

Clinical Impact of Connexin 43 Deregulation on Myocardial Infarction

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ABSTRACT

Introduction: Coronary artery disease (CAD) is a major and multifaceted health problem but also the first cause of death in modern Western societies. Furthermore, myocardial infarction (MI) constitutes a challenge for analysis in the field of molecular mechanisms, early diagnosis and therapeutic approaches, as its incidence increases every year worldwide. Concerning the histopathological diagnosis in the corresponding cases, a variety of immunohistochemistry (IHC) markers and methods are available to support conventional histology diagnosis. Immunohistochemistry techniques are effective for use in forensic pathology, expanding the limits of differential diagnoses in borderline cases, as they can be applied to tissue samples fixed in formalin and embedded in paraffin.

Objective: The purpose of the current review was to explore the role of connexin 43 (gene locus: 6q22.31) as a reliable biomarker of myocardial disease/infarction and its impact on MI pathology.

Material and method: A systematic review of the literature was carried out based on the international database PubMed. The majority of medical data referred to articles published after the year 2020, whereas specific references of great importance and value were also included. The following keywords were used: coronary, artery, myocardial, infarction, connexin and immunohistochemistry.

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Results: A pool of 38 significant articles focused on the mechanisms and novel experimental biomarkers was selected for the present study at the basis of combining molecular knowledge with new clinical features in CAD, and MI histodiagnosis.

Conclusions: The role of connexin 43 – as a significant gap junction intermediate protein – in MI pathology, clinical symptoms and prognosis is critical because its dysfunction is involved in myocardial conduction and the onset of ventricular arrhythmias due to a crucial interruption of the intra-cardiomyocyte's conjunction.

Keywords: artery, coronary, connexin 43, infarction, immunohistochemistry, myocardium.

ABBREVIATIONS

ATP: adenosine triphosphate
 BRAF: B-raf proto-oncogene
 Cnx 43: connexin 43
 CAD: coronary artery disease
 IHC: immunohistochemistry
 cLA: linoleic acid
 MI: myocardial infarction
 NOX: NADPH oxidase
 PDMS: polydimethylsiloxane
 ROS: reactive oxygen species

INTRODUCTION

Ischemic heart disease is one of most common causes of heart failure and death worldwide and is expected to remain the number one cause in the next years (1). The term ischemic heart disease refers to a group of clinical syndromes which are characterized by the appearance of myocardial ischemia due to an acute disturbance of the balance between the blood supply and consumption of oxygen in the myocardium. Ischemia can result from conditions of stress like tachycardia or hypertension. In the majority of patients, ischemic heart disease is due to obstructive atherosclerotic disease which causes reduction in coronary blood flow leading to myocardial ischemia (2). In fact, it is characterized by the presence of lesions that critically affect the arterial wall, causing stenosis or sudden occlusion of the lumen of a coronary artery. The result of these histopathological modifications is the myocardial ischemia combined or not with necrosis of the myocardium (3).

Persistent coronary artery disease (CAD) is characterized by a chronic myocardial irritation, stable angina and silent ischemia, based on a

progressive atherosclerotic plaque development. It is also the most common heart disease in forensic practice and postmortem diagnosis of early myocardial infarction (MI). Concerning the histopathological diagnosis in the corresponding cases, a variety of immunohistochemistry (IHC) markers and methods have been added to conventional histology. Immunohistochemistry techniques have proven to be effective for use in forensic pathology expanding the limits of differential diagnoses in borderline cases, as they can be applied to tissue samples fixed in formalin and embedded in paraffin (4). In the current review we explored the role and impact of connexin 43 – a gap junction protein in human myocardium – on the clinical significance and severity of MI lesion, describing also its molecular characteristics and potential novel targeted therapeutic strategies.

Pathogenetic factors in coronary artery disease and myocardial infarction

Atherosclerosis is a disease of large and medium-sized arteries. It is the main cause of ischemic heart disease and is characterized by the accumulation of lipids and calcium in the intima of the vessels, while endothelial dysfunction and vascular inflammation coexist. Atherosclerosis begins after the damage to the endothelial cells, which causes endothelial dysfunction and inflammation of the arterial wall (5, 6).

Atherosclerosis is a process that begins in the first decades of life and leads to the formation of atherosclerotic plaque, vascular remodeling and acute or chronic occlusion of the vascular lumen. Multiple risk factors are involved in the atherosclerosis process such as smoking, diabetes mellitus, obesity, dyslipidemia and arterial

hypertension. The main mechanisms involved in the formation of early atherosclerotic lesions are the disturbance of lipid metabolism, activation and dysfunction of the endothelium, and chronic inflammation.

Endothelial damage is provoked by the increased oxidation of low-density lipoprotein (LDL), arterial hypertension, diabetes mellitus and elevated levels of homocysteine in plasma. Endothelium loses its ability to prevent the passage of circulating lipoproteins into the arterial wall. Endothelial dysfunction is also characterized by adhesion of monocytes and platelets to the endothelial layer and hyperplasia of the cellular elements of the arterial wall (7). As a result of increased endothelial permeability, LDL particles enter the arterial wall and become trapped in it; there, they are further oxidized and absorbed by macrophages, into which they enter through special receptors (scavenger receptors). The entry of LDL into the macrophages facilitates the accumulation of cholesterol esters and the conversion of macrophages into foam cells. The fatty streaks, which consisted of monocytes, foam cells and T lymphocytes, are the earliest visible sign of atherosclerosis.

Chronic inflammation plays an important role in the pathogenesis of atherosclerosis through the interaction between endothelial cells, monocytes, T-lymphocytes and platelets, and it is seen in areas where the blood flow is characterized by low shear stress and is turbulent (8). These flow conditions, in combination with the presence of modified LDL, induce the expression of specific molecules (selectins) on the surface of endothelial cells with subsequent adhesion of monocytes and T lymphocytes to them. Monocytes and T lymphocytes then cross the endothelial layer and migrate to the interior of the arterial wall. Their interaction promotes cellular hyperplasia. An integral element of the inflammatory reaction and atherogenesis is the adhesion of platelets to the dysfunctional endothelium, exposed collagen and macrophages, resulting in thrombus formation. Platelets release secondary aggregators, including thromboxane A2 and serotonin, which contribute to the contraction of the smooth muscle fibers of the vessels and to fibrin accumulation. If the inflammatory reaction does not neutralize the causes that lead to the dysfunction of the endothelium, inflammation continues with the result of the migration of smooth muscle fi-

bers from the tunica media to the inflamed area of the tunica intima and the production by the smooth muscle fibers of collagen, elastin and glycoproteins that constitute the atherosclerotic plaque (9). The ongoing inflammatory reaction contributes to the proliferation of macrophages and lymphocytes in the area of damage and causes increased damage.

During the process of atherosclerosis, in the initial stages the atheromatous plaque does not affect the blood flow. Then, the remodeling of the arterial wall, that accompanies the development of the damage, comes from the production of smooth muscle cells by proteinases specialized in the degradation of the components of the extracellular space. Gradually, the atheromatous plaque grows towards the arterial lumen and leads to the formation of plaques that restrict blood flow. Destabilization of atherosclerotic plaque is due to metalloproteinase secretion (10). The disturbance of coronary flow, especially in situations of increased oxygen demand, causes ischemia and angina pectoris.

Rupture of the atherosclerotic plaque is the most common cause of acute thrombosis of the coronary arteries, causing myocardial infarction. These specific plaques usually have large fatty cores surrounded by a thin fibrous capsule and also called vulnerable plaques which means that are prone to rupture (11). On the contrary, plaques with limited lipid accumulation and a denser fibrous capsule are called stable. Inflammatory processes inhibit the synthesis of interstitial collagen by smooth muscle cells, disrupting their ability to maintain the framework of the fibrous capsule. Activated inflammatory cells can also activate collagenases that are specialized in breaking down basic structural elements of the fibrous capsule. Plaque rupture exposes its contents to the blood, with clotting factors such as tissue factor causing acute thrombosis. As a result of coronary artery occlusion, myocardial blood supply is obstructed leading to myocardial cells necrosis (12). Ischemia that lasts 20-40 minutes causes irreversible injury and myocyte death.

The erosion of the atheromatous plaque constitutes a different mechanism of causing ischemic events. Eroded atheromatous plaques usually have a rich extracellular space without a thin fibrous capsule, with few leukocytes and a lower percentage of lipids. The erosion of the atherosclerotic plaque is usually accompanied by

non-occlusive thrombus and myocardial infarction without elevation of ST segment (NSTEMI), which is characterized by rupture of vulnerable atherosclerotic plaque or erosion of the endothelium with non-occlusive thrombus formation. Unstable angina has the same pathological substrate with acute MI with non-elevation of the ST segment. Acute myocardial infarction with ST segment elevation (STEMI) is characterized by rupture of vulnerable atherosclerotic plaque and is accompanied by occlusive thrombus formation. Coronary angiography is the best examination for the confirmation of diagnosis of acute MI as with the help of special catheters and the injection of intravenous dye illustrates the anatomy as well as the lesions of the coronary vessels. Postmortem coronary angiography might also be helpful but is not indicated and it is not included as a recommendation in the European and American guidelines of cardiology (13, 14).

The coronary arteries consist of three layers: the tunica intima, tunica media and tunica adventitia. Tunica intima contains the endothelium and the basement membrane. Tunica media is composed of smooth muscle fibers and matrix. Tunica adventitia contains collagen, fibroblasts and a small amount of smooth muscle fibers. The hallmark during histological examination of the coronary arterial wall is the presence of atherosclerotic changes, which are responsible for the development of the arterial wall thrombus (15). In some cases, coronary thrombosis might be caused by Kawasaki disease, a form of vasculitis where blood vessels throughout the body become inflamed (16). In extremely rare cases, cardiac contusion as a result of blunt trauma can lead to MI due to coronary thrombus formation (17).

Connexin 43: structure and functions

Connexin 43 (Cnx 43) or Gap junction alpha-1 (GJA1) protein is a member of the connexin gene family. The encoded protein, a component of gap junctions, is encoded by the GJA1 gene (gene locus: 6q22.31). Connexin 43 – as a 43.0 kDa protein – includes a series of 382 amino acids (18). More specifically, it is characterized by an elongated C-terminal domain, an N-terminal domain, whereas centrally located trans-membrane domains are also recognized. According to its stereo-chemical formation, C- and N-terminal

are endo-cytoplasmic domains, whereas its trans-membrane part penetrates multiple times the main phospholipid cell membrane. Biochemically, the C-terminal is involved in a variety of post-translational modifications that critically affect transcription factors. Additionally, the N-terminal regulates channels assembly – provided also by the C-terminal – and oligomerization/switching of them (19). Gap junction channel development and formation is mediated by the trans-membrane domains. In fact, intact gap junction channels are composed by the interaction of disulfide bonds in formatted hexamers. These intercellular channels join adjacent cells and are responsible for the circulation of low-weight molecules and ions that regulate intra-cellular homeostasis. Inside the cytoplasmic environment, Cnx 43 is implicated in microtubule network stability, whereas in mitochondria it enhances cell survival by blocking the corresponding apoptotic pathway and preventing the destructive cataract of oxidative stress elevated stress (20).

Connexin 43 induces the inter-cellular gap junction, also regulating crucial cellular functions, including embryonic tissue development, proliferation, differentiation, and also cell death. Cnx 43 is involved in muscle contraction and myocardium mobility providing the basis for intercellular communication in the cardiovascular system (21). Based on this ability, Cnx 43 regulates maintenance of the normal cardiac rhythm, the vascular tone, endothelial function as well as metabolic interchange between the cells. The expression of gap junctional and mitochondrial Cnx 43 can be influenced by several conditions such as hypertension, hypertrophy, hypercholesterolemia, ischemia and heart failure (22). Harmful stimuli such as ischemia induce dephosphorylation and redistribution of Cnx 43 to the cytoplasm or lateral cell borders of cardiomyocytes. Experimental studies have shown that, in the event of MI, this marker dephosphorylates and accumulates in the intercalary disks (23).

Connexin 43 in myocardial infarction

Connexin 43 deregulation seems to critically affect the cell microenvironment in myocardium leading to MI onset and development. Concerning the interactions with other molecules, NADPH oxidase enzymes – increased by the conjugated linoleic acid (cLA) – regulate the le-

vels of reactive oxygen species (ROS). A study group reported a simultaneous decreased Cnx 43 and increased NADPH oxidase (NOX)/ROS expression in isolated left ventricles after cLA-based treatment. This mechanism is potentially involved in a high risk for a sudden arrhythmic death in MI patients and for this reason Cnx 43 demonstrates an important clinical significance (24). Interestingly, another experimental study based on rat-MI models analyzed the role of an active monomer of heart-protecting musk pill, the muscone, in the deregulation of Cnx 43 in the corresponding myocardium epithelia. The authors observe that this factor decreases fibrosis and ventricular inflammation and enhances Cx43 normal expression by preventing inflammasome activation that follows MI in the corresponding laboratory animals (25). Similarly, another study explored the role of another agent – the conductive hydrogel patch – in experimental mouse models and found a positive effect of this material in Cnx 43 by inducing its leading also in a significant MI tissue repair *in vivo* (26). In contrast to these factors, hygrothermal stress – as a result of myocardium heat waves exposure – negatively affects the normal expression and function of Cnx 43. In fact, Cnx 43 downregulation is responsible for the disruption of the intercardiomyocyte gap junctions leading clinically to the onset of malignant arrhythmias (27). These severe ventricular arrhythmias – which are major causes of sudden cardiac death worldwide – are closely related to specific Cnx 43 post-translational modifications that reduce its normal regulation in gap junctions in myocardium (28). Investigating the deregulation of Cnx 43 molecule, a study group analyzed the role of a B-raf proto-oncogene (BRAF) specific mutation (BRAF-V600E); their conclusion was that activation of the mutated BRAF kinase reduces Cnx 43 normal expression in cardiac tissues (29). Additionally, point mutations in another molecule – the casein kinase 1 – also negatively affect the normal Cnx 43 activity. A molecular analysis showed that specific phosphatase-resistant mutations at three casein kinase 1 phosphorylated sites were responsible for the Cnx 43 alterations in cardiomyocytes (30). In contrast to the previous referred negative influences in Cnx 43 normal expression and function, specific novel tyrosine kinase inhibitors released from hydrogels seem to provide a beneficial effect. More specifically,

saracatinib induces Cnx 43 activity leading to an enhanced cardiomyocyte-based interaction (31). Furthermore, the role of Cnx 43 nuclear accumulation in cardiomyocyte/cardio myoblasts is of major importance. A study group reported that translocated Cnx 43 to the nucleus created new intra-nuclear envelope channels improving and accelerating the rhythm of cardio myoblasts biogenesis and cardiomyocyte differentiation (32). In order to evaluate and modify the differences in Cnx 43 expression levels in abnormal myocardial tissues, a variety of modern sophisticated methods have been developed and applied, including electron and super-resolution light microscopy for nanostructures, highly electroactive tissue engineering scaffolds based on nanocellulose (33, 34). Additionally, the combination of topographical, conductive and mechanical stimulation based on a grooved polydimethylsiloxane (PDMS) membrane in a silver nanowires substrate or the use of novel multitissue microdissection techniques are power tools for analyzing immunohistochemically or by immunofluorescence the corresponding Cnx 43 protein expression (35-37).

Concerning the differences of Cnx43 deregulation in stable or unstable CAD, and also in the two main MI types – the acute ST-elevation MI (STEMI) and the unstable angina with normal/minimally elevated cardiac enzymes (NSTEMI) – there are very limited data. Interestingly, a molecular study based on the combined analysis of Cnx 43 and zonula occludens-1 (ZO1) molecule revealed the potential significance of their decreased mRNA levels in the early MI diagnosis in both previous referred MI types and most importantly in the sudden cardiac death event (38).

In conclusion, in the current review study we emphasized the importance of Cnx 43 in CAD/MI development and progression by describing mechanisms of its deregulation in the corresponding tissue substrates. This study includes new data in the field of molecular and targeted therapeutic strategies regarding the protein. Cnx 43 is the most significant molecule in normal myocardial formation and function due to its central regulation of gap junctions in the inter-cardiomyocyte communication. Concerning its pathophysiological significance in MI, progressive loss of its expression leads to functional inactivation in MI cardiac tissues is a negative biochemical event that is correlated with an

advance clinical image in the corresponding patients. Finally, approaching its potential prognostic and therapeutic value in the corresponding patients, enhancement of Cnx 43 expression is a most promising target for novel therapeutic agents and strategies in order to prevent or partially heal the MI traumatic and dysfunctional epithelia. $\square$

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REFERENCES

1. Sun X, Yao B, Shi K, et al. The impact of chest pain center on treatment delay of STEMI patients: a time series study. *BMC Emerg Med* 2021;21:129.
2. Jäger B, Farhan S, Rohla M, et al. Clinical predictors of patient related delay in the VIENNA ST-elevation myocardial infarction network and impact on long-term mortality. *Eur Heart J Acute Cardiovasc Care* 2017;6:254-261.
3. Islas F, Ramchandani B, Cuesta-Corral M, et al. Methodology for the induction of myocardial infarction and cardiac function evaluation. *Methods Cell Biol* 2024;185:151-164.
4. Moldovan R, Ichim VA, Beliş V. Recent perspectives on the early expression immunohistochemical markers in post-mortem recognition of myocardial infarction. *Leg Med (Tokyo)* 2023;64:102293-102301.
5. Chen B, Chang YM. CPR with assisted extracorporeal life support. *Lancet* 2008;372:1879-1880.
6. Thiagarajan RR, Brogan TV, Scheurer MA, et al. Extracorporeal membrane oxygenation to support cardiopulmonary resuscitation in adults. *Ann Thorac Surg* 2009;87:778-785.
7. Valenzuela TD, Roe DJ, Cretin S, et al. Estimating effectiveness of cardiac arrest interventions: a logistic regression survival model. *Circulation* 1997;96:3308-3313.
8. Ibrahim N, Eilenberg W, Neumayer C, Brostjan C. Neutrophil Extracellular Traps in Cardiovascular and Aortic Disease: A Narrative Review on Molecular Mechanisms and Therapeutic Targeting. *Int J Mol Sci* 2024;25:3983-3992.
9. Spearpoint KG, Gruber PC, Brett SJ. Impact of the Immediate Life Support course on the incidence and outcome of in-hospital cardiac arrest calls: an observational study over 6 years. *Resuscitation* 2009;80:638-643.
10. Hosmane VR, Mustafa NG, Reddy VK. Survival and neurologic recovery in patients with ST-elevation myocardial infarction resuscitated from cardiac arrest. *J Am Coll Cardiol* 2009;53:409-415.
11. Suran MC, Siliste C, Vinereanu D. Cardiac Memory – from Theory to Clinical Practice. *Maedica (Bucur)* 2019;14:135-139.
12. Bohiltea RE, Turcan N, Muresian H, et al. Postpartum Aortic Bifurcation Thrombosis on the Background of Thrombophilic Disorder. *Maedica (Bucur)* 2016;11:241-244.
13. Arrivé L, Pichereau C, Monnier-Cholley L, et al. Postmortem coronary CT angiography. *Intensive Care Med* 2016;42:1293-1294.
14. Rotzinger DC, Magnin V, van der Wal AC, et al. Coronary CT angiography for the assessment of atherosclerotic plaque inflammation: postmortem proof of concept with histological validation. *Eur Radiol* 2024;34:1755-1763.
15. Konishi T, Virmani R, Jinnouchi H, et al. Plaque histological characteristics in individuals with sudden coronary death. *Vascul Pharmacol* 2023;153:107240-107251.
16. Ohashi R, Fukazawa R, Shimizu A, et al. M1 macrophage is the predominant phenotype in coronary artery lesions following Kawasaki disease. *Vasc Med* 2019;24:484-492.
17. Biffi WL, Fawley JA, Mohan RC. Diagnosis and Management of Blunt Cardiac Injury: What You Need to Know. *J Trauma Acute Care Surg* 2023;96:685-693.
18. Zhang M, Wang ZZ, Chen NH. Connexin 43 Phosphorylation: Implications in Multiple Diseases. *Molecules* 2023;28:4914-4921.
19. Pun R, Kim MH, North BJ. Role of Connexin 43 phosphorylation on Serine-368 by PKC in cardiac function and disease. *Front Cardiovasc Med* 2022;9:1080131-1080142.
20. Sedovy MW, Leng X, Leaf MR, et al. Connexin 43 across the Vasculature: Gap Junctions and Beyond. *J Vasc Res* 2023;60:101-113.
21. Tsuji Y, Ogata T, Mochizuki K, et al. Myofibroblasts impair myocardial impulse propagation by heterocellular connexin43 gap-junctional coupling through micropores. *Front Physiol* 2024;15:1352911-1352927.
22. Schultz F, Swiatlowska P, Alvarez-Laviada A, et al. Cardiomyocyte-myofibroblast contact dynamism is modulated by connexin-43. *FASEB J* 2019;33:10453-10468.
23. Leybaert L, De Smet MA, Lissani A, et al. Connexin hemichannels as candidate targets for cardioprotective and anti-arrhythmic treatments. *J Clin Invest* 2023;133:e168117-161126.
24. Kelm NQ, Solinger JC, Piell KM, Cole MP. Conjugated Linoleic Acid-Mediated Connexin-43 Remodeling and Sudden Arrhythmic Death in Myocardial Infarction. *Int J Mol Sci* 2023;24:11208-11219.
25. Yang S, Bi Y, Wei Y, et al. Muscone attenuates susceptibility to ventricular arrhythmia by inhibiting NLRP3 inflammasome activation in rats after myocardial infarction. *J Biochem Mol Toxicol* 2023;37:e23458.
26. Mei T, Cao H, Zhang L, et al. 3D Printed Conductive Hydrogel Patch Incorporated with MSC@GO for Efficient Myocardial Infarction Repair. *ACS Biomater Sci Eng* 2024;10:2451-2462.
27. Chi J, Wu N, Li P, et al. Hygrothermal stress increases malignant arrhythmias susceptibility by inhibiting the LKB1-AMPK-Cx43 pathway. *Sci Rep* 2024;14:5010.
28. Yang F, Zhang XL, Liu HH, et al. Post translational modifications of connexin 43 in ventricular arrhythmias after myocardial infarction. *Mol Biol Rep* 2024;51:329.
29. Strash N, DeLuca S, Janer Carattini GL, et al. Time-dependent effects of BRAF-V600E on cell cycling, metabolism,

- and function in engineered myocardium. *Sci Adv* 2024;10:eadh2598.
30. **Al-Attar R, Jargstorf J, Romagnuolo R, et al.** Casein Kinase 1 Phosphomimetic Mutations Negatively Impact Connexin-43 Gap Junctions in Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Biomolecules* 2024;14:61-68.
31. **Zheng L, Shi W, Liu B, et al.** Evaluation of Tyrosine Kinase Inhibitors Loaded Injectable Hydrogels for Improving Connexin43 Gap Junction Intercellular Communication. *ACS Appl Mater Interfaces* 2024;16:1985-1998.
32. **Martins-Marques T, Witschas K, Ribeiro I, et al.** Cx43 can form functional channels at the nuclear envelope and modulate gene expression in cardiac cells. *Open Biol* 2023;13:230258.
33. **Mezache L, Soltisz AM, Johnstone SR, et al.** Vascular Endothelial Barrier Protection Prevents Atrial Fibrillation by Preserving Cardiac Nanostructure. *JACC Clin Electrophysiol* 2023;9:2444-2458.
34. **Sun C, Xie Y, Zhu H, et al.** Highly Electroactive Tissue Engineering Scaffolds Based on Nanocellulose/ Sulfonated Carbon Nanotube Composite Hydrogels for Myocardial Tissue Repair. *Biomacromolecules* 2023;24:5989-5997.
35. **Kim J, Shanmugasundaram A, Lee CB, et al.** Enhanced cardiomyocyte structural and functional anisotropy through synergetic combination of topographical, conductive, and mechanical stimulation. *Lab Chip* 2023;23:4540-4551.
36. **Kovacs S, Racz B, Sotonyi P, Bakos Z.** Morphological and histological investigation of the conduction system in the equine atrial muscle sleeve of pulmonary veins. *Equine Vet J* 2023;1:1-9.
37. **Villgrater HE, Xia R, Sharma Chivukula A, et al.** Microdissection and Immunofluorescence Staining of Myocardial Sleeves in Murine Pulmonary Veins. *J Vis Exp* 2023;201:65836-65842.
38. **Xue Y, Zhao R, Du SH, et al.** Decreased mRNA levels of cardiac Cx43 and ZO1 in sudden cardiac death related to coronary atherosclerosis: a pilot study. *Int J Legal Med* 2016;130:915-922.

