 77, pp. 1426–1435, 2021.
[9] A. Schindler, R. Schinner, N. Altaf, A.A. Hosseini, R.J. Simpson, L. Esposito-Bauer, N. Singh, R.M. Kwee, Y. Kurosaki, S. Yamagata, et al., "Prediction of stroke risk by detection of hemorrhage in carotid plaques: meta-analysis of individual patient data", *JACC: Cardiovascular Imaging*, vol. 13, pp. 395–406, 2020.
[10] D. Bos, D.H.K. van Dam-Nolen, A. Gupta, L. Saba, D. Saloner, B.A. Wasserman and A. van der Lugt, "Advances in multimodality carotid plaque imaging: AJRexpert panel narrative review", *American Journal of Roentgenology*, vol. 217, pp. 16–26, 2021.
[11] L. Saba, T. Saam, H.R. Jager, C. Yuan, T.S. Hatsukami, D. Saloner, B.A.Wasserman, L.H. Bonati and M. Wintermark, "Imaging biomarkers of vulnerable carotid plaques for stroke risk prediction and their potential clinical implications", *Lancet Neurology*, vol. 18, pp. 559–572, 2019.
[12] D.H.K. van Dam-Nolen, A.C. van Dijk, G. Crombag, C. Lucci, M.E. Kooi, J. Hendrikse, P.J. Nederkoorn, M. Daemen, A.F.W. van der Steen, P.J. Koudstaal, et al., "Lipoprotein(a) levels and atherosclerotic plaque characteristics in the carotid artery: the Plaque at RISK (PARISK) study". *Atherosclerosis*, vol. 329, pp. 22–29, 2021.
[13] N. Altaf, S.T. MacSweeney, J. Gladman and D.P. Auer, "Carotid intraplaque hemorrhage predicts recurrent symptoms in patients with high-grade carotid stenosis", *Stroke*, vol. 38, pp. 1633–1635, 2007.
[14] D.H.K. van Dam-Nolen, M.T.B. Truijman, A.G. van der Kolk, M.I. Liem, F. Schreuder, E. Boersma, M. Daemen, W.H. Mess, R.J. van Oostenbrugge, A.F.W. van der Steen, et al., "Carotid plaque characteristics predict recurrent ischemic stroke and TIA: the PARISK (Plaque At RISK) Study", *JACC: Cardiovascular Imaging*, vol. 15, pp. 1715–1726, 2022.
[15] T. Han, P. Paramsothy, J. Hong, D. Isquith, D. Xu, H. Bai, M. Neradilek, E. Gill and X.Q. Zhao, "High-resolution MRI assessed carotid atherosclerotic plaque characteristics comparing men and women with elevated ApoB levels", *The International Journal of Cardiovascular Imaging*, vol. 36, pp. 481–489, 2020.
[16] M. Glisic, B. Mujaj, O.L. Rueda-Ochoa, E. Asllanaj, J.S.E. Laven, M. Kavousi, M.K. Ikram, M.W. Vernooij, M.A. Ikram, O.H. Franco, et al., "Associations of endogenous estradiol and testosterone levels with plaque composition and risk of stroke in subjects with carotid atherosclerosis", *Circulation Research*, vol. 122, pp. 97–105. 2018.
[17] M.K. Kapral, M. Ben-Yakov, J. Fang, D.J. Gladstone, G. Saposnik, A. Robertson and F.L. Silver, "Gender differences in carotid imaging and revascularization following stroke", *Neurology*, vol. 73, pp.1969–1974, 2009.
[18] J.W. Song, Q. Cao, J.E. Siegler, J.M. Thon, J.H. Woo and B.L. Cucchiara. "Sex differences in carotid plaque composition in patients with embolic stroke of undetermined source", *Journal of the American Heart Association*, vol. 10:e020143, 2012.
[19] J.J. Man, J.A. Beckman and I.Z. Jaffe, "Sex as a biological variable in atherosclerosis", *Circulation Research*, vol. 126, pp.1297–1319, 2020.
[20] J. Krejza, M. Arkuszewski, S.E. Kasner, J. Weigele, A. Ustymowicz, R.W. Hurst, B.L. Cucchiara and S.R. Messe. "Carotid artery diameter in men and women and the relation to body and neck size", Stroke, vol. 37, pp. 1103–1105, 2006.
[21] V. Fernández-Alvarez, M. Linares Sánchez, F. López Alvarez, C. Suárez Nieto, A.A. Mäkitie, K.D. Olsen and A. Ferlito, "Evaluation of Intima-Media Thickness and Arterial Stiffness as Early Ultrasound Biomarkers of Carotid Artery Atherosclerosis", *Cardiology and Therapy*, vol.11, n. 2, pp. 231-247, 2022.
[22] P. Appelros, B. Stegmayr and A. Terent, "Sex differences in stroke epidemiology: a systematic review", *Stroke*, vol. 40, pp. 1082–1090, 2009.
[23] Z. Wang, W. Li, W. Liu and J. Tian, "Gender is a determinant of carotid artery stiffness independent of age and blood pressure*", British Journal of Radiology*, vol. 94: 20200796, 2021. doi:10.1259/bjr.20200796.
[24] D.H.K. van Dam-Nolen, N.C.M van Egmond, K. Dilba, K. Nies, A.G. van der Kolk, M.I. Liem, M.E. Kooi, J. Hendrikse, P.J. Nederkoorn, P.J. Koudstaal, et al. "Sex differences in plaque composition and morphology among symptomatic patients with mild-to-moderate carotid artery stenosis", *Stroke*, vol. 53, pp. 370–378, 2022.
[25] N. Singh, A.R. Moody, B. Zhang, I. Kaminski, K. Kapur, S. Chiu and P.N. Tyrrell. "Age-specific sex differences in magnetic resonance imaging-depicted carotid intraplaque hemorrhage", Stroke, vol. 48, pp. 2129–2135, 2017.
[26] L. Zhang, L. Zhu, M. Lu, X. Zhao, F. Li, J . Cai, C. Yuan and investigators C-I, "Comparison of carotid plaque characteristics between men and women using magnetic resonance vessel wall imaging: a Chinese atherosclerosis risk evaluation study", *Journal of Magnetic Resonance Imaging*, vol. 54, pp. 646–654, 2021.
[27] N. Kandiyil, N. Altaf, A.A. Hosseini, S.T. MacSweeney and D.P. Auer, "Lower prevalence of carotid plaque hemorrhage in women, and its mediator effect on sex differences in recurrent cerebrovascular events". *Public Library of Science (PLOS)* One, 7, e47319. doi: 10.1371/journal.pone.0047319, 2012.
[28] H. Ota, M.J. Reeves, D.C. Zhu, A. Majid, A. Collar, C. Yuan and J.K. DeMarco, "Sex differences of high-risk carotid atherosclerotic plaque with less than 50% stenosis in asymptomatic patients: an in vivo 3T MRI study", *American Journal of Neuroradiology*, vol. 34, pp.1049–55, S1, 2013.

[29] J.E. van der Toorn, O.L. Rueda-Ochoa, N. van der Schaft, M.W. Vernooij, M.A. Ikram, D. Bos and M. Kavousi, "Arterial calcification at multiple sites: sex-specific cardiovascular risk profiles and mortality risk-the Rotterdam Study", *BMC Medicine*, vol. 18, pp. 263, 2020.
[30] K. Gasbarrino, D. Di Iorio and S.S. Daskalopoulou, "Importance of sex and gender in ischaemic stroke and carotid atherosclerotic disease", *European Heart Journal*, vol. 43, pp. 460–473, 2022.
[31] C.S.P. Lam. "How to incorporate sex and gender into the design of cardiovascular clinical trials", *Circulation*, vol. 145, pp. 499–501, 2022.
[32] E. Touze and P.M. Rothwell, "Sex differences in heritability of ischemic stroke: a systematic review and meta-analysis", *Stroke*, vol. 39, pp.16–23, 2008.
[33] G. Sangiorgi, S. Roversi, G. Biondi Zoccai, M.G. Modena, F. Servadei, A. Ippoliti and A. Mauriello, "Sex-related differences in carotid plaque features and inflammation", *Journal of Vascular Surgery*, vol. 57, pp. 338–344, 2013.
[34] U.G. Schulz and P.M. Rothwell, "Sex differences in carotid bifurcation anatomy and the distribution of atherosclerotic plaque", *Stroke*, vol. 32, pp.1525– 1531, 2001.
[35] D. Ditomasso, M.R. Carnethon, C.M. Wright and M.A. Allison, "The associations between visceral fat and calcified atherosclerosis are stronger in women than men". *Atherosclerosis*, vol. 208, pp. 531–536, 2010.
[36] R.J.G. Hartman, M. Mokry, G. Pasterkamp and H.M. den Ruijter, "Sex-dependent gene co-expression in the human body", *Scientific Reports*, vol. 11, pp. 18758, 2021.
[37] R.J.G. Hartman, K. Owsiany, L. Ma, S. Koplev, K. Hao, L. Slenders, M. Civelek, M. Mokry, J.C. Kovacic, G. Pasterkamp, et al., "Sex-stratified gene regulatory networks reveal female key driver genes of atherosclerosis involved in smooth muscle cell phenotype switching", *Circulation*, vol. 143, pp. 713–726, 2021.
[38] J.E. van der Toorn, D. Bos, B. Arshi, M.J.G. Leening, M.W. Vernooij, M.A. Ikram, M.K. Ikram and M. Kavousi, "Arterial calcification at different sites and prediction of atherosclerotic cardiovascular disease among women and men" *Atherosclerosis*, vol. 337, pp. 27–34, 2021.
[39] S. Ahmed and J.D. Spence, "Sex differences in the intestinal microbiome: interactions with risk factors for atherosclerosis and cardiovascular disease", *Biology of Sex Differences*, vol. 12, pp. 35, 2021.
[40] J.G, Cannon, G. Sharma, G. Sloan, C. Dimitropoulou, R.R. Baker, A. Mazzoli, B. Kraj, A. Mulloy and M. Cortez-Cooper, "Leptin regulates CD16 expression on human monocytes in a sex-specific manner", *Physiological Reports*, vol. 2:e12177, 2014.