

Mönckeberg's media sclerosis; vascular calcification disorder sui generis

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Abstract

Vascular calcifications (VC) of the intima are primarily related to atherosclerosis and VC of the media are related to a host of disorders classically described by Mönckeberg in the arteries of the extremities, now termed Mönckeberg's media sclerosis (MMS). MMS related VC represent frequent clinical findings in the elderly and in patients with type II diabetes and chronic renal disease. Based on current understanding, MMS related VC result from active biological processes causing precipitation and crystallization of ionic intra- and extra- cellular forms. In this article we review the current state of knowledge about the clinical pathology, molecular biology and diagnostics of MMS, expand on potential mechanisms responsible for poor prognosis in patients with MMS and expose some of the directions for future research in this area.

Key Words

Mönckeberg; media sclerosis; vascular calcifications; vascular molecular biology and genetics; vascular function

Introduction

Vascular calcifications (VC) are of similar composition to bone minerals. In Mönckeberg's media sclerosis (MMS) VC are, in principal, deposits of hydroxyapatite with high degree of crystallization (1). Initially vascular calcifications were thought to be the result of passive degenerative processes, however, recent studies illustrate that vascular calcification is an active process initiated via a variety of molecular signaling pathways (2). While considerable progress elucidating the signaling pathways regulating VC formation has been achieved, the exact molecular basis of VC still remains elusive (3).

With incoming new research data the already large number of molecular mechanisms suggested to contribute to VC formation continues to grow. It appears that while deposition of hydroxyapatite represents the ultimate common pathway of VC in MMS, different initiating and propagator molecular mechanisms as well as different crystalline composition of calcium apatite crystals may be present in other forms of VC (4). For example, it seems likely that vascular calcification processes associated with atheroma formation may be triggered by specific biochemical cascades that are altogether different from the cascades initiated by primary damage to elastic fibers; however both ultimately result in ectopic VC. While experimental conditions may replicate parts of the calcification process in a given model they may not provide the whole picture in even any of these specific conditions. Thus, some of the emerging molecular complexity of VC may be possibly accounted for by differences in experimental designs as well as disregard for specific etiologies of VC types.

We believe that one of the obstacles to the progress of understanding molecular biology of VC can be found in the various differences in experimental designs and tendencies to unite a host of different findings associated with different types of VC under a single umbrella hypothesis. Misappropriation of MMS as a form of atherosclerosis spans more than 150 years of medical research and provides a textbook example of this glaring inaccuracy (see, Appendix A - Historical perspective). Furthermore, because of the paucity of stenotic changes most cases of MMS were, until recently, considered a secondary signature of numerous diseases as opposed to a distinct pathological process of its own. It was only in the early 1980's that the first reports concerning the negative prognostic value of MMS in diabetics and patients with end-stage renal disease were published (5, 6). Nevertheless, the exact mechanisms responsible for poor prognosis of patients with MMS have not yet been fully clarified. To date physicians attribute the gradual stiffening of the large conduit vessels as the cause of poor clinical outcomes (7).

In this article we review the current state of knowledge about the clinical pathology, molecular biology and diagnostics of MMS, expand on potential mechanisms responsible for poor prognosis in patients with MMS and expose some of the directions for future research.

Prevalence and incidence

The prevalence of MMS in the general population is not known. Peripheral Atherosclerotic Occlusive Disease (PAOD) is classified as having an ankle-brachial index (ABI) of <0.9 ; the prevalence of PAOD ranges between 3-5% for persons 50 years old and 15-40% among persons 80 years old. Comparatively, the prevalence of MMS in the general population, based on $ABI >1.3$, has been estimated to be 0.5%; it is more prevalent in men compared to women (3:2) and the highest prevalence of MMS has been observed in type II diabetics ranging between 4-9% (8), depending on age and duration of diabetes (9). In patients with type II diabetes and chronic renal disease MMS has been also recognized as an independent risk factor for cardiovascular events (5-7, 9, 10). Less frequently MMS has been reported in association with a number of other diseases (see, Appendix B). MMS has also been reported in absence of other diseases or any other known risk factors (11), and is frequently associated with aging (12).

Topography

Originally, as described by Mönckeberg, MMS has been located in the muscular arteries of the lower and upper extremities (13). Subsequently, MMS lesions confirmed by histology were also observed in large elastic type arteries (ascending aorta), medium-sized visceral or kidney arteries, small arteries (coronary, temporal, uterine, ovarian, parathyroid, mammary gland and other) with diameter of at least 0.5 mm (14-17). A systemic distribution of MMS appears uncommon (18), yet the true incidence remains unknown.

Histopathology

The VC seen in MMS is clearly distinct from the pathology of atherosclerosis, where VC is primarily localized in the intima. MMS lesions, whether in elastic, transitional or muscular type arteries, appear identical when examined microscopically. The four stages of lesions progression distinguish the extent and severity of MMS. In *stage 1*, calcifications appear on haematoxylin-eosin, (H&E), staining as irregular blue or violet deposits embedded within the media. In the absence of atherosclerosis the intima shows subendothelial hyperplasia. On high resolution light microscopy (40 x – 1000 x magnification) using H&E, Elastica-van-Gieson, von Kossa or Alizarin staining deposits consisting of fine granulations, which increase in size and become confluent with time are revealed (Figure 1a).

Both intra- and extracellular deposits are present. Intracellular deposits are located in vascular smooth muscle cells (VSMC); extracellular deposits are largely associated with damaged and fractured elastic fibers embedded within the extracellular matrix (Figure 1b). In some cases, MMS lesions are also associated with the internal elastic membrane (19). In muscular and transitional arteries, on HE-staining granular calcifications develop alongside the internal elastic membrane (Figure 1c). These bands of calcium-rich deposits may thicken becoming solid plates extending deep into the inner layer of the media. With further progression of the disease calcifications may distort the junctions of the innermost and outermost layers of the media spanning up to three quadrants of the cross-section (*stage 2*, Figure 1d) or it may occur involving the entire circumference (*stage 3*, Figure 1e). In *stages 2 and 3* large conglomerates of calcifications may form solid plates and sheaths progressively distorting the architecture of the media; intrusions upon the intima are then common (20). In *stage 4* of MMS foci of bone formation within the arterial media may be found, calcifications may undergo osseous metaplasia giving rise to true bony trabeculae. These structures delineate true medullary spaces harboring hematopoietic cells interspersed with adipocytes (21); Virchow, Mönckeberg, and other pathologists made these observations over the course of the 19th and early 20th century.

In the arterial wall, calcification deposits associated with MMS may be perceived as foreign bodies and induce granuloma formation; these structures often contain multinucleated giant cells. Other inflammatory components such as foam cells, lymphocytes, and mast cells may be also present, yet in contrast to atherosclerosis they are absent in early stages and they do not represent the key findings. Large calcifications may induce subendothelial hyperplasia characterized by an increase of cellularity (e.g. myofibroblasts, fibroblasts, fibrocytes), ulcerate into the intima or even protrude into the lumen. These particular lesions may become niduses for thrombosis or they may detach and cause peripheral embolism (22). It should be noted that MMS lesions do not regress and the clinical complications may occur according to the site and the amount of calcification. In addition, presence of MMS is not necessarily indicative of an occlusive atherosclerotic peripheral artery disease, yet the co-incidence of both entities particularly in diabetics is common. Anatomically, MMS lesions also involve the microcirculation, e.g. arterioles (10 to 500 μm in diameter). However, neither the extent of MMS lesions in the periphery of the arterial tree or the potential effects of MMS on microcirculation or endothelial function has been sufficiently studied. Calciphylaxis, presence of tissue necrosis involving mostly skin and skeletal muscle in patients with end-stage renal disease due to massive media calcifications of the large and small arteries including arterioles (23), may represent just a snippet of a larger number of more common, less pronounced varieties of VC involving both, macro- and microcirculation.

Biochemistry and Molecular Biology

Since the early description of “endarteritis deformans” by Virchow, medial VC was considered a passive process of calcium phosphate deposition. However, twenty years ago, the pioneering work of Demer’s group (24) first described the expression of an osteoblast-related gene (BMP2, a bone morphogenetic protein) in calcified human atherosclerotic lesions, a finding that was subsequently corroborated using additional osteogenes and calcification models (e.g. 25, 26) as well as in human MMS lesions. These discoveries initiated the view that VC is an active process of ossification, a pathogenic observation that was reinforced with the description of bone forming events, including the production of matrix vesicles and apoptotic bodies (27, 28). In parallel to this pathogenic view, results from several groups supported an alternate hypothesis that calcification is the consequence of the loss of local and/or circulating calcification inhibitors, such as matrix-Gla protein, (MGP), (29), pyrophosphate, fetuin A (30) and osteoprotegerin (31). Animal knockout studies further showed that loss of a single inhibitor could result in extensive medial calcification independent of atherosclerosis. While both pathogenic models are not mutually exclusive (32), a logical consequence of such dichotomy was the interpretation of the expression of bone-related genes as either the cause or the consequence of the calcification/deposition of calcium phosphate (e.g. 33). Clarification of this controversy is still pending, and is a question of major importance in the field of VC because therapies will differ depending on the true mechanism initiating the process of calcification.

Over the last fifteen years, several proposals have tried to explain both, the active bone forming and the loss-of-inhibitor mechanisms of calcification, but so far none has been successful in explaining completely ectopic calcification (for excellent reviews that shows the evolution of theories see 32, 34, 35). This is likely due to the complexity of the initiation events, signalling pathways and mechanisms that lead to VC particularly in MMS that forms over a long period of time. In addition, the use of different *in vitro* and *in vivo* laboratory models, cell culture conditions for growth and calcification/precipitation assays, animal strains and species, as well as experimental confusion between calcification versus mineral precipitation are among the controversies that slow the advance of knowledge on the pathological causes of the disease. The main consequence of this slow progress is the absence of efficacious treatments to prevent and treat this degenerative process.

In recent years, intriguing experimentation from several groups using new VC disease models of chronic kidney disease (CKD)-related hyperphosphatemia is providing findings that strongly support some of the former hypothesis for the pathogenesis of VC. Patients with hyperphosphatemia induced by CKD develop rapid and extensive MMS, in part due to mineral dysregulation stemming from the primary renal disorder. Under these morbid conditions, vasculotoxicity of excess inorganic phosphate relies in part on the formation of nanocrystals with calcium and other ions, which nucleate and deposit in soft tissues, including the vascular wall (36). In the presence of physiological concentrations of

inorganic phosphate (~1 mM Pi), calcification is only observed in specific genetic conditions that cause calcification (see below) and after long periods of exposure, either *in vitro* (33, 37, 38) or *in vivo* as in ageing (39, 40). However, in the presence of pathological hyperphosphatemic conditions calcium phosphate deposition is accelerated and easily observed. This strongly suggests that deposition is actively prevented by the presence and activity of calcification inhibitors, and that calcification is therefore accelerated when the limited capability of calcification inhibitors is overcome (33, 41).

In contrast to bone, calcium phosphate in the arteries is not predominantly deposited on type I collagen but rather on the amorphous elastin that comprises the elastic lamellae (42). This process is accelerated when elastin is degraded by elastase and other proteases (43). Elastin is the main component of the connective tissue, which in combination with VSMCs, comprises the medial layer and spans the vessel wall circumference. While the elastin fibers can calcify both *in vitro* and *in vivo* as in elastocalcinosis (39, 43) the role of the VSMCs and accompanying cells is more complicated. There are at least three key aspects to be considered and elucidated in these cells regarding the pathogenesis of VC: i) the role of transformation into an osteochondrogenic phenotype, ii) the generation of nucleating structures for calcium phosphate deposition, and iii) the endocytosis and toxicity of calcium phosphate nanocrystals. On the one hand, the phenotypic plasticity of the mesenchymal VSMC leads them to *trans*-differentiate *in vitro* into a “synthetic” phenotype, as indicated above, with osteochondrogenic characteristics and the expression of bone-related transcription factors (Cbfa1/Runx2, Msx2), BMP2, alkaline phosphatase, osteopontin, etc., as evidenced *in vitro* and *in vivo* (e.g. 44, 45). The causes, consequences and identity of the specific cells that *trans*-differentiate still need to be elucidated (e.g. 46). Multiple factors that activate these osteogenic pathways have been identified *in vitro*, however the pathways that dominate *in vivo* are not clearly defined. Recent findings that persistent DNA damage signalling that is associated with cellular senescence can activate osteogenic pathways in VSMCs is of significance, as ageing is the most dominant risk factor for the progression of media sclerosis (47, 48). In addition to these senescence associated osteogenic pathways, recent evidence strongly supports the view that osteogene expression is also a consequence of the nucleation of calcium phosphate crystals, as bone-related gene expression can be completely prevented with calcification inhibitors like pyrophosphate or phosphonoformic acid, even in the presence of high concentrations of calcium or phosphate (37, 38). In order to activate osteogenic gene expression, nanoparticles of 30-500 nm need to be endocytosed and accumulated in the lysosomes, where the crystals are then dissolved. Increased calcium concentration in the cytosol can additionally be a cause of apoptosis and necrosis (37, 49).

VSMCs also participate in the nucleation of calcium phosphate crystals through the formation of extracellular vesicles. Classification of these vesicles is a controversial area of research; recent attempts have been made to normalize nomenclature and classification (50). Matrix vesicles (30–300 nm in diameter) and fragmented apoptotic bodies (50 to 5000 nm in diameter) are the most studied

vesicular structures in calcification (51). Matrix vesicles seem to be released by macrophages and VSMCs in calcified atheromas (52), but their role in medial calcification has been studied in less detail (53). Apoptotic bodies released by VSMCs can act as nucleating agents for the deposition of crystals in the aortic wall (27). As shown *in vitro* (28) and *ex vivo* (52) the production of apoptotic bodies and even matrix vesicles is dependent on the concentrations of calcium and phosphate, while normal vesicles contain inhibitors of calcification, especially in the presence of serum and do not initiate calcification (52, 53). Apoptosis of VSMCs is also associated with calcification and medial degeneration (54), and cell death can be caused by internalization of calcium phosphate crystals smaller than 1 μm and accumulation and dissolution in the lysosomes (49). Cell death is not restricted to apoptosis, and necrotic cell debris also has been shown to induce calcification in a mesenchymal *in vitro* model (54).

Recently an additional cellular phenomenon has been described that counteract the pro-calcific effect of apoptosis: autophagy (55). In this process, proteins and organelles are engulfed into membrane vesicles named autophagosomes, which are delivered to lysosomes and degraded and recycled. It is an important mechanism to increase cell survival during stress and nutrient deprivation. Dai and collaborators found that blockade of autophagy increases calcification in response to very high phosphate concentrations, even in the presence of apoptosis inhibitors, while activating autophagy decreases calcification and the release of matrix vesicles. Recent findings suggest that the initiating events and the early pathogenic steps in the various ectopic calcification disorders may be different. Calcifying atherosclerosis, for example, occurs in the absence of hyperphosphataemia, and the strong inflammatory component is sufficient to create the conditions for nucleating calcium phosphates (56). The macrophage infiltrating response during atheroma formation is accompanied by the release of several pro-calcifying cytokines, which favours the osteogenic trans-differentiation of VSMC and/or increases apoptosis of VSMC, macrophages, etc. Matrix vesicles and apoptotic bodies then serve as nucleating sites for the deposition of calcium phosphate crystals. This localized inflammatory response has not been observed in MMS, in particular the macrophage accumulation in areas of medial calcification. During chronic kidney disease (a major cause of MMS), a generalized, non-localized pro-inflammatory status is observed, with overexpression of TNF α , which could activate the osteochondrogenic programme in VSMCs.

Many other general cellular mechanisms have been related to vascular calcification, such as microRNA control of gene expression (57), endoplasmic reticulum stress (58), and activation of the inflammasome (59) etc. In contrast to Virchow's early views, this apparently simple process of ectopic calcification has turned out to be much more complicated and complex than originally expected. Elucidation of the main regulatory mechanisms and differentiating them from secondary phenomena is one of the urgent pending questions that must be addressed. Specifically related to the involvement of

VSMC in VC, we must identify which cell types are involved in the osteochondrogenic *trans*-differentiation and under what conditions; why do some cells respond apoptotically to endocytosis of nanocrystals, while other cells simply modify gene expression? And what are the specific conditions that determine apoptosis, necrosis, and other types of cell death as well as autophagy in the vessel wall?

The success of on-going and future studies will enhance our understanding of these specific questions and add to our knowledge of VC pathogenesis. Figure 2 shows potential molecular pathogenic pathways associated with MMS. The triggering event responsible for precipitation of hydroxyapatite from the metastable supersaturated solution of calcium and phosphate within the environment of the media, i.e. VSMC and matrix, may result from both, presence of toxins, genetic predisposition and/or acquired deficiency in calcification inhibitors.

Molecular Genetics

There are several genetically defined vascular diseases associated with primary vascular media calcification and understanding the mechanisms regulating these diseases may shed light on the genes and mechanism involved in MMS. One monogenetic autosomal recessive disease that seems most closely to resemble the classical description of MMS is Arterial Calcification due to Deficiency of CD73 (ACDC), a rare vascular disease with medial calcifications (60, 61). In retrospect, it appears that ACDC patients were described as having atypical MMS, however, genetic confirmation on these patients cannot be pursued (62-64). ACDC patients develop massive vascular calcification in the medial layer of muscular type arteries primarily located in the lower-extremities (61). These patients have no indication of classic atherosclerosis, impaired kidney function or diabetes. ACDC patients have diminished lower limb perfusion with progressive, intermittent claudication. The ankle-brachial index (ABI) in ACDC patients is extremely low - below 0.3 - which clearly distinguishes it from MMS, where a typical ABI ranges between 1.1 and 2.0. Histological evaluations of affected vessels from ACDC patients demonstrate destruction of the medial wall with circumferential calcifications localized alongside broken elastic fibers within vessel wall. Medial disease progression leads to lumen occlusion due to medial dysplasia. ACDC is caused by loss-of-function mutations in the gene encoding for CD73 protein. This cell membrane bound extracellular enzyme generates adenosine and inorganic phosphate (Pi) from AMP, and is the primary enzyme to generate extracellular adenosine from purine precursors. The role of adenosine in vascular calcification appears intriguing and the discovery of ACDC opens up a new field of research as to the role of adenosine signaling on this disease phenotype. Experimental data suggest that adenosine signaling regulates tissue non-specific alkaline phosphatase (TNAP or ALPL), which is known as a known regulator of bone formation and

modulator of ectopic calcifications. Recently, the role of nucleosides and nucleotides in calcification prevention has been addressed and compared to PPi (65).

Generalized Arterial Calcification in Infants (GACI, also referred to as Idiopathic Infantile Arterial Calcification) is another rare vascular disease with progressive and systemic vascular calcifications that develop *in utero* and during the early postnatal period (66). This fast-progressing vascular calcification disease has a high mortality rate early on in life. Histologically it is characterized by medial calcifications that lead to neointimal formation and vessel occlusion. This systemic vascular proliferative disease causes multi-organ failure due to non-atherosclerosis related myocardial infarction as early as 3 months of age. GACI is an autosomal recessive disease caused by mutations in the gene ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). This cell membrane bound extracellular enzymes generates AMP and pyrophosphate (PPi) from ATP and is therefore directly upstream of CD73, the causal gene for ACDC, in the extracellular purine metabolic pathway. PPi is a potent physiological inhibitor of TNAP and the lack of PPi and subsequent increase in TNAP activity is believed to be the main driver for this pathological malignant form of primary medial calcification.

Familial idiopathic basal ganglia calcification (IBGC, also commonly known as Fahr's disease) is third rare disease with a wide spectrum of neuropathological symptoms that have recently been attributed to the medial calcification of small blood vessel that supply the area of the basal ganglia (67). Genetic evaluations of a subset of IBGC families identified variants in the gene sodium-dependent phosphate transporter 2 (SLC20A2 or PiT-2). PiT-2 is a Na⁺/Pi co-transporter that plays an important role in Pi homeostasis and may be functionally linked to ENPP1 and CD73 in extracellular purine metabolism. Involvement of PDGF signaling has been also recently reported (68).

Overall these three distinct genetic vascular diseases, all with medial calcification as their common primary pathology, highlight the importance of extracellular purine metabolism and downstream adenosine signaling and phosphate homeostasis in the development of vascular media calcifications. The clinical presentation of primary MMS is clearly distinct from the devastating pathologies seen in patients suffering from ACDC, GACI and IBGC, however. It is therefore important to genetically evaluate this disease so that the full spectrum of the genetic causes of primary vascular media calcifications can be identified and understood. This will not only aid in the accurate diagnosis of patients with vascular calcification disorders, but will further the development of treatment strategies targeting vascular media calcifications.

Functional Implications

Arterial compliance (C) and distensibility (C/A) are given by the slope of the non-linear relation between the transmural pressure (p) and the luminal cross-sectional area (A), an expression of the elastodynamic coupling between the blood flow dynamics and vessel wall mechanics. The speed of the pressure wave, which is inversely proportional to the square root of the wall distensibility, can be also computed using the Moens-Korteweg equation (69). As calcium apatite deposits lowers the arterial distensibility, the pressure wave velocity (PWV) rises and causes premature wave reflection, thus impairing the left ventricular function (69). Altered left ventricular (LV) loading conditions result in LV hypertrophy and predispose to LV failure due to diastolic dysfunction while systolic function may remain preserved (70). Pathophysiological consequences of an increased pulsatility are less well understood. In a recent review arterial wall stiffening has been a strong independent predictor of future cardiovascular events and all-cause mortality (71).

Based on the available evidence patients with MMS are likely to suffer from more profound vascular dysfunction of in addition to the frequently reported arterial wall stiffening and associated alterations of the LV loading. However, the precise nature of this vascular dysfunction remains unclear. We suggest three additional pathogenic principles potentially responsible for negative prognostic significance of MMS. First, the ring-like calcifications present in advanced MMS are likely to interfere with positive arterial remodeling in presence of atherosclerosis as described by Glagov et al. (72). In these cases would MMS clearly accelerate the co-incident atherosclerosis and worsen the organ perfusion. Second, stage 4 MMS with secondary invasion of the intima increases the risk of thromboembolic events. Third, MMS arteriolar lesions may be associated with alterations in mechanotransduction resulting in disturbances of autoregulation governing peripheral tissue perfusion. Here, sustained increase in systolic and pulse pressures in patients with MMS shall predictably cause arteriolar constriction, reduction of luminal cross-sectional area, augmented flow resistance and rise in blood pressure. Rise in blood pressure exceeding the upper limits of autoregulation, i.e. maximum vasoconstriction, would then increase, and while blood pressure falling below the lower limit of autoregulation, such as seen in patients with developing heart failure, would decrease peripheral tissue perfusion reflected by reciprocal changes in blood flow velocity in conduit vessels. To determine the true clinical relevance of MMS in addition to the measurements of PWV (73) also concurrent measurements of microcirculatory and endothelial functions employing for example the digital pulse volume measurement technology (74, 75) in clinical settings are needed. Furthermore, serial measurements of arterial dimensions in patients with MMS associated with POAD and/or diabetic macrovasculopathy are critical to determine the effects of MMS on compensatory positive arterial remodeling. It is one of the objectives of this review to stimulate such clinical research.

Diagnostic evaluations

MMS is suggested by measuring the ankle-brachial index; MMS is diagnosed with an ABI ≥ 1.1 . ABI readings of 1.1-1.3, 1.3-1.5 and >1.5 has been proposed to denote an early, intermediate and late MMS, respectively (76). In patients with suggested MMS (ABI ≥ 1.1) the toe-brachial index (TBI) has been proposed to improve the specificity of segmental blood pressure measurements (77). However, questions have been raised about the validity of this approach and the role of TBI in diagnostics of MMS remains uncertain (78). Nevertheless, despite limitations ABI remains the most important screening tool to suggest the presence of both, POAD and MMS (79).

In clinical settings MMS is frequently identified accidentally from x-ray studies. In native x-ray images e.g. of lower extremities MMS lesions are visualized as symmetric narrowly-spaced, finely granular radiopaque rings often spanning the full diameter and the entire length of the artery. Similarity with railroad tracks was noted by Mönckeberg citing two other sources (13) and is also referred to in a more recent literature (80). With progressing disease the granulations become coarser and less regular.

Figure 3a & 3b shows typical radiographic appearance of MMS lesions on native and angiographic x-ray images. Figure 3c & 3d shows the progression of MMS after a 16 years follow-up in the same patient.

Vascular ultrasound imaging allows clear differentiation of MMS and the atherosclerosis related lesions. In B-mode ultrasound images in patients with MMS distinct echogenic granular pattern located in the abluminal layers of the arterial walls and intact endothelial interfaces are seen. Depending on the stage of the disease MMS lesions can be small dots or larger confluent zones of echogenicity eventually spanning the entire vessel circumference on cross-sectional images (Figure 4a). Visualization of MMS lesions on ultrasound can be optimized by focusing on the image layer containing the lesions (media) and employing Gaussian filtering at 6MHz (81). Employing the color flow duplex ultrasound both MMS calcifications and associated flow patterns can be visualized (Figure 4b). In patients with MMS undergoing endovascular interventions MMS-related lesions may be easily visualized employing intravascular ultrasound (IVUS) or optical coherence tomography (OCT). . On IVUS MMS lesions are seen as highly echogenic zones located within the media. Due to the presence of fibrotic tissue typically no acoustic “shadowing” is seen (Figure 5a). In contrast, in calcifications of the intima as seen in atherosclerosis acoustic “shadowing” is frequent. Compared to IVUS OCT provides higher resolution und substantially better visualization of the innermost layers of arterial walls. MMS lesions are distinctly visualized within the tunica media (Figure 5b).

While imaging studies employing conventional x-ray or ultrasound allow unequivocal definition of MMS, laboratory chemistries are non-specific; they are employed to assess the presence of associated diseases such as diabetes, chronic renal and endocrine disorders, and to assess the metabolic risk of atherosclerosis (Table 1). To date, there is no known biomarker for MMS or other vascular calcification disorders.

Clinical Management

In absence of causal therapy directed at prevention or treatment of MMS- associated lesions at present the therapeutic measures are directed at the co-existent diseases such as diabetes type II, chronic renal disease and atherosclerosis. Metabolic and hormonal control of diseases associated with altered handling of body calcium and phosphates appears critical to control the disease process.

Perspectives

To fully understand the pathophysiology, clinical significance and therapy for MMS patients, we propose the following steps:

First: standard nomenclature of VC is needed. Cases of VC with **unequivocally documented calcifications of the media** should be separated from intimal forms. In this group, patients with identifiable risk factors (e.g. diabetes, chronic renal disease) and those without any such known risk factors (e.g. primary MMS) should be distinguished. Protocols to evaluate human vascular tissues (e.g. atherectomy specimen) from both groups of patients implementing genetic and molecular technology should be developed.

Second: morphological studies should be conducted to determine the systemic incidence of MMS in different vascular beds, including microcirculation.

Third: determine the clinical impact of MMS on cardiovascular health. Comprehensive evaluations and serial measurements of arterial dimensions, arterial wall stiffness and peripheral tissue perfusion in affected patients belonging to different subsets are required.

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Figure legends

Figure 1a: MMS *stage 1*. Deposits consisting of fine granulations, which increase in size and become confluent with time are shown (H & E-stain, light microscope, x 200).

Figure 1b: Ultrastructure of calcified deposits. Ultrastructurally, calcified deposits are largely associated with damaged and fractured elastic fibers embedded within the extracellular matrix (Electron microscopy, x 50000, figure with permission of Perfusion, Janzen et al. 2006).

Figure 1c: MMS *stage 1*. Granular calcifications alongside the internal elastic membrane giving rise to a blue band are demonstrated. (Differential eosin fluorescence pattern, laser scanning microscope, x 400).

Figure 1d: MMS *stage 2*. Medial calcifications may distort the junctions of the innermost and outermost layers spanning up to three quadrants of the cross-section and consisting of calcified plates. (H & E-stain, light microscope, x 40).

Figure 1e: MMS *stage 3*. Calcified plates involving the entire arterial circumference are demonstrated. (H & E-stain, light microscope, x 40).

Figure 2 Pathogenesis of MMS. Shown are molecular pathways potentially associated with initiation and propagation of MMS.

Figure 3a, b. X-ray image of a femoral bifurcation with typical appearance of MMS of the superficial femoral (SFA) and deep femoral arteries on native x-ray (left) and selective needle angiogram of the same bifurcation (right). Typical appearance of Mönckeberg's media sclerosis showing "railroad trucks" pattern on native x-ray images can be appreciated.

Figure 3c, d. Progression of MMS over a period of 16 years. Shown are native x-ray images of the left upper thigh of a 46 years old male (left) and of the same male on 16 years follow-up. Coarser and less regular pattern of MMS calcifications can be appreciated.

Figure 4a. Shown are ultrasound images of patients with MMS without (upper panels and left lower panel) and with (right lower panel) atherosclerosis. Granular pattern of calcification can be clearly seen on longitudinal and cross-sectional (insert) views (left upper panel). Formation of solid calcific plates (upper right panel) and irregular continuous calcifications (left lower panel) are also demonstrated. Concurrent presence of MMS lesions (abluminal echogeneities) and atherosclerotic lesions (intraluminal echogeneities) is also visualized (right lower panel) (courtesy C. Garn).

Figure 4b. Shown are ultrasound images of a patient with MMS employing B-mode and color Doppler mode techniques. Medial calcifications (both images) and unperturbed intraluminal blood flow are clearly seen (right).

Figure 5a. Intravascular cross-sectional ultrasound image of the proximal superficial femoral artery in a patient with MMS is shown. Dense tunica media calcification (arrows), absence of acoustic shadowing and freedom from intimal atherosclerotic disease should be noted (Eagle Eye catheter, Volcano, 20MHz, lateral resolution 200-250 μ , axial resolution 80-100 μ).

Figure 5b. Optical coherence tomography cross-sectional image of the proximal superficial femoral artery with circumferential media-calcinosis (white arrows) interrupted by a bigger side-branch located at 3 o'clock. The yellow arrows mark intraluminal prolapsing fibrotic plaque. § = OCT catheter, * 0.0.14 inch guidewire (Akquise, St. Jude Medical, C7 Dragonfly catheter, lateral resolution 20 μ , axial resolution 10 μ).

Appendix

A. Historical perspective

Diseases of arterial walls associated with focal calcifications have been noted by pathologists for centuries. However, the first systematic review can likely be attributed to Johann Friedrich Lobstein. In the second volume of his textbook of general and special pathology he devoted 56 pages to the diseases involving arterial walls and termed arterial diseases associated with thickening and hardening of arteries arteriosclerosis (Lobstein JCCFM. Traite d'Anatomie pathologique. 2 volumes. Paris:F-G Levault, 1829-1833; Lobstein JCCFM. Lehrbuch der pathologischen Anatomie. Band 1 und 2. Stuttgart:F. Brodhag, 1834-1835). Roughly half a century later and for similar group of arterial diseases Felix Marchand has coined the term atherosclerosis (Marchand F. Ueber Arteriosklerose. Verhandlungen des Congresses für Innere Medicin. Einundzwanzigster (21.) Congress. Gehalten zu Leipzig, vom 18. - 21. April 1904. Mit 11 Tafeln und 15 Textabbildungen. (Beiträge von/über: den Wert der Blutdruckmessung für die Behandlung der Ateriosklerose, Anatomische Befunde am Circulations-Apparate, speziell den Arterien bei Typhus abdominalis, Zur Bestimmung der Leistungsfähigkeit des gesunden und kranken Herzens durch Muskelarbeit, u.a.). Wiesbaden: JF Bergmann, 1904;21:23-59).

In his treatises, Virchow noted the difference between calcifications of the arterial intima and media, and stated that calcifications of the intima appeared of inflammatory origin while those of media did not and pointed out similarities between calcifications of arteries and ossifications of the bone in these calcification (Virchow R. Cellularpathologie; in ihrer Begründung auf physiologische und pathologische Gewebelehre. 4 Auflage, Berlin: A. Hirschwald, 1871; p. 452-453). In 1903 Mönckeberg conducted at the behest of his teacher, Eugen Fränkel study of medial vascular calcifications and confirmed the distinction between the calcific diseases of the media and intima (Mönckeberg JG. Über die reine Mediaverkalkung der Extremitätenarterien und ihr Verhalten zur Arteriosklerose. Virchows Archiv für pathologische Anatomie und Physiologie, und für klinische Medicin, Berlin, 1903, 171: 141-167). Based on macroscopic examinations of arteries of the lower and upper extremities from 130 cases with additional light microscopic evaluation in 86 of these cases Mönckeberg found calcification of the tunica media with no evidence of atherosclerosis in 55 cases (age 35-85 years; 39 males, 16 females). On hematoxylin-eosin staining, H&E, Mönckeberg found granular calcium deposits in the media; in more advanced stages calcific buckles, plates and rings were found corresponding to the macroscopic appearance and roentgenographic images of the *Gänsetrachea* (windpipe of the goose). Depending of the amount and degree of calcifications of the tunica media Mönckeberg observed secondary stretching, bulging and perforations of the internal and external elastic laminae along with destruction of smooth muscle layer. In most advanced cases the arteries have been transformed into rigid pipes. Interestingly, in four cases, all males, with isolated calcifications of the media the affected individual were younger than 50 years old with two of the men

being less than 40 years old. Overall, calcifications of the tunica media were most frequent in femoral arteries (42 cases).

Subsequently, vascular calcifications primarily located in the arterial tunica media were termed Mönckeberg' Media Sclerosis (MMS), Mönckeberg's later attempted to defend and to justify the nosologic distinctness of the intima- and media- related vascular calcification (Mönckeberg JG. Mediaverkalkung und Atherosklerose. Virch Arch Pathol Anat Physiol klin Med 1914;216:408-416) have remained a matter of debate until present (Micheletti RG, Fishbein GA, Currier JS, Fishbein MC. Mönckeberg sclerosis revisited; a clarification of the histologic definition of Mönckeberg's sclerosis. Arch Pathol Lab Med 2008;132:43-47). In consequence the nomenclature of calcific arterial disorders remains unsettled.

B. Diseases reported to be associated with Mönckeberg's media sclerosis

Diseases other than diabetes mellitus and chronic renal disease associated with Media Sclerosis Mönckeberg have been summarized in the table.

Diabetes mellitus type II	Edmonds ME, Morrison N, Laws JW, Watkins PJ. Medial arterial calcification and diabetic neuropathy. Br Med J Clin Res Ed 1982;284:928-30.
Chronic renal disease	Chen NX, Moe SM. Uremic vascular calcification. J Invest Med 2006;54:380-384.
Osteoporosis	Rubin MR, Silverberg SJ. Vascular Calcification and Osteoporosis—The Nature of the Nexus. J Clin Endocrinol Metab 2004, 89:4243–4245.
Hyperparathyroidism	Terai K, Nara H, Takahura K, Mizukami K, Sanagi M, Fukushima S, Fujimori A, Itoh H, Okada M. Vascular calcification and secondary hyperparathyroidism of severe chronic kidney disease and its relation to serum phosphate and calcium levels. British J Pharmacol 2009;156:1267–1278.
Vitamin D hyper- and hypovitaminoses	Drüeke TB, Massy ZA. Role of vitamin D in vascular calcification: bad guy or good guy? Nephrol Dial Transplant 2012;27:1704-1707; Malick NP, Berlyne GM. Arterial calcification after vitamin-D therapy in hyperphosphatemic renal failure. Lancet 1968;ii:1316-

	1319
Autonomic neuropathy	Goebel FD, Fuessl HS. Monckeberg's sclerosis after sympathetic denervation in diabetic and non-diabetic patients. <i>Diabetologia</i> 1983;24:347-350
Ehlers-Danlos syndrome	McKusick VA. Heritable disorders of connective tissue. St. Louis: CV Mosby, 1972.
Pseudoxanthoma elasticum	Lebwohl M, Halperin M, Phelps RG. Occult pseudoxanthoma elasticum in patients with premature cardiovascular disease. <i>N Engl J Med</i> 1993;329:1237-1239.
Systemic sclerosis	Son CN, Jung KH, Song SY, Jun JB. Monckeberg's sclerosis in a patient with systemic sclerosis. <i>Rheumatol Int</i> 2009;30:105-107.
β -Thalassemia	Aessopos A, Samarkos M, Voskaridou E, Papaioannou D, Kavoukis E, Vasopoulos G, Stamatelos G, Loukopoulos D. Arterial calcifications in beta-thalassemia. <i>Angiology</i> 1998;49:137-143.
Kawasaki-disease	Ino T, Shimazaki S, Akimoto K, Park I, Nishimoto K, Yabuta K, Tanaka A. Coronary artery calcifications in Kawasaki disease. <i>Pediatr Radiol</i> 1990;20:520-523.
Singelton-Merten-Syndrom	Singleton EB, Merten DF. An unusual syndrome of widened medullary cavities of the metacarpals and phalanges, aortic calcification and abnormal dentition. <i>Pediatr Radiol</i> 1973;1:2-7
Generalized arterical calcification of infants	Maayan C, Pele O, Eyal F, Mogle P, Rosenman E, Ziv JB. Idiopathic infantile arterial calcification: a case report and review of the literature. <i>Eur J Pediatr</i> 1984;142:211-215.
Arterial Calcification due to Deficiency of CD73	St Hilaire C, Ziegler SG, Markello TC, Brusco A, Groden C, Gill F, Carlson-Donohoe H, Lederman RJ, Chen MY, Yang D, Siegenthaler MP, Arduino C, Mancini C, Freudenthal B, Stanescu HC, Zdebik AA, Chaganti RK, Nussbaum RL, Kleta R, Gahl WA, Boehm M. Nt5e mutations and arterial calcifications. <i>N Engl J Med</i> 2011;364:432-442
Idiopathic basal ganglia calcification	Wang C, Li Y, Shi L, Ren J, Patti M, Wang T, de Oliveira JR, Sobrido MJ, Quintans B, Baquero M, Cui X, Zhang XY, Wang L, Xu H, Wang J, Yao J, Dai X, Liu J, Zhang L, Ma H, Gao Y, Ma X, Feng S, Liu M, Wang QK, Forster IC, Zhang X, Liu JY. Mutations in slc20a2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. <i>Nat Genet.</i> 2012;44:254-256

Rheumatoid arthritis treated with cortisone	Amos RS, Wright V. Mönckeberg's arteriosclerosis and metabolic bone disease. Lancet 1980;ii:248-249.
Elderly and aged persons	Johnson RC, Leopold JA, Loscalzo J. Pathobiological mechanisms and clinical implications. Circ Res 2006;99:1044-1059

Tables

Table 1. Conditions associated with Mönckeberg's media sclerosis.

Condition	Laboratory examination	Comments
Atherosclerosis	Lipoprotein and Glucose metabolism analysis	Screening (Duplex carotis, ABI), polyvascular disease?
Diabetes	Glucose (fasting, profile) HbA1c	Endorgan involvement?
Renal disease	Creatinine, Glomerular filtration rate, Urea, Phosphate, Calcium	Osteoporosis?
Parathormon related disorders	Calcitriol (1,25[OH]2D3, intact parathormon (iPTH)	Thyroid disease, hypophyseal disorders
β -thalasemia	Hb, Ferritin, Hb-electrophoresis	
Vitamin D3 related disorders	Calcitriol (1,25[OH]2D3	
Kawasaki disease	TNF α , IL-1, IL-6, B/T lymphocytes	childhood
Administration of corticosteroids or cortison related disorders	Cortisone	Rheumatic diseases?
Pseudoxanthoma elasticum	Skin biopsy, genetic testing for ABCC6, recessive	Adolescence, habitus, Clinical overlap with GACI
Arterial Calcification due to Deficiency of CD73	Genetic testing for CD73 variants, recessive	Early adulthood, decreased ABI, decreased limb perfusion
Idiopathic basal ganglia calcification	Genetic testing for SLC20A2, PDGF-B	Cerebral vascular calcifications, neurological defects
Singleton-Mertens syndrome		Childhood, adolescence, habitus, teeth dyplasias
Idiopathic arterial calcification	Genetical testing	Newborn, early childhood
Generalized arterical	Genetical testing for ENPP1	Newborn, early childhood,

calcification of infants	variants, recessive	clinical overlap with PXE
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*TNF – tumor necrosis factor, IL- interleukin