Vulnerable Atherosclerotic Plaque

Subjects: Cardiac & Cardiovascular Systems
Contributor: Roxana Mihaela Chiorescu , Mihaela Mocan , Andreea Ioana Inceu , Andreea Paula Buda , Dan Blendea , Sonia Irina Vlaicu

Atherosclerosis and its clinical manifestations, coronary and cerebral artery diseases, are the most common cause of death worldwide. The main pathophysiological mechanism for these complications is the rupture of vulnerable atherosclerotic plaques and subsequent thrombosis. Pathological studies of the vulnerable lesions showed that more frequently, plaques rich in lipids and with a high level of inflammation, responsible for mild or moderate stenosis, are more prone to rupture, leading to acute events. Identifying the vulnerable plaques helps to stratify patients at risk of developing acute vascular events. Traditional imaging methods based on plaque appearance and size are not reliable in prediction the risk of rupture. Intravascular imaging is a novel technique able to identify vulnerable lesions, but it is invasive and an operator-dependent technique.

atherosclerosis vulnerable plaque inflammatory biomarkers proteomics micro-RNAs oxidative stress

1. Introduction

Atherosclerosis and its clinical manifestations, coronary and cerebral artery diseases, are the most common cause of death in developed countries. Atherosclerosis is an inflammatory process resulting in subintimal lipid accumulation that may cause lumen stenosis. The plaque rupture and subsequent thrombosis results in acute complications, such as myocardial infarction (MI) or a stroke. An atherosclerotic plaque with an increased risk for rupture and thrombosis is called a vulnerable plaque [1].

When studying the association between the appearance of the atherosclerotic plaques and their risk of rupture, it was found that minor plaques, but rich in lipids, are more likely to become unstable due to the inflammatory reaction maintained by the interaction between lipoproteins, monocytes, macrophages, T lymphocytes, and vascular wall cells. Therefore, traditional imaging techniques that characterize the plaque by its appearance and size are not enough to predict the risk of rupture and the development of an acute thrombotic event. Thus, it is necessary to identify novel biomarkers and imaging methods, such as intravascular imaging, that are correlated with the instability of the atheroma plaques [2][3][4].

Therefore, the risk stratification for patients susceptible to an acute vascular event is very important in therapy management and it becomes necessary to use a multimarker strategy based on biomarkers involved in different pathways of atherosclerosis.

2. Pathophysiological Mechanisms and Molecular Events during the Development of Vulnerable Atherosclerotic Plaques: Plaque Erosion versus Plaque Fissure

The endothelial lining of the vascular tree is cardinal in preserving homeostasis in the cardiovascular system, since it governs flow-mediated vasodilation, vascular permeability, and recruitment of immune cells in the subendothelial space [5]. Healthy vascular endothelial cells (EC) exposed to uniform laminar shear stress, found in a basal antiinflammatory quiescent non-proliferative state, have been noted to display an atheroprotective phenotype [6]. Contrastingly, exposure to enzymatically modified LDL, oxidative stress, advanced glycation end products, and disturbed shear stress will activate EC. Endothelial dysfunction implies EC with an NF-κB signaling driven atheroprone phenotype: Accelerated cellular turnover, enhanced cell surface expression of adhesion molecules (VCAM-1, ICAM-1, and P-selectin), and production of proinflammatory receptors (toll-like receptor 2—TLR2), chemokines (MCP-1 and IL-8), and potent prothrombotic molecules (tissue factor and plasminogen activator inhibitor 1) [7][8][9].

In atherosclerosis, susceptible regions are characterized by compromised endothelial barrier integrity—deriving from diminished expression of endothelial nitric oxide synthase and superoxide dismutase SOD [6]. Thereupon, fatty streak lesion formation, the earliest event in the pathogenic sequence of atherogenesis, consists of the focal permeation and trapping of circulating modified (via oxidation, glycation, enzymatic) lipoprotein particles in the subendothelial space. Circulating monocytes are recruited into the intima, where they will withstand differentiation into macrophages—the source for later transformation into lipid-laden foam cells [7][9].

Phenotype M1 macrophages are proinflammatory and their presence is correlated with vulnerable plaques, whereas M2 macrophages are rather antiinflammatory and preclude foam cell formation.

Recent data from Edsfeldt et al. showed that in vulnerable carotid plaques transcription factor interferon regulatory factor-5 drives CD11c+ proinflammatory macrophage activation, subsequent production of chemokines CCL2 and CCL4, and concomitant inhibition of the efficient efferocytosis apparatus [10]. Previously, the same group had reported the association between CD163+ macrophages and vulnerable plaque phenotype in a large cohort of 200 human carotid plaques [11].

Oxidized or enzymatically modified LDL prompts not only the participation of the innate immune system through scavenger-receptor internalization of the modified lipid particles, signal pattern recognition receptors (PRRs), or mast cells, but also the contribution of the connectors between the innate and the adaptive immune systems—the dendritic cells. In addition, trapping of circulating modified LDL particles in the subendothelial space abets the involvement of adaptive immunity: subsets of T lymphocytes (proinflammatory THELPER1 and antiinflammatory TREGULATORY cells) and B lymphocytes (atheroprotective B-1 cells and proatherogenic B-2 cells) [12][13].

In symptomatic human atherosclerotic carotid plaques, T cells were found to be more prominent, more activated, differentiated, and exhausted (expressing high levels of PD-1—marker of T cell exhaustion) compared to their

blood counterpart [9].

A more complex lesion—the fibromuscular plaque—arises after chemokines secreted by ECs and macrophages incite the migration of smooth muscle cells (SMCs) from the media into the intima, where they will proliferate and synthesize extracellular matrix (ECM) macromolecules (interstitial collagen, elastin, proteoglycans, and glycosaminoglycans) or, in other words, the fibrous cap [9]. A defective efferocytosis process elicits the formation of the necrotic, lipid-rich core in parallel. Mature atherosclerotic plaques can either be stable lesions (featuring a thick fibrous cap and less lipid and inflammatory cell content) or display structural plaque instability, through the proteolytic modification of its ECM components. The evolution of vulnerable plaques potentially culminates in thrombus formation due to frank plaque rupture or due to superficial plaque erosion [14]. Culprit lesions of fatal thrombi in coronary arteries display decreased VSMC synthesis and increased breakdown (by enhanced levels of collagen-degrading enzymes overexpressed by macrophages) of the collagen fibrils that would have shielded the plaque from rupture [15]. However, uncommonly, acute coronary syndromes (ACS) can also occur without apparent thrombus [16].

The vulnerability index, a calculated histological ratio, was proven an efficient tool to discern between vulnerable and stable atherosclerotic lesions; this index measures the existing elements decisive for plaque rupture: plaque destabilizers (macrophages, hemorrhage, and lipids) and stabilizers (smooth muscle cell and collagen) [17].

An even more complex classification of vulnerable plaques discriminates between disrupted fibrous cap lesions, represented by (i) plaque ruptures and (ii) calcified nodules, and intact fibrous cap lesions (plaque erosions) [16].

The pathogenesis of superficial plaque erosion and that of ruptured plaque (also known as thin-capped fibroatheroma (TCFA)) are dominated by distinct molecular events and cellular participants. TCFAs possess large lipid cores covered by a thin fibrous cap (under 60 microns) and a fibrin-rich thrombus [14][18], which are usually infiltrated by inflammatory cells (mostly macrophages) provoking proteolytic activity, and hence, ECM degradation [19]. VSMC senescence and apoptosis is seen in the “shoulder” regions of such atherosclerotic plaques, with VSMC senescence being steered by decreased expression of telomeric repeat-binding factor-2 (TRF2), followed by telomere dysfunction and DNA damage [20].

In contrast to TCFAs, in lesions with superficial erosion there is a scarcity of macrophages, a substantial supply of dedifferentiated VSMCs and ECM (hyaluronan, versican, type III collagen), little or no lipid core and no disruption of the fibrous cap [21]. In the superficial plaque erosion, the center-stage belongs to endothelial cells and to granulocytes, according to in vitro data provided by Quillard and colab.; they hypothesized a “two hit” molecular sequence in the superficial plaque erosion, the first consisting of TLR2 mediated EC injury, EC detachment, or apoptosis [8]. The second hit is represented by the recruitment and adherence of granulocytes to the intimal injured site and subsequent release of reactive oxygen species, DNA, histones or—in other words—the formation of neutrophil extracellular traps (NETs). NETs are able to forge a nidus, amplifying thrombosis and expanding the local inflammatory response [8][18].

Most patients with plaque erosion will have a non-ST-segment elevation ACS as clinical presentation, while ST-segment elevation myocardial infarction (STEMI) will occur in most of the individuals with coronary ruptured TFCAs [13]. Plaque erosion was also associated more with women than with men. The formidable efficacy of lipid lowering therapy in the last decade has led to a shift in the clinical presentation of ACS: From a preponderance of STEMI to an ascendency of NSTEMI. Harmoniously, a synchronicity in the mechanistic phenomenon was also observed: The rise of plaque erosion cases (which now account for a third of ACS—namely the residual burden despite a quality lipid control) in parallel with the regression of TCFA ruptures [18].

Microcalcifications in the atherosclerotic plaques begin with matrix vesicles and apoptotic bodies released during the death of macrophages and proliferative phenotype SMCs [22][23]. Microcalcifications then fuse into sizable masses, expanding from the deeper region of the necrotic core into the surrounding ECM, and ultimately assemble into calcified sheets or plates. Patients with larger calcifications are oftentimes asymptomatic [22][23]. The extent of calcification is inversely correlated to macrophage infiltration. Vulnerable plaques prone to complicate rupture or intraplaque hemorrhage usually display either multiple and superficial calcifications (small spherical calcification or arc-shaped calcification) or calcified nodules [23][24].

3. Imaging Biomarkers of Vulnerable Plaques

Imaging atherosclerosis has evolved along with morphological findings in vulnerable plaques. There are at least four anatomopathological features strongly associated with the risk of rupture and worth targeting by different imaging methods. These risk features include fibrous cap micro-calcifications, cholesterol crystals, apoptosis of intraplaque macrophages, and endothelial shear stress distribution [25]. Imagery of vulnerable plaque comprises anatomical imaging and molecular imaging. Anatomical imaging is classified as invasive and non-invasive.

Catheter-based contrast angiography (CCA) is the gold standard for the coronary vessels' invasive evaluation, having the advantage of high spatial and temporal resolution. Currently, fractional flow reserve (FFR) is the most powerful tool in assessing the potential ischemic risk of lesions and in reducing future clinical events in patients with symptoms [25]. This method uses the intracoronary pressure to establish whether or not the flow is limited by the presence of a plaque and diameter reduction. It represents the difference between the pressure upstream and downstream of the lesion after administration of a vasodilator to augment flow [14][26]. Unfortunately, FFR is not a good predictor of acute events. Studies have shown that the vulnerability of the plaque is strongly determined by its propensity to rupture and with individual systemic characteristics, such as inflammation [26]. Thus, other supplementary intravascular imaging techniques, called catheter-based imaging techniques were develop to further characterize the plaque and its surroundings [27]. Optical coherence tomography (OCT) provides detailed images of the fibrous cap using near infrared light delivered via a fiber optic wire. The images provided by OCT correlate well with histological features high signals in the fibrous cap signify macrophage adherence and thrombus formation [28][29]. Near infrared spectroscopy (NIRS) is another catheter-based invasive technique. NIRS does not require a blood free field and uses wave scatter to produce a gradient map corresponding to the probability of adjacent lipid. The resultant lipid-core burden index describes the ratio of high lipid content in adjacent structures against the total study area [30]. Modern probes are combined with intravascular ultrasound (IVUS) to provide structural context to

the morphological data. IVUS is a more established and, consequently, cost-effective technique than OCT and NIRS [31]. Calcification could be easily detected by IVUS combined with virtual histology or MicroPure IVUS which is rather useful for carotid plaques [32].

Non-invasive classical imaging methods reliably assess atherosclerotic plaques in clinical practice with little additional effort. Coronary computed tomography angiography (CCTA) or cardiac magnetic resonance imaging (CMR) are safe and offer a reliable alternative to catheter angiography [27]. These imaging modalities can simultaneously image the vessel wall and any surrounding atherosclerosis. Similarly, ultrasound (US) can identify the plaque location and characteristic and is a reliable method to quantify the degree of stenosis, rather useful to assess peripheral arteries than coronary territory.

Characteristics of vulnerable plaques as identified by CCTA are synthesized as follows: low attenuation plaque (<30 Hounsfield units), napkin-ring sign (NRS), spotty calcium, and positive remodeling [33]. Low-attenuation plaque is associated with a necrotic core rich in lipids, because of necrosis and apoptosis of macrophage foam cells [33]. A thin fibrotic cap above a necrotic core is often described as an NRC on CCTA, manifesting as a low-attenuation area surrounded by a high-attenuation rim, which are characteristics of high-risk plaques [34]. Finally, spotty calcification identifies inflamed areas of confluent coronary calcification and microcalcification and are the consequence of an inflammatory microenvironment [34]. MR spectroscopy (MRS) combines the spatial imaging obtained from MRI with spectral analysis to detect the chemical composition and metabolic state of cardiovascular tissue. MRS is able to detect a range of atoms, including 1-Hydrogen (1H), 31-Phosphorus (31P), and 13-Carbon (13C) and identifies holesteryl esters as the major class of lipids found in the lipid-rich necrotic core of vulnerable [35]. Non-invasive imaging is useful for the clinical decision making, risk stratification and establish the opportunity for intervention by a multidisciplinary team [27].

Morphological imaging characteristics of vulnerable plaques are the progression of plaque volume over time, and its morphology with irregular surface and the presence of calcifications, ulcerations, and intraplaque hemorrhage [31]. Non-invasive imaging methods of vulnerable plaques classified by the targeted mechanism of vulnerability are mentioned in **Table 1**.

Table 1. Non-invasive imaging of vulnerable plaque classified by the mechanisms of vulnerability.

Mechanism of Plaque Vulnerability	CTCA	PET Techniques
Inflammatory cell infiltration (macrophage activation)	Fat attenuation index (FAI) CTCA Radiomics [36] Radiotranscriptomics	18F-fluorodeoxyglucose (18F-FDG)
		68Ga-somatostatin receptor subtype 2 (68Ga-DOTATATE) [37]
		11C-translocator protein (11C-PK11195)
		18F-fluorocholine (18F–FCH)

Mechanism of Plaque Vulnerability	CTCA	PET Techniques
	FRP CTCA	89Zr-DFO-Gal3-F(ab')2 mAb [38]
		In-DOTA-JR11PET/CT [39]
Neo-angiogenesis		18F-glycoprotein IIb/IIIa platelet receptor (18F-GP1)
Hypoxia		18F-fluoromisonidazole (18F-FMISO)
		18F-HX4
Apoptosis	'Napkin-ring sign' on CCTA	18F-annexin *V*
Calcification and microcalcification	Spotty calcification on CCTA	18F-NaF and 18F-FDG hybrid PET and MR [40]
Hemodynamics and shear stress	CFD CCTA	18F-NaF PET [40]
Intraplaque hemorrhage	FLECT NIR-AF [41]	18F-NaF PET [40]

CCTA, coronary computed tomography angiography; CFD, computational flow dynamics; FLECT, fluorescence emission computed tomography; NIR-AF, Near infrared auto-fluorescence; PET, positron emission tomography; 11c, carbon-11; 68Ga, gallium-68; 18F, fluorine-18; 18F-NaF, 18F-sodium fluoride; 124i, iodine-124.

ues were contribute significantly to the assessment of several crucial mechanisms of plaque vulnerability, such as inflammatory cell infiltration, neo-angiogenesis, hypoxia, apoptosis, and calcifying activity [42]. Htun et al. showed that in vivo monitoring of intraplaque hemorrhage is possible by using fluorescence emission computed tomography (FLECT) and detecting near infrared autofluorescence [41].In order to improve risk assessment accuracy, combined methods using artificial intelligence and imagery were developed. Radiomics represents the process of extraction of quantitative characteristics for radiological images and the conversion into datasets that could be exploited using data extraction and deep learning algorithms which integrate clinical and genetical information from a multidimensional database, useful not only for statistical analysis of general population, but also for individual risk assessment for each and every patient [36]. Radiomic features can be calculated using both the original images as well as mathematical transformations of the original data, such as wavelet decompositions. Wavelet transformation decomposes the data into high- and low-frequency components, which describe the pattern and rate at which attenuation changes along spatial directions [43].

Unfortunately, imagistic biomarkers do not fulfill ideal the ideal biomarker features, having drawbacks such as their invasive character, interpretation being visually performed, being subjective, being influenced by experience, high costs, and low availability [44].

References

1. Björkegren, J.L.; Lusis, A.J. Atherosclerosis: Recent developments. Cell 2022, 185, 1630–1645.
2. Vivanco, F.; Martín-Ventura, J.L.; Duran, M.C.; Barderas, M.G.; Blanco-Colio, L.; Dardé, V.M.; Mas, S.; Meilhac, O.; Michel, J.B.; Tuñón, J.; et al. Quest for Novel Cardiovascular Biomarkers by Proteomic Analysis. J. Proteome Res. 2005, 4, 1181–1191.
3. Lynch, M.; Barallobre-Barreiro, J.; Jahangiri, M.; Mayr, M. Vascular proteomics in metabolic and cardiovascular diseases. J. Intern. Med. 2016, 280, 325–338.
4. Chiorescu, R.M.; Mocan, M.; Barta, A.; Stoia, M.A.; Anton, P.; Mocan-Hognogi, D.L.; Cerasela, M.; Goidescu, A.D.F. Hypoalbuminemia—Prognostic factor for the short-term evolution of patients with unstable angina. Hum. Vet. Med. 2017, 11, 160–165.
5. Hirase, T.; Node, K. Endothelial dysfunction as a cellular mechanism for vascular failure. Am. J. Physiol. Circ. Physiol. 2012, 302, H499–H505.
6. Hansson, G.K.; Libby, P. The immune response in atherosclerosis: A double-edged sword. Nat. Rev. Immunol. 2006, 6, 508–519.
7. Gimbrone, M.A.; García-Cardeña, G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ. Res. 2016, 118, 620–636.
8. Quillard, T.; Araújo, H.A.; Franck, G.; Shvartz, E.; Sukhova, G.; Libby, P. TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: Implications for superficial erosion. Eur. Hear. J. 2015, 36, 1394–1404.
9. Vlaicu, S.I.; Tatomir, A.; Rus, V.; Mekala, A.P.; Mircea, P.A.; Niculescu, F.; Rus, H. The role of complement activation in atherogenesis: The first 40 years. Immunol. Res. 2015, 64, 1–13.
10. Edsfeldt, A.; Swart, M.; Singh, P.; Dib, L.; Sun, J.; E Cole, J.; Park, I.; Al-Sharify, D.; Persson, A.; Nitulescu, M.; et al. Interferon regulatory factor-5-dependent CD11c+ macrophages contribute to the formation of rupture–prone atherosclerotic plaques. Eur. Hear. J. 2022, 43, 1864–1877.
11. Bengtsson, E.; Hultman, K.; Edsfeldt, A.; Persson, A.; Nitulescu, M.; Nilsson, J.; Goncalves, I.; Björkbacka, H. CD163+ macrophages are associated with a vulnerable plaque phenotype in human carotid plaques. Sci. Rep. 2020, 10, 1–9.
12. Hansson, G.K.; Hermansson, A. The immune system in atherosclerosis. Nat. Immunol. 2011, 12, 204–212.
13. Fernandez, D.M.; Rahman, A.H.; Fernandez, N.F.; Chudnovskiy, A.; Amir, E.D.; Amadori, L.; Khan, N.S.; Wong, C.K.; Shamailova, R.; Hill, C.A.; et al. Single-cell immune landscape of human atherosclerotic plaques. Nat. Med. 2019, 25, 1576–1588.
14. Libby, P.; Buring, J.E.; Badimon, L.; Hansson, G.K.; Deanfield, J.; Bittencourt, M.S. Atherosclerosis. Nat. Rev. Dis. Prim. 2019, 5. Available online: http://www.nature.com/articles/s41572-019-0106-z (accessed on 16 August 2019).

15. Hansson, G.K.; Libby, P.; Tabas, I. Inflammation and plaque vulnerability. J. Intern. Med. 2015, 278, 483–493.
16. Partida, R.A.; Libby, P.; Crea, F.; Jang, I.-K. Plaque erosion: A new in vivo diagnosis and a potential major shift in the management of patients with acute coronary syndromes. Eur. Heart J. 2018, 39, 2070–2076.
17. Goncalves, I.; Sun, J.; Tengryd, C.; Nitulescu, M.; Persson, A.F.; Nilsson, J.; Edsfeldt, A. Plaque Vulnerability Index Predicts Cardiovascular Events: A Histological Study of an Endarterectomy Cohort. J. Am. Hear. Assoc. 2021, 10.
18. Libby, P.; Pasterkamp, G.; Crea, F.; Jang, I.K. Reassessing the Mechanisms of Acute Coronary Syndromes: The “vulnerable Plaque” and Superficial Erosion. Circ. Res. 2019, 124, 150–160.
19. Buono, M.F.; Slenders, L.; Wesseling, M.; Hartman, R.J.; Monaco, C.; Ruijter, H.M.D.; Pasterkamp, G.; Mokry, M. The changing landscape of the vulnerable plaque: A call for fine-tuning of preclinical models. Vasc. Pharmacol. 2021, 141, 106924.
20. Guo, Y.; Tang, Z.; Yan, B.; Yin, H.; Tai, S.; Peng, J.; Cui, Y.; Gui, Y.; Belke, D.; Zhou, S.; et al. PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) Triggers Vascular Smooth Muscle Cell Senescence and Apoptosis: Implication of Its Direct Role in Degenerative Vascular Disease. Arter. Thromb. Vasc. Biol. 2022, 42, 67–86.
21. Kolodgie, F.D.; Burke, A.P.; Farb, A.; Weber, D.K.; Kutys, R.; Wight, T.N. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: Insights into plaque erosion. Arterioscler. Thromb. Vasc. Biol. 2002, 22, 1642–1648.
22. Nakahara, T.; Dweck, M.R.; Narula, N.; Pisapia, D.; Narula, J.; Strauss, H.W. Coronary Artery Calcification From Mechanism to Molecular Imaging. JACC Cardiovasc. Imaging 2017, 10, 582–593.
23. Shi, X.; Gao, J.; Lv, Q.; Cai, H.; Wang, F.; Ye, R. Calcification in Atherosclerotic Plaque Vulnerability: Friend or Foe? Front Physiol. 2020, 11, 1–12.
24. Buffinton, C.M.; Ebenstein, D. Effect of Calcification Modulus and Geometry on Stress in Models of Calcified Atherosclerotic Plaque. Cardiovasc. Eng. Technol. 2014, 5, 244–260.
25. Lee, K.Y.; Chang, K. Understanding Vulnerable Plaques: Current Status and Future Directions. Korean Circ. J. 2019, 49, 1115–1122.
26. Libby, P. The changing landscape of atherosclerosis. Nature 2021, 592, 524–533.
27. Syed, M.B.; Fletcher, A.; O Forsythe, R.; Kaczynski, J.; E Newby, D.; Dweck, M.R.; Van Beek, E.J. Emerging techniques in atherosclerosis imaging. Br. J. Radiol. 2019, 92, 20180309.
28. Milzi, A.; Lemma, E.D.; Dettori, R.; Burgmaier, K.; Marx, N.; Reith, S.; Burgmaier, M. Coronary plaque composition influences biomechanical stress and predicts plaque rupture in a morpho-

mechanic oct analysis. Elife 2021, 10, 1–16.

29. Prati, F.; Romagnoli, E.; Gatto, L.; Manna ALa Burzotta, F.; Ozaki, Y. Relationship between coronary plaque morphology of the left anterior descending artery and 12 months clinical outcome: The CLIMA study. Eur. Heart J. 2020, 41, 383–391.
30. Muller, J.; Madder, R. OCT-NIRS Imaging for Detection of Coronary Plaque Structure and Vulnerability. Front. Cardiovasc. Med. 2020, 7.
31. Tomaniak, M.; Katagiri, Y.; Modolo, R.; de Silva, R.; Khamis, R.Y.; Bourantas, C.V.; Torii, R.; Wentzel, J.J.; Gijsen, F.J.H.; van Soest, G.; et al. Vulnerable plaques and patients: State-of-the-art. Eur. Hear. J. 2020, 41, 2997–3004.
32. Yang, S.; Cai, J.; He, Y.; Sun, C.; Lian, X. Usability of Ultrasonic MicroPure Imaging for Evaluating the Vulnerability of Carotid Atherosclerotic Plaques. J. Ultrasound Med. 2021, 40, 2727–2734.
33. Puchner, S.B.; Liu, T.; Mayrhofer, T.; Truong, Q.A.; Lee, H.; Fleg, J.L. High-risk plaque detected on coronary CT angiography predicts acute coronary syndromes independent of significant stenosis in acute chest pain: Results from the ROMICAT-II trial. J. Am. Coll. Cardiol. 2014, 64, 684–692.
34. Liu, T.; Maurovich-Horvat, P.; Mayrhofer, T.; Puchner, S.B.; Lu, M.T.; Ghemigian, K.; Kitslaar, P.H.; Broersen, A.; Pursnani, A.; Hoffmann, U.; et al. Quantitative coronary plaque analysis predicts high-risk plaque morphology on coronary computed tomography angiography: Results from the ROMICAT II trial. Int. J. Cardiovasc. Imaging 2017, 34, 311–319.
35. Canu, M.; Broisat, A.; Riou, L.; Vanzetto, G.; Fagret, D.; Ghezzi, C.; Djaileb, L.; Barone-Rochette, G. Non-invasive Multimodality Imaging of Coronary Vulnerable Patient. Front. Cardiovasc. Med. 2022, 9.
36. Oikonomou, E.K.; Williams, M.C.; Kotanidis, C.P.; Desai, M.Y.; Marwan, M.; Antonopoulos, A.S. A novel machine learning-derived radiotranscriptomic signature of perivascular fat improves cardiac risk prediction using coronary CTangiography. Eur. Heart J. 2019, 40, 3529–3543.
37. Hyafil, F.; Vigne, J. Imaging inflammation in atherosclerotic plaques: Just make it easy! J. Nucl. Cardiol. 2018, 26, 1705–1708.
38. Varasteh, Z.; De Rose, F.; Mohanta, S.; Li, Y.; Zhang, X.; Miritsch, B.; Scafetta, G.; Yin, C.; Sager, H.B.; Glasl, S.; et al. Imaging atherosclerotic plaques by targeting Galectin-3 and activated macrophages using (89Zr)-DFO- Galectin3-F(ab')2 mAb. Theranostics 2021, 11, 1864–1876.
39. Meester, E.J.; Krenning, B.J.; de Blois, E.; de Jong, M.; van der Steen, A.F.W.; Bernsen, M.R. Imaging inflammation in atherosclerotic plaques, targeting SST2 with In-DOTA-JR. J. Nucl. Cardiol. 2021, 28, 2506–2513.

40. Karakatsanis, N.A.; Abgral, R.; Trivieri, M.G.; Dweck, M.R.; Robson, P.M.; Calcagno, C. Hybrid PET- and MR-driven attenuation correction for enhanced 18F-NaF and 18F-FDG quantification in cardiovascular PET/MR imaging. J Nucl. Cardiol. 2020, 27, 1126–1141.
41. Htun, N.M.; Chen, Y.C.; Lim, B.; Schiller, T.; Maghzal, G.J.; Huang, A.L.; Elgass, K.D.; Rivera, J.; Schneider, H.G.; Wood, B.R.; et al. Near-infrared autofluorescence induced by intraplaque hemorrhage and heme degradation as marker for high-risk atherosclerotic plaques. Nat. Commun. 2017, 8, 75.
42. Giannakou, S.; Angelidis, G.; Tsougos, I.; Valotassiou, V.; Kappas, K.; Georgoulias, P. Pet tracers for vulnerable plaque imaging. Ann. Nucl. Med. 2020, 34, 305–313.
43. Oikonomou, E.K.; Siddique, M.; Antoniades, C. Artificial intelligence in medical imaging: A radiomic guide to precision phenotyping of cardiovascular disease. Cardiovasc. Res. 2020, 116, 2040–2054.
44. Fedewa, R.; Puri, R.; Fleischman, E.; Lee, J.; Prabhu, D.; Wilson, D.L.; Vince, D.G.; Fleischman, A. Artificial Intelligence in Intracoronary Imaging. Curr. Cardiol. Rep. 2020, 22, 1–15.