FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2014/2015

Tiago João Martins Oliveira

Calciphylaxis:

from the disease to the diseased

março, 2015

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Tiago João Martins Oliveira

Calciphylaxis:
from the disease to the diseased

Mestrado Integrado em Medicina

Área: Nefrologia

Trabalho efetuado sob a Orientação de:

Doutor João Miguel Machado Dória Frazão

Elaborado segundo as normas de Publicação da revista

"Journal of Nephrology"

março, 2015

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Tiago João Martins Oliveira, abaixo assinado, nº mecanográfico 200900121, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 16/03/2015

Assinatura conforme cartão de identificação:

Tiago João Martins Oliveira

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Tiago João Martins Oliveira

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)	E-MAIL	TELEFONE OU TELEMÓVEL
13914125	tiagojoao92@gmail.com	915084345

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
200900121	16/03/2015

DESIGNAÇÃO DA ÁREA DO PROJECTO

Nefrologia

TÍTULO ~~DISSERTAÇÃO~~/MONOGRAFIA (riscar o que não interessa)

Calciphylaxis: from the disease to the diseased

ORIENTADOR

Doutor João Miguel Machado Dória Frazão

COORIENTADOR (se aplicável)

_____

É autorizada a reprodução integral desta ~~Dissertação~~/Monografia (riscar o que não interessa) para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 16/03/2015

Assinatura conforme cartão de identificação: Tiago João Martins Oliveira

Title:

Calciphylaxis: from the disease to the diseased

Authors:

Tiago M. Oliveira[1], João M. Frazão[1,2]

[1] School of Medicine of Porto University, Porto, Portugal

[2] Nephrology Department, Centro Hospitalar de São João, EPE, Porto, Portugal
and
Nephrology and Infectiology Research and Development Group, INEB, Portugal

Correspondence Address:

João M. Frazão, Serviço de Nefrologia, Faculdade de Medicina da Universidade do Porto, Al. Prof. Hernâni Monteiro, 4200-451 Porto, Portugal.

Phone: +351 919568100

Fax: +351 225512228

Email: jmmdfrazao@netcabo.pt

Conflict of interest:

The authors declare that they have no conflict of interest.

Abstract

Calciphylaxis, or calcific uremic arteriolopathy, is a vascular ossification-calcification disease involving cutaneous or visceral arterioles, with ischemic damage of the surrounding tissues, usually in the setting of chronic kidney disease. Pathogenesis is still unclear and probably comprises the participation of vascular smooth muscle cells, endothelial cells and macrophages surrounded by a uremic and/or pro-calcifying environment. According to the original concept of calcific uremic arteriolopathy coined by Hans Selye, risk factors may be divided into sensitizers and challengers and their knowledge is useful in clinical practice to pre-emptively identify both uremic and non-uremic 'at risk' patients and guide treatment. Systemic calcific uremic arteriolopathy is a rarity. Cutaneous calcific uremic arteriolopathy is more frequent and clinically presents as a first phase of cutaneous hardening and erythema, followed by a second phase of ulcerations and scars; these two phases are probably associated with the initial development of arteriolar lesion and tissue ischemic damage, respectively. Clinical history, physical examination, laboratory, histology and imaging are the main tools to exclude important differential diagnoses and obtain a definitive diagnosis. Treatment is generally unrewarding and consists of rigorous control of comorbid conditions, anti-oxidant, anti-inflammatory and antithrombotic strategies, avoidance of iatrogeny and wound and pain management. Prognosis remains poor in terms of morbidity and mortality. Efforts should be made towards a greater awareness of calcific uremic arteriolopathy, development of better therapies and improvement of clinical outcomes.

Keywords calcific uremic arteriolopathy · calciphylaxis · chronic kidney disease – mineral and bone disorders · vascular ossification-calcification · soft tissue calcification

Introduction

Chronic kidney disease (CKD) comprises a variety of homeostatic imbalances arising from a severely compromised renal function, as well as the influence of cardiometabolic comorbidities. Uremic syndrome is an umbrella term for the clinical consequences of these phenomena throughout the body. In particular, dysregulation of calcium (Ca) and phosphate (P) metabolism plays a central role in the development of

changes in bone (renal osteodystrophy) and extraosseous calcification involving vessels, extraosseous soft tissues and other structures [1].

Calcification of the vascular tree, also known as vascular ossification-calcification, is a highly prevalent complication of CKD. It is a result of multifactorial, coordinated and active osteochondrogenic processes occurring in the vessels. Chronic injurious stimuli in certain chronic diseases, namely CKD, produce an environment of metabolic toxicity in the vasculature favourable to mineralization [2, 3].

Calciphylaxis, or calcific uremic arteriolopathy (CUA), is a systemic pathology of extraosseous calcification. Usually, but not always [4], it occurs in the context of CKD and affects dermal, subcutaneous and/or visceral arterioles and soft tissues, according to histopathologic and functional studies [5, 6]. Some authors view CUA as a pure small-artery disease which should be clearly differentiated from soft tissue calcification [7], but CUA is also regarded as one of the five subtypes of *calcinosis cutis* [8].

As a middle ground between these concepts, CUA may be perceived as a vascular ossification-calcification disease with other associated phenomena in the arterioles and surrounding tissues, leading to significant ischemic damage and other dermatologic, soft tissue and/or visceral manifestations [6].

This review aims to describe the pathway from the pathophysiologic roots of the disease to the strategies used in the diagnosis and treatment of the CUA patient.

Methods

Searches were performed in the electronic PubMed database with combinations of the following words: “calciphylaxis”, “calcific uremic arteriolopathy”, “renal osteodystrophy”, “vascular calcification”, “visceral calciphylaxis” and “systemic calciphylaxis”. Related articles mentioned in the reference sections of some of the retrieved articles were also considered. Articles not written in English or without accessible full texts were excluded.

A multi-layered pathogenesis

Active vascular ossification-calcification processes in the tunica media of the arterioles are the anchor of the development of CUA [9-11]. However, they are not enough to explain the whole clinical picture, correctly establish the diagnosis or justify some of the therapeutic strategies currently used [12].

Neither a step-by-step pathogenic pathway nor the relative weight of all the factors involved are known. It is thought that CUA is a multifactorial disease ensuing from an intricate interaction of events happening in all the arteriolar layers, arteriolar lumen and surrounding skin, soft tissues, and/or visceral parenchyma. This leads to medial calcification without associated intimal calcification or vasculitic changes and one or more of the following alterations: extracellular matrix (ECM) remodelling, subintimal fibrosis, thrombus formation and tissue inflammation, calcification, ischemia and necrosis [3, 5, 13].

Figure 1 outlines some possible pathophysiological mechanisms of CUA. The main cellular protagonists are vascular smooth muscle cells (VSMCs) of the arteriolar media and endothelial cells of the arteriolar intima. Macrophages are the main cellular expression of a prominent inflammatory response observed in CUA [13]. All these cells are part of a very specific environment along serum and ECM.

Serum and ECM set the ground for CUA

Serum is the vehicle of major systemic factors strongly associated with CUA, making ECM and cellular events happen, as described below. It reflects unique characteristics and comorbidities of the CUA patient (described in “Clinical aspects”).

The balance between pro- and anti-calcification factors in the serum is particularly critical in vascular ossification-calcification diseases [14], including CUA. Raised serum Ca-P product (CaXP) and the protein fetuin-A promote and inhibit vascular ossification-calcification, respectively. In general, CaXP is increased and fetuin-A is decreased in CUA; however, in a subset of patients, CUA may occur without these changes [13, 15].

Raised CaXP may be a result of increased serum levels of Ca, P or both. It is a marker of impaired Ca and P metabolism in CKD, as well as an independent risk factor of extraskeletal calcification, strongly associated with increased mortality [16]. High CaXP was thought to trigger passive precipitation of calcium-phosphate crystals in the vessel wall [17]. However, more recent studies refute this simplistic view,

showing that, instead of eliciting a passive metastatic calcification process, raised Ca and P levels have an active role in changing VSMCs phenotype [15], as detailed later.

Fetuin-A (α-Heremans Schmid glycoprotein, AHSG) is a circulating inhibitor of vascular ossification-calcification synthesized in the liver. Due to its high affinity to hydroxyapatite, it tends to accumulate in calcification sites. Its transient effects are mediated by "calciprotein particles", which clear circulating Ca and P and therefore selectively inhibit vascular ossification-calcification without interfering in bone mineralization [18]. Fetuin-A is also a TGF-β antagonist, acting at a cellular level and interacting with inflammatory mediators involved in osteogenesis. As a negative acute-phase reactant, fetuin-A was shown to be downregulated in inflammatory states, CKD and CUA patients, which is favourable to vascular ossification-calcification [13, 19, 20].

Besides serum, ECM also makes a contribution towards a pro-calcifying milieu. ECM remodelling is now seen as an early event of CUA: according to recent autopsy studies of skin lesions of CUA, there is a marked upregulation of several ECM proteins indicating extensive matrix remodelling in the subcutis [21]. These proteins, such as osteopontin, collagen I, fibronectin and laminin, create a pro-calcifying ECM environment as they are involved in biomineralization [5]. Another critical ECM protein in CUA is the matrix GLA protein (MGP), which may be seen as the ECM equivalent of fetuin-A.

MGP is a constitutive ECM protein that inhibits vascular ossification-calcification. Like fetuin-A, it is particularly present where vascular ossification-calcification happens, and it is synthesised in VSMCs. Active MGP forms complexes with fetuin-A and inhibits BMP-2-induced osteogenic differentiation [18, 22]. γ-Carboxylation is a vitamin K-dependent enzymatic reaction necessary for MGP activity [23]. Thus, vitamin K deficiency leads to increased uncarboxylated (inactive) MGP in calcification sites, with a corresponding decrease in its circulating fraction. According to ELISA-based studies, inactive MGP is systematically depleted in the serum of CUA patients [24].

VSMCs actually make calcification happen

Arteriolar biomineralization basically consists of crystal nucleation and propagation with hydroxyapatite deposition in the arteriolar media [3, 5] and is actively performed by VSMCs. VSMCs are pluripotent [9] and may undergo transdifferentiation from a contractile to an osteochondrocytic phenotype, necrosis or apoptosis [25]. These different cellular transformations can promote medial calcification in dif-

ferent ways. Recently, numerous molecules and signalling pathways have been studied in the VSMCs of CUA and CKD patients [15, 26, 27]; their extensive analysis goes beyond this review.

VSMCs shift into an osteochondrocytic phenotype makes them capable of active osteogenesis. This transformation is mainly attributed to inorganic phosphate and presumably does not happen in its absence [28]. In addition, uremic toxins, reactive oxygen species, proinflammatory cytokines, hypercalcemia, BMP-2 upregulation in endothelial cells and decreased levels of constitutive calcification inhibiting proteins are thought to play a concurrent role by themselves or through facilitation of phosphate action [9, 13]. Phosphate enters VSMCs via Pit-1 (NaPOC), a sodium-dependent phosphate cotransporter. Through this protein, elevated serum phosphate levels translate into increased cytosolic phosphate, which in turn upregulates the transcription factor Runx2 (Cbfa-1), promoting differentiation of VSMCs into a secretory phenotype. A prominent feature of these cells is the ability to release mineralization-competent matrix vesicles [29].

Thus, the secretory phenotype corresponds to a proper adaptation of VSMCs to a high phosphate environment. VSMCs that maintain their contractile phenotype are said not to be adapted.

Besides phosphate, systemic hypercalcemia and a pro-calcifying local and systemic milieu seem to explain further changes in both adapted and non-adapted VSMCs [30].

As a result of these stimuli, secretory (adapted) VSMCs undergo osteochondrocytic differentiation, with increased osteopontin and decreased α-actin expression [9, 31]. Consequently, production and release of matrix vesicles is increased and nanocrystals are endocyted and released into the pro-calcific ECM. Release of matrix vesicles is a protective mechanism by preventing intracellular calcium overload, but induces ECM calcification [3]. Finally, uptake of nanocrystals by VSMCs induces their senescence and apoptosis and triggers new cycles of osteogenic differentiation via BMP-2 and osteopontin upregulation [30, 32].

On the contrary, contractile (non-adapted) VSMCs cannot defend themselves from calcium overload, which, in the context of high phosphate, triggers their necrosis. Apoptotic and necrotic VSMCs are thought to be a nidus for calcification, enhancing the whole process [25].

It should be reemphasized that vascular ossification-calcification, albeit necessary, is not enough to cause tissue necrosis [12]. Along with medial calcification, VSMCs may promote arteriolar stenosis and occlusion through mechanisms interconnected with endothelial cells: intimal hyperplasia and sloughing of vascular cells.

Intimal hyperplasia is the universal healing response of the vessel wall to insults. Endothelial cells dysfunction and injury trigger an inflammatory reaction with release of mediators inducing VSMCs migration to the intima, proliferation and synthesis of ECM. The consequence of this process is the formation of a permanently thickened neointima, causing arteriolar stenosis [13].

Sloughing of vascular cells (VSMCs and endothelial cells) into the vessel lumen may be favoured by increased osteopontin expression by VSMCs [31] and results in non-thrombotic arteriolar occlusion.

Endothelial cells add up to the problem

The effect of oscillatory shear stress, inflammatory cytokines and reactive oxygen species (ROS) cause BMP-2 upregulation in endothelial cells inducing VSMCs' osteochondrogenic transdifferentiation [5].

Endothelial cells contribute to arteriolar stenosis and occlusion through dysfunction, injury, necrosis and hyperplasia.

Endothelial dysfunction consists of complex changes in endothelial cells biology and occurs in a broader context of chronic cardiometabolic comorbidities and inflammation. The synthesis of the vasodilator nitric oxide is decreased, which causes vasoconstriction; there is also a preferential synthesis of procoagulant molecules [15]. Increased ROS formation potentiates nitric oxide depletion and leads to injury of endothelial cells [9].

Injury of endothelial cells arises from endothelial dysfunction and vascular ossification-calcification. Injured endothelial cells eventually suffer necrosis and slough into the vascular lumen, promoting subintimal fibrosis, non-thrombotic occlusion and thrombogenesis. Histologic studies found destruction of the endothelial layer, detached endoluminal CD31+ endothelial cells and signs of total occlusion in subcutaneous arterioles of CUA patients [5].

Hyperplasia of endothelial cells (not to be confounded with intimal hyperplasia) is an associated histological finding of CUA [13] which might cause further arteriolar stenosis. BMP-4 may be one of the culprits of this proliferative activity of endothelial cells [5].

Surrounding tissues may also calcify: the Selye's CUA

Simultaneously to medial arteriolar calcification, there may be calcified deposits in the extravascular structures, which in general is associated with more severe CUA [8].

One of the possible explanations for the presence of ectopic calcium deposits involves the adipocytes. Altered adipocyte biology is one of the hallmarks of chronic inflammation with increased production of adipocytokines, boosting local and systemic inflammation [13].

Inflammation and associated necrosis of subcutaneous adipose tissue (panniculitis) are thought to disseminate calcium deposition [5], producing deposits in adipose lobules, interadipocyte spaces and subcutaneous septa.

Rare cases of CUA have been described where the most prominent pathologic features are soft tissue calcification and necrosis. These patients belong to an extreme of the spectrum of CUA termed "tissue calciphylaxis" or "calcifying panniculitis". Another interesting fact about these cases is the well-defined cause-effect relationship between specific precipitating factors, such as injection of iron-dextran or calcium heparinate, and CUA [33, 34]. This way of inducing the disease is strikingly similar to the concept of calciphylaxis first introduced by Hans Selye, who performed experiments in animals exposed to a set of sensitization and challenging factors [11, 35, 36].

Despite the differences between experimental and human CUA, Selye's focus on sensitizing and challenging factors may be useful to understand some links between the disease and the complexity of the CUA patient.

Clinical aspects

Risk factors: seeing the patient as a whole

According to the original definition of CUA as a condition of anaphylactic hypersensitivity, this disease would result from the exposure of a previously sensitized ("allergic") patient to external challengers ("allergens") [35, 37]. Even though human CUA is not currently regarded as an allergic disease, this

model remains useful in clinical practice: in the CUA patient, a timely recognition and removal of both sensitizing and challenging etiologic agents is key to treatment [12, 38].

It is unclear whether some factors involved in this disease are actually causative or only associated. This distinction is relevant because associated factors help to identify patients at risk of developing CUA, but only true causative factors guide treatment [21, 39]. For instance, hypoalbuminemia is a marker for malnutrition and mortality in CKD patients with a strong association with CUA, because it is linked to chronic inflammation, dermal loss in ulcerated lesions and reduced fetuin-A levels. However, this factor probably does not participate in the pathogenesis of CUA [9, 20]. Randomized controlled trials would provide the most accurate confirmation of causality; however, the design of such studies would be a challenge, considering the rarity of CUA. Due to this limitation, several case-control studies have been performed to identify statistically significant risk factors for the development of CUA. Some of the variables consistently associated with CUA include white race, obesity, warfarin and calcitriol therapy and elevated serum calcium levels; CUA registries may be helpful in the verification of these associations [40-43]. Also, some therapies are risk factors: iatrogeny is thought to play a prominent role in the development of CUA [20].

Sensitizers confer predisposition to CUA by creating a systemic pro-calcification and pro-inflammatory milieu. These agents define the patient's clinical profile: CUA is found in both uremic and non-uremic patients, who differ in their clinical backgrounds and sensitizers.

Uremic CUA occurs in patients with advanced CKD, mainly stage V on chronic dialysis or after renal transplantation, and represents the majority of the cases [6, 12, 13]. Hyperphosphatemia, hypercalcemia, increased CaXP and secondary hyperparathyroidism are thought to play a central role in the development of CUA [38, 44]. High calcium and phosphate diet, treatment with calcium-containing phosphate binders and active vitamin D analogs may exacerbate this type of systemic sensitization [13, 44, 45]. Therefore, a tight metabolic control is an important effort for successful treatment [13].

Non-uremic CUA has been increasingly recognized and affects patients with preserved renal function [4, 9, 13]. It is more commonly described in primary hyperparathyroidism. This non-uremic CUA has also been reported in patients with autoimmune diseases, chronic inflammatory states, such as Crohn disease, connective tissue diseases, sarcoidosis, alcoholic liver disease, and malignant neoplasms [6, 46, 47]. In patients with autoimmune diseases, CUA is often incorrectly diagnosed as a vasculitic process [47]. It should be noted that autoimmune conditions may also contribute to the development of CUA in

uremic patients [48]. Thus, anti-inflammatory and antioxidant approaches should be part of the treatment of both uremic and non-uremic CUA [13].

Uremic and non-uremic CUA patients may share other sensitizers not directly related with inflammatory diseases. Obesity, type II diabetes mellitus, female gender and hypercoagulability may elicit CUA by distinct mechanisms. Obesity may compromise arteriolar blood flow to skin and subcutaneous tissues and potentiates arteriolar thrombosis. Type II diabetes mellitus is associated with kidney disease, inflammation and platelet aggregation, which contribute to renal function impairment, endothelial dysfunction and arteriolar thrombosis, respectively [9, 38]. Females have a predominantly proximal adipose tissue distribution, where CUA is more common, and hormonal influences (estrogen and leptin) may play a role [39]. Hypercoagulability is a systemic influence towards arteriolar thrombosis arising from protein C or S deficiency, obesity or antiphospholipid syndrome [6]. Low molecular weight heparin and tissue plasminogen activator have been shown to successfully heal skin lesions in CUA patients supporting the role of hypercoagulability [49].

In a sensitized uremic or non-uremic patient, challengers act as external acute triggers which precipitate or aggravate clinical manifestations of CUA [20]. Subcutaneous injections, corticosteroids, warfarin, antineoplastic drugs, iron salts, erythropoietin and albumin are some potential iatrogenic causes [6, 9, 35, 46, 50]. All these etiologies are plausible in practice because, in most cases, CUA patients are thoroughly followed by nephrologists or internists and, therefore, potentially exposed to overtreatment [20, 39].

Warfarin, a vitamin K antagonist commonly used in dialysis patients, inhibits γ-carboxylation reactions, required for the activity of MGP, an anti-calcifying protein located in the ECM. Consequently, warfarin increases vascular ossification-calcification processes, which may explain the strong association between warfarin use and the development of CUA [20, 40, 41, 44].

This division of risk factors into sensitizers and challengers underscores the importance of preventive strategies in the management of CUA, but fails to explain why very few uremic patients actually develop the disease even if exposed to challenging factors [12, 39].

It is clinically relevant to identify the very distinct uremic and non-uremic individuals who may potentially develop CUA. Moreover, diagnosis is even more difficult in non-uremic patients, requiring a high index of clinical suspicion [51]. However, non-uremic patients ultimately develop the identical clinical presentation and histopathologic findings of classic CUA [13].

Painting the clinical picture and ruling out differential diagnoses

Due to the clinical heterogeneity of CUA in both uremic and non-uremic patients, subgroups of the disease with distinct prognoses have been proposed [20, 39]. Skin involvement is more frequent; rare cases of CUA affecting internal organs have also been described [52].

Visceral CUA has been identified in post-mortem examinations of critically ill patients. Reported clinical presentations include acute respiratory failure [52, 53], gastrointestinal bleeding [54] and widespread visceral involvement in a patient with primary autoimmune myelofibrosis [51]. A recent investigation has questioned the actual existence of non-cutaneous CUA, pointing the lack of definitive histopathologic evidence in some of these autopsy cases [55]. Furthermore, some systemic manifestations associated with cutaneous CUA may result from extracutaneous calcification processes not necessarily related to visceral CUA [36]. Taking these ideas into consideration, visceral CUA will not be further discussed in this review.

Cutaneous CUA typically presents as a biphasic process [44, 56]. Each phase may be understood as a set of clinical consequences of specific histologic lesions belonging to a continuum towards tissue necrosis. ECM remodelling, medial calcification and arteriolar stenosis could be considered “primary lesions”, whereas thrombus formation and luminal obstruction leading to tissue infarction have been postulated as “secondary lesions” [13, 39]. Primary and secondary lesions might be associated with the first and second clinical phases, respectively.

Phase one usually starts with areas of skin leather-like induration with superimposed pruritic and excruciatingly painful erythematous nodules, plaques or livedo reticularis. These lesions are more prone to appear at adipose tissue sites and become progressively deeper and more extensive.

Phase two consists of painful ischemic necrosis which manifests as non-healing ulcerations and black deep eschars. Infection, abscess formation and gangrene frequently follow the appearance of these lesions [6, 9, 10, 35, 36, 38, 56].

Acral, distal and penile lesions have a more favourable clinical course, whereas lesions with proximal distribution carry a worse prognosis [20, 35, 36, 39, 50].

Clinical conditions with prominent vascular involvement and dermatologic manifestations similar to CUA belong to an extensive list of differential diagnoses. Some of these diseases are highly prevalent or carry a fatal prognosis and must be promptly excluded, namely peripheral artery disease, autoimmune

vasculitis, diabetes mellitus-related lower extremity involvement and cholesterol embolization [6, 9, 35, 39, 44, 57, 58]. In some situations, these diseases may coexist with CUA and should be treated accordingly.

In a patient presenting with skin lesions suggesting CUA, a thorough clinical history should be obtained, with particular emphasis on risk factors, and complementary exams need to be performed in order to rule out differential diagnoses and establish a definitive diagnosis of CUA [6]. Biochemistry, histopathology and imagiology are the three main vectors of diagnostic investigation, but negative results should not be regarded as a warranty of exclusion of the disease [36].

Laboratory findings are unspecific, variable and dependent on underlying pathologies. Hyperphosphatemia, increased CaXP, hyperparathyroidism and slight hypercalcemia may be found in uremic patients; elevated canalicular enzymes and albumin are other possible findings in all CUA patients. However, both uremic and non-uremic CUA patients may present without these biochemical changes: usage of medications in an attempt to correct these metabolic parameters may be one of the reasons for this clinical fact [15, 17, 36, 59].

Deep skin and subcutaneous tissue incisional biopsy with calcification-sensitive (von Kossa) staining is the gold standard for definitive diagnosis [6, 9, 10, 13, 58]. There is controversy on whether biopsy should be routinely performed, because this procedure may induce novel non-healing ulcers, existing lesions may become more difficult to heal and easier to superinfect and there may be false negative results [6, 8, 20, 44, 58, 60]. Our institution adopts a very restrictive approach to the use of skin biopsy, whose execution is decided on a case-by-case basis, according to the clinical experience of each nephrologist. Histopathologic findings in CUA, albeit specific, are not pathognomonic and may include one or more of the following: medial arteriolar calcification without associated intimal calcification or vasculitic changes, intimal hyperplasia, ECM remodelling, soft tissue calcification, thrombosis, epidermal ulceration and dermal or subcutaneous necrosis [6, 13, 20, 36].

Imaging modalities may be useful to support histologic results, avoid biopsy or monitor response to treatment [61]. Plain soft tissue radiographs may show a typical net-like pattern of vascular calcification and irregularity of the soft tissues [8, 61]. Mammography technique is thought to be superior to plain-soft tissue x-ray, because it is safe, inexpensive and able to delineate high-contrast microcalcifications which cannot be identified in other radiography exams; the main disadvantage of this exam is that it requires the compression of the lesions between two plates, causing intense pain [6, 58]. Xeroradiography is described

by some authors as the optimal way of studying arteriolar and soft tissue calcifications [17, 36, 44]. Bone scintigraphy with Tc99m methylene diphosphate detects increased soft tissue uptake of bone tracer in areas with noticeable clinical expression, but lacks sensitivity and specificity [8, 39, 44, 57, 60].

Once the clinical picture is fully identified and a definitive diagnosis is obtained, patients should promptly receive specialized care. However, therapy should ideally begin early in the stage of the disease and, many times, before definitive diagnosis.

Therapy: to be started in the early stages of CUA

Treatment of CUA remains a series of experimental interventions lacking clinical evidence, with unrewarding results and controversial impacts on morbidity and mortality [11, 20, 39]. The various pathogenic mechanisms involved in CUA support a multimodal approach targeted at distinct aspects of the disease, although uremic and non-uremic patients are treated similarly.

Active treatment of established CUA tends not to be enough. Despite the impressive clinical picture of advanced CUA, most of the therapeutic measures are not focused on healing visible lesions but rather on treating underlying disease processes and removing iatrogeny [11, 39], which can be done before overt CUA develops. In CUA, therapy may be seen as a continuum starting from prevention, which is a very important proactive strategy for 'at risk' patients [62].

Withdrawal of iatrogeny and management of underlying disease processes are the mainstay of prevention and treatment of CUA

Certain medications that may aggravate systemic sensitization or act as challenging factors in the development of CUA should be discontinued, namely vitamin D analogs, subcutaneous injections and warfarin [44, 57]; the latter may be replaced with "safe" anticoagulants, namely low-molecular weight heparin, and administration of vitamin K [9, 20, 39]. Corticosteroids belong to a "grey zone" between iatrogeny and therapy. On the one hand, prednisolone may be an etiologic agent of CUA and increases infection risk of ulcerated lesions; on the other hand, this drug decreases tissue inflammation and has already been shown to be effective in some cases [44, 46, 57].

As far as underlying disease processes are concerned, nutritional status and comorbid conditions of uremic and non-uremic CUA patients require careful monitoring and treatment [6, 44, 46, 63]. In particular, control of metabolic parameters is an essential strategy and may be achieved with interventions targeted at phosphate, calcium, CaXP and/or parathormone (PTH) levels, in order to prevent and treat hyperphosphatemia, hypercalcemia and/or hyperparathyroidism, respectively.

Hyperphosphatemia and hypercalcemia require consideration of more intensive dialysis with low calcium dialysate, replacement of oral calcium phosphate binders with non-calcium phosphate binders and withdrawal of calcium supplementation [9, 10, 13, 39, 50, 57]. Control of hyperphosphatemia should be prioritized over control of hypercalcemia, because phosphate is a requirement for VSMCs to perform their active role of arteriolar calcification. In theory, however, induction of transient hypocalcemia could also prove beneficial to normalize CaXP and remove calcium from tissues [44].

Hyperparathyroidism may be treated medically or surgically. Cinacalcet is a calcimimetic, suppressing PTH secretion and rapidly correcting calcium and phosphate levels [6, 9, 10, 13, 46]. The EVOLVE clinical trial (EValuation Of Cinacalcet Hydrochloride (HCl) Therapy to Lower CardioVascular Events), an event-driven cardiovascular outcomes study, involved the randomization of 3883 hemodialysis patients with secondary hyperparathyroidism to receive cinacalcet or placebo. 24 patients developed CUA, 18 in the placebo group and 6 in the cinacalcet group. According to a post-hoc analysis of the results of the EVOLVE trial, reduction of serum PTH levels with cinacalcet therapy reduced CUA incidence by 70%, with a hazard ratio (cinacalcet versus placebo) of 0.25 [64]. For patients with unsatisfactory response to medical treatment and evidence of high bone turnover, parathyroidectomy has been proposed [9, 10, 13, 20, 46, 57, 65, 66]. Despite the risks of the procedure and unproven benefits on survival, this surgery may improve wound healing and tissue oxygenation, especially in patients with very high PTH levels [6, 9, 11, 12, 44].

As outlined above, CUA may happen in the context of a normal Ca-P metabolism. Thus, systemic therapies with antioxidant, anti-inflammatory and antithrombotic mechanisms of action are also used in both uremic and non-uremic patients [13].

Sodium thiosulfate: a step forward in therapy

Sodium thiosulphate (STS) is currently regarded as the first-line treatment of patients without hyperparathyroidism [6, 13, 46, 50, 57].

The mechanism of action of STS is unknown; dissolution of calcium deposits, chelation of calcium ions, antioxidant effects and potent vasodilation are some possible effects [6, 9, 11, 13, 39, 46, 57, 67].

In 2004, Cicone et al. reported the first successful treatment of CUA with STS, which took inspiration from previously reported cases of tumoral calciosis responsive to STS. Dramatic improvements in algic complaints, subcutaneous lesions and technetium 99 scans were observed. Also, STS did not produce any effects on Ca-P metabolism, which suggests the use of this drug in tandem with a rigorous metabolic control. According to these authors, STS could be particularly beneficial in patients unresponsive to other therapeutic measures [68].

Potential adverse effects of long-term usage of STS remain a concern, especially bone demineralization due to STS-induced metabolic acidosis [20]. Other side effects, namely nausea, vomiting, headache, hypotension andelectrocardiographic abnormalities, have also been described [9, 13, 20, 46, 57].

Other strategies also belong to the unfinished equation of therapy

Bisphosphonates are widely used to treat osteoclast-mediated bone loss and have been shown to be capable of treating tumoral calcinosis and preventing experimental CUA. These assumptions motivated the first use of pamidronate by Monney et al. in the treatment of a patient with rapidly aggravating CUA and CKD. Forty-eight hours after the initiation of treatment with pamidronate, a marked improvement in the clinical course, with pain reduction and ulcer healing, has been observed [69]. Subsequent studies also demonstrated favourable clinical outcomes, which may be mediated by a decrease in inflammation, inhibition of arteriolar calcification and reduction of serum calcium levels through suppression of osteoclastic activity. Bisphosphonates have been shown to be effective and well-tolerated, but their use should be cautiously considered due to potentially deleterious effects on bone metabolism and renal function [6, 9, 12, 13, 20, 39, 46, 57, 63].

Hyperbaric oxygen (HBO) therapy consists of breathing 100% oxygen in a pressurized environment, increasing the amount of dissolved oxygen in the plasma, counteracting local tissue hypoxia and improv-

ing wound healing. Also, HBO is directly bactericidal and bacteriostatic and stimulates neutrophil bactericidal activity, angiogenesis and fibroblast proliferation. Middle ear barotrauma, pulmonary and central nervous system oxygen toxicity, claustrophobia and high monetary costs are some potential problems of HBO therapy. Despite its limitations, HBO therapy has been shown to be beneficial in several case reports and retrospective case reviews, especially in patients with distal CUA without secondary hyperparathyroidism, without surgical conditions or refractory to parathyroidectomy [6, 9, 13, 39, 44, 46, 70].

According to a matched case-control study, statins may prevent CUA development in dialysis patients. Anti-inflammatory, antithrombotic and anticalcification properties of these drugs may underlie this association between statin use and CUA. Should this association be corroborated, statins may become an important strategy for CUA prevention [41].

Besides systemic therapies, advanced CUA lesions also require intensive local wound care and pain management. Some therapeutic options include debridement of gangrenous tissue, use of broad-spectrum antibiotics for superimposed bacterial infection, sterile dressings and pain schedule with opiates [6, 9-11, 13, 17, 39, 44, 46, 56, 57, 60].

Conclusion

Prognosis of CUA remains poor. Local and systemic infectious complications are responsible for short survival and 5-year mortality rates around 60-70% in CUA patients, even when aggressively treated with large-spectrum antibiotherapy [9, 10, 17, 36, 57, 66].

In this increasingly recognized disease, there is still much to be elucidated on the pathogenesis. According to our revision of the literature, vascular ossification-calcification phenomena in CUA are basically similar with common uremic vascular ossification-calcification events; however, these mechanisms might not explain vascular ossification-calcification in non-uremic patients, where inflammation probably assumes a prominent role. As far as diagnosis is concerned, an early identification of ‘at risk’ uremic and non-uremic patients is a crucial step for a more successful treatment. Targeted therapies have been mentioned in the literature as a promising innovation in the management of CUA [6, 10]. However, it is likely that prevention, multidisciplinary care and avoidance of iatrogeny should remain the foundations in the management of CUA [11, 20, 45, 50, 56, 66].

In summary, many questions on fundamental aspects of this complex disease are yet to be answered, deserving further attention by investigators and clinicians.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Prabahar, M.R., et al., *Severe extraossous calcification in chronic kidney disease.* Dialysis & Transplantation, 2006. **35**(12): p. 772-776.
2. Hayden, M., et al., *Vascular ossification - calcification in metabolic syndrome, type 2 diabetes mellitus, chronic kidney disease, and calciphylaxis - calcific uremic arteriolopathy: the emerging role of sodium thiosulfate.* Cardiovascular Diabetology, 2005. **4**(1): p. 4.
3. Demer, L.L. and Y. Tintut, *Vascular Calcification: Pathobiology of a Multifaceted Disease.* Circulation, 2008. **117**(22): p. 2938-2948.
4. Kalajian, A.H., et al., *Calciphylaxis with normal renal and parathyroid function: Not as rare as previously believed.* Archives of Dermatology, 2009. **145**(4): p. 451-458.
5. Kramann, R., et al., *Novel insights into osteogenesis and matrix remodelling associated with calcific uraemic arteriolopathy.* Nephrol Dial Transplant, 2013. **28**(4): p. 856-68.
6. Ng, A.T. and D.H. Peng, *Calciphylaxis.* Dermatologic Therapy, 2011. **24**(2): p. 256-262.
7. Hafner, J., et al., *Uremic small-artery disease with medial calcification and intimal hyperplasia (so-called calciphylaxis): a complication of chronic renal failure and benefit from parathyroidectomy.* J Am Acad Dermatol, 1995. **33**(6): p. 954-62.
8. Reiter, N., et al., *Calcinosis cutis: part I. Diagnostic pathway.* J Am Acad Dermatol, 2011. **65**(1): p. 1-12; quiz 13-4.
9. Rogers, N.M., D.J. Teubner, and P.T. Coates, *Calcific uremic arteriolopathy: advances in pathogenesis and treatment.* Semin Dial, 2007. **20**(2): p. 150-7.
10. Wollina, U., *Update on cutaneous calciphylaxis.* Indian J Dermatol, 2013. **58**(2): p. 87-92.
11. Magro, C.M., R. Simman, and S. Jackson, *Calciphylaxis: a review.* J Am Col Certif Wound Spec, 2010. **2**(4): p. 66-72.
12. Bhambri, A. and J.Q. Del Rosso, *Calciphylaxis: a review.* J Clin Aesthet Dermatol, 2008. **1**(2): p. 38-41.
13. Sowers, K.M. and M.R. Hayden, *Calcific uremic arteriolopathy: pathophysiology, reactive oxygen species and therapeutic approaches.* Oxid Med Cell Longev, 2010. **3**(2): p. 109-21.
14. Giachelli, C.M., *Vascular Calcification: In Vitro Evidence for the Role of Inorganic Phosphate.* Journal of the American Society of Nephrology, 2003. **14**(suppl 4): p. S300-S304.
15. Weenig, R.H., *Pathogenesis of calciphylaxis: Hans Selye to nuclear factor kappa-B.* J Am Acad Dermatol, 2008. **58**(3): p. 458-71.

16. Shroff, R.C., et al., *Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis.* Circulation, 2008. **118**(17): p. 1748-57.
17. Mathur, R.V., J.R. Shortland, and A.M. El Nahas, *Calciphylaxis.* Postgraduate Medical Journal, 2001. **77**(911): p. 557-561.
18. Heiss, A., et al., *Structural basis of calcification inhibition by alpha 2-HS glycoprotein/fetuin-A. Formation of colloidal calciprotein particles.* J Biol Chem, 2003. **278**(15): p. 13333-41.
19. Ketteler, M., et al., *Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study.* Lancet, 2003. **361**(9360): p. 827-33.
20. Brandenburg, V.M., et al., *Calcific uraemic arteriolopathy: a rare disease with a potentially high impact on chronic kidney disease-mineral and bone disorder.* Pediatr Nephrol, 2014.
21. Brandenburg, V.M., et al., *Calciphylaxis in CKD and beyond.* Nephrology Dialysis Transplantation, 2012. **27**(4): p. 1314-1318.
22. Bostrom, K., et al., *Matrix GLA protein modulates differentiation induced by bone morphogenetic protein-2 in C3H10T1/2 cells.* J Biol Chem, 2001. **276**(17): p. 14044-52.
23. Schurgers, L.J., E.C. Cranenburg, and C. Vermeer, *Matrix Gla-protein: the calcification inhibitor in need of vitamin K.* Thromb Haemost, 2008. **100**(4): p. 593-603.
24. Cranenburg, E.C.M., et al., *The Circulating Inactive Form of Matrix Gla Protein (ucMGP) as a Biomarker for Cardiovascular Calcification.* Journal of Vascular Research, 2008. **45**(5): p. 427-436.
25. Shroff, R.C., et al., *Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification.* J Am Soc Nephrol, 2010. **21**(1): p. 103-12.
26. Giachelli, C.M., *The emerging role of phosphate in vascular calcification.* Kidney Int, 2009. **75**(9): p. 890-7.
27. Thompson, B. and D.A. Towler, *Arterial calcification and bone physiology: role of the bone-vascular axis.* Nat Rev Endocrinol, 2012. **8**(9): p. 529-43.
28. Jablonski, K.L. and M. Chonchol, *Vascular calcification in end-stage renal disease.* Hemodialysis International, 2013. **17**: p. S17-S21.
29. Kapustin, A.N. and C.M. Shanahan, *Calcium regulation of vascular smooth muscle cell-derived matrix vesicles.* Trends Cardiovasc Med, 2012. **22**(5): p. 133-7.
30. Proudfoot, D. and C.M. Shanahan, *Nanocrystals seed calcification in more ways than one.* Kidney Int, 2011. **79**(4): p. 379-82.
31. Ahmed, S., et al., *Calciphylaxis is associated with hyperphosphatemia and increased osteopontin expression by vascular smooth muscle cells.* Am J Kidney Dis, 2001. **37**(6): p. 1267-76.
32. Sage, A.P., et al., *Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells in vitro.* Kidney Int, 2011. **79**(4): p. 414-22.
33. Anderson, D., W. Stewart, and D. Piercy, *CALCIFYING PANNICULITIS WITH FAT AND SKIN NECROSIS IN A CASE OF URÆMIA WITH AUTONOMOUS HYPERPARATHYROIDISM.* The Lancet, 1968. **292**(7563): p. 323-325.
34. Richens, G., M.W. Piepkorn, and G.G. Krueger, *Calcifying panniculitis associated with renal failure. A case of Selye's calciphylaxis in man.* J Am Acad Dermatol, 1982. **6**(4 Pt 1): p. 537-9.

35. Edwards, R.B., et al., *Calciphylaxis: a rare limb and life threatening cause of ischaemic skin necrosis and ulceration.* Br J Plast Surg, 2000. **53**(3): p. 253-5.
36. Dauden, E. and M.J. Onate, *Calciphylaxis.* Dermatol Clin, 2008. **26**(4): p. 557-68, ix.
37. Weenig, R.H., et al., *Calciphylaxis: natural history, risk factor analysis, and outcome.* J Am Acad Dermatol, 2007. **56**(4): p. 569-79.
38. Budisavljevic, M.N., D. Cheek, and D.W. Ploth, *Calciphylaxis in chronic renal failure.* J Am Soc Nephrol, 1996. **7**(7): p. 978-82.
39. Brandenburg, V.M., M. Cozzolino, and M. Ketteler, *Calciphylaxis: a still unmet challenge.* J Nephrol, 2011. **24**(2): p. 142-8.
40. Hayashi, M., et al., *A case-control study of calciphylaxis in Japanese end-stage renal disease patients.* Nephrol Dial Transplant, 2012. **27**(4): p. 1580-4.
41. Nigwekar, S.U., et al., *Statin use and calcific uremic arteriolopathy: a matched case-control study.* Am J Nephrol, 2013. **37**(4): p. 325-32.
42. Zacharias, J., B. Fontaine, and A. Fine, *Calcium use increases risk of calciphylaxis: a case-control study.* Peritoneal Dialysis International, 1999. **19**(3): p. 248-252.
43. Bleyer, A.J., et al., *A case control study of proximal calciphylaxis.* Am J Kidney Dis, 1998. **32**(3): p. 376-83.
44. Wilmer, W.A. and C.M. Magro, *Calciphylaxis: Emerging Concepts in Prevention, Diagnosis, and Treatment.* Seminars in Dialysis, 2002. **15**(3): p. 172-186.
45. Rezaie, W., et al., *Calciphylaxis in chronic renal failure: An approach to risk factors.* Indian J Nephrol, 2009. **19**(3): p. 115-8.
46. Vedvyas, C., L.S. Winterfield, and R.A. Vleugels, *Calciphylaxis: a systematic review of existing and emerging therapies.* J Am Acad Dermatol, 2012. **67**(6): p. e253-60.
47. Lee, J.L., et al., *Recognizing calcific uremic arteriolopathy in autoimmune disease: an emerging mimicker of vasculitis.* Autoimmun Rev, 2008. **7**(8): p. 638-43.
48. Slough, S., et al., *Association between calciphylaxis and inflammation in two patients on chronic dialysis.* Adv Perit Dial, 2006. **22**: p. 171-4.
49. Harris, R.J. and T.G. Cropley, *Possible role of hypercoagulability in calciphylaxis: review of the literature.* J Am Acad Dermatol, 2011. **64**(2): p. 405-12.
50. Rogers, N.M. and P.T. Coates, *Calcific uraemic arteriolopathy: an update.* Curr Opin Nephrol Hypertens, 2008. **17**(6): p. 629-34.
51. Nichols, B., P. Saadat, and M.S. Vadmal, *Fatal systemic nonuremic calciphylaxis in a patient with primary autoimmune myelofibrosis.* Int J Dermatol, 2011. **50**(7): p. 870-4.
52. Kim, N.R., et al., *Pulmonary calciphylaxis associated with acute respiratory and renal failure due to cryptogenic hypercalcemia: an autopsy case report.* Korean J Pathol, 2012. **46**(6): p. 601-5.
53. Li, Y.J., et al., *Fulminant pulmonary calciphylaxis and metastatic calcification causing acute respiratory failure in a uremic patient.* Am J Kidney Dis, 2006. **47**(4): p. e47-53.
54. Brown, D.F., C.F. Denney, and D.K. Burns, *Systemic calciphylaxis associated with massive gastrointestinal hemorrhage.* Arch Pathol Lab Med, 1998. **122**(7): p. 656-9.

55. Andersen, L.K., J.S. Lehman, and M.D. Davis, *Calciphylaxis is a cutaneous process without involvement of internal organs in a retrospective study of postmortem findings in three patients.* Acta Derm Venereol, 2014. **94**(3): p. 298-302.
56. Tsolakidis, S., et al., *Calciphylaxis - a challenging & solvable task for plastic surgery? A case report.* BMC Dermatol, 2013. **13**: p. 1.
57. Smith, J.R., et al., *The role of sodium thiosulphate in the treatment of calciphylaxis.* Portuguese Journal of Nephrology & Hypertension, 2012. **26**: p. 245-254.
58. Bleibel, W., B. Hazar, and R. Herman, *A case report comparing various radiological tests in the diagnosis of calcific uremic arteriolopathy.* Am J Kidney Dis, 2006. **48**(4): p. 659-61.
59. Kyttaris, V.C., et al., *Calciphylaxis: a pseudo-vasculitis syndrome.* Semin Arthritis Rheum, 2007. **36**(4): p. 264-7.
60. Kumar, V.A., *Calcific uremic arteriolopathy: an underrecognized entity.* Perm J, 2011. **15**(2): p. 85-7.
61. Shmidt, E., et al., *Net-like pattern of calcification on plain soft-tissue radiographs in patients with calciphylaxis.* J Am Acad Dermatol, 2012. **67**(6): p. 1296-301.
62. Khalpey, Z., et al., *The importance of prevention of calciphylaxis in patients who are at risk and the potential fallibility of calcimimetics in the treatment of calciphylaxis for patients with secondary hyperparathyroidism.* NDT Plus, 2010. **3**(1): p. 68-70.
63. Torregrosa, J.V., et al., *Successful treatment of calcific uraemic arteriolopathy with bisphosphonates.* Nefrologia, 2012. **32**(3): p. 329-34.
64. Floege, J., et al. *The Effect of Cinacalcet on Calciphylaxis Events in Haemodialysis Patients in the EVOLVE Clinical Trial*. in *American Society of Nephrology Kidney Week 2014*. 2014. Philadelphia, PA.
65. Coates, T., et al., *Cutaneous necrosis from calcific uremic arteriolopathy.* Am J Kidney Dis, 1998. **32**(3): p. 384-91.
66. Kang, A.S., et al., *Is calciphylaxis best treated surgically or medically?* Surgery, 2000. **128**(6): p. 967-71;discussion 971-2.
67. O'Neill, W.C., *Sodium thiosulfate: mythical treatment for a mysterious disease?* Clin J Am Soc Nephrol, 2013. **8**(7): p. 1068-9.
68. Cicone, J.S., et al., *Successful treatment of calciphylaxis with intravenous sodium thiosulfate.* Am J Kidney Dis, 2004. **43**(6): p. 1104-8.
69. Monney, P., et al., *Rapid improvement of calciphylaxis after intravenous pamidronate therapy in a patient with chronic renal failure.* Nephrology Dialysis Transplantation, 2004. **19**(8): p. 2130-2132.
70. Rogers, N.M., et al., *Hyperbaric oxygen as effective adjuvant therapy in the treatmentof distal calcific uraemic arteriolopathy.* NDT Plus, 2008. **1**(4): p. 244-249.

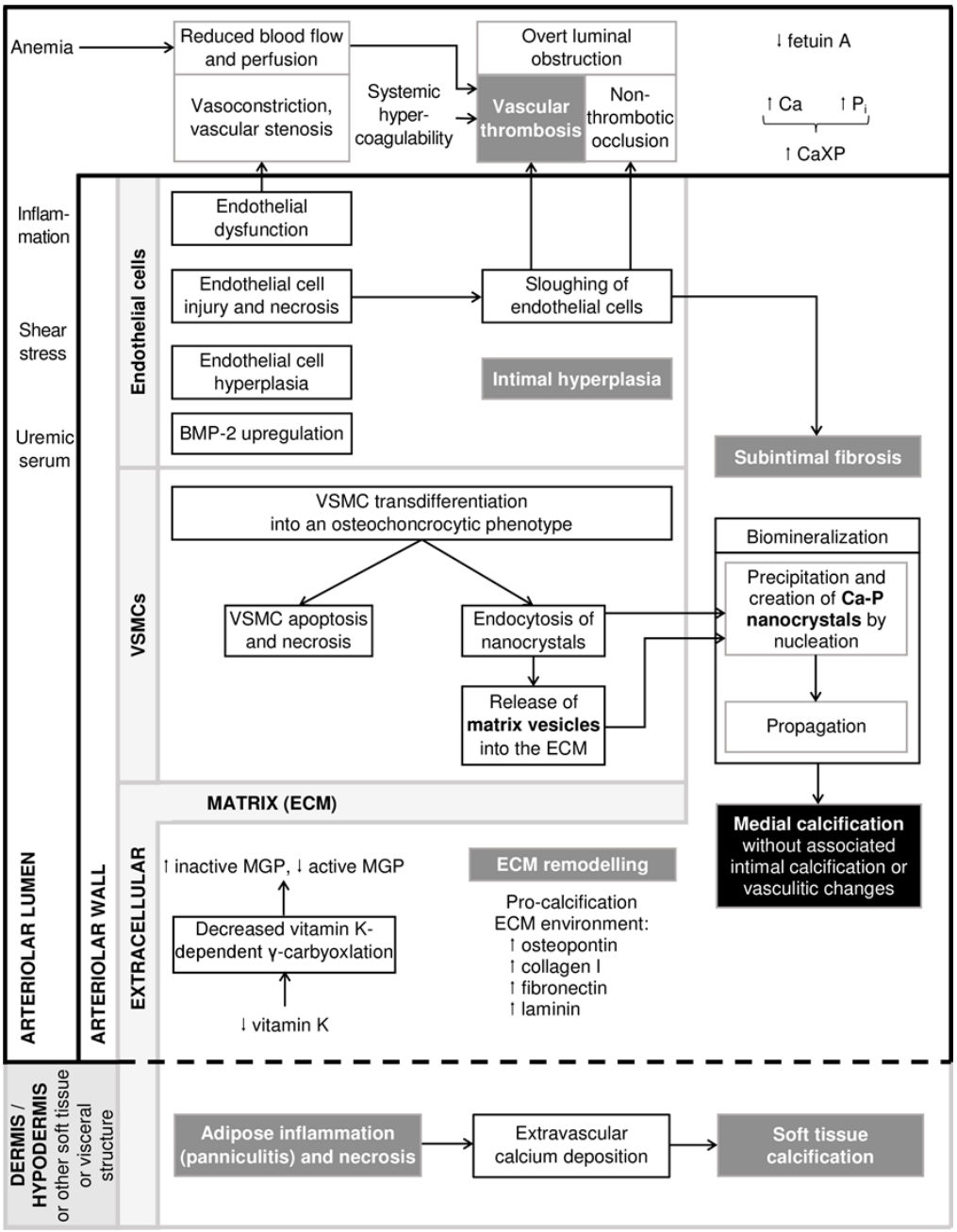


Fig. 1 Calcific uremic arteriolopathy: a multi-layered pathogenesis. This diagram outlines the main events in the arteriole and adjacent tissues. From the top to the bottom, like a transverse section of an arteriole: lumen; tunica intima; tunica media; extracellular matrix of the arteriole; adipocytes and extracellular matrix of the surrounding dermis, hypodermis or other soft tissue or visceral structure. Various influences between systemic and local factors and events were not depicted for clarity. The black box signals the main event and histologic finding of calcific uremic arteriolopathy. Gray boxes mark associated structural changes which can be found in calcific uremic arteriolopathy. See text for further details. *Ca* calcium, P_i inorganic phosphate, *CaXP* calcium-phosphate product, *VSMCs* vascular smooth muscle cells, *MGP* matrix GLA protein, *BMP* bone morphogenetic protein

Instructions for Authors – "Journal of Nephrology"

Types of Papers

Types of manuscripts accepted:

- Editorials dealing with subjects of general informative interest are invited. They should not exceed 1500 words, not include abstract and keywords, not include more than 1 illustration and references should be limited to 10.
- Commentaries are solicited by the Editor-in-Chief and are related to published material, raise challenging questions, or explore controversies. They should not exceed 1500 words, not include abstract and keywords, not include more than 1 illustration and references should be limited to 10.
- Review articles are papers by recognized authorities on special topics of general interest, which must be initially agreed with the Editor-in-Chief. Unsolicited reviews will also be considered. Reviews should not exceed 5000 words, not be signed by more than 3 authors, not include more than 6 illustrations and references should be limited to 70.
- Original articles should present original observations, or observations deriving from a relevant experience in a specific field. The text should be divided into the following sections: Abstract (and up to 4 keywords) Introduction, Methods, Results, and Discussion. Original articles normally should not exceed 3000 words, not include more than 4 illustrations and references should be limited to 30.
- Position papers and Guidelines are written by groups of experts. They should not exceed 5000 words, not include more than 4 illustrations and references should be limited to 100.
- Case reports New/interesting/very rare cases can be reported. Cases with clinical significance or implications will be given priority. The paper should not exceed 1500 words, not be signed by more than 5 authors, not include more than 3 illustrations and references should be limited to 15. The manuscript is arranged as follows: Abstract (and up to 4 keywords), Introduction, Case report, Discussion, References and Legends.
- Letters to the Editor are considered for publication provided they do not contain material that has been submitted or published elsewhere. Only letters in reference to a Journal article are considered and must be received within three months after publication online of the article. They must not exceed 200 words (and 5 references) with one table or figure and without abstracts, and not be signed by more than 3 authors. They should be addressed to the Editor-in-Chief. Submitted letters will be subject to shortening and editorial revision.

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out.

The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Authors should submit their manuscripts online. Electronic submission substantially reduces the editorial processing and reviewing times and shortens overall publication times. Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Title page

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.

- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:
1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
The entries in the list should be numbered consecutively.

- Journal article

  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

- ISSN.org LTWA

If you are unsure, please use the full journal title.
For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 2 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer's LaTeX macro package.

Tables

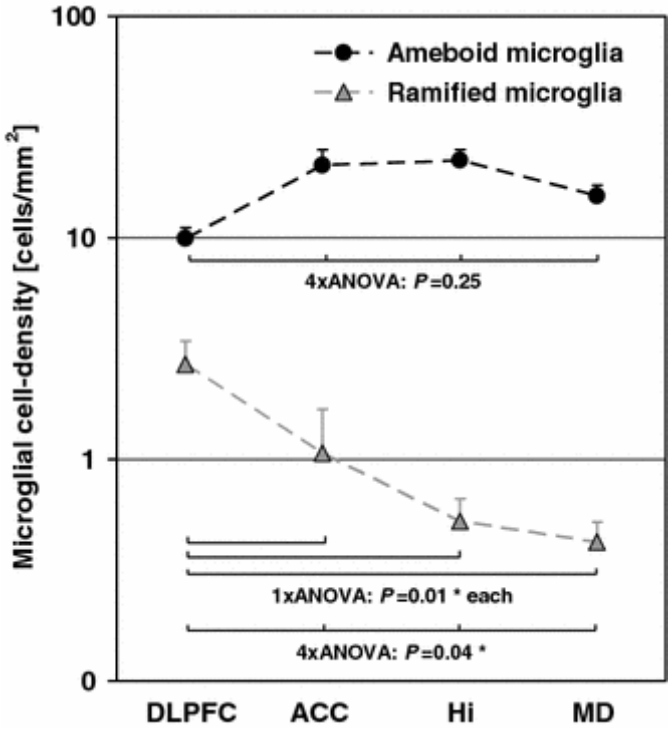
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

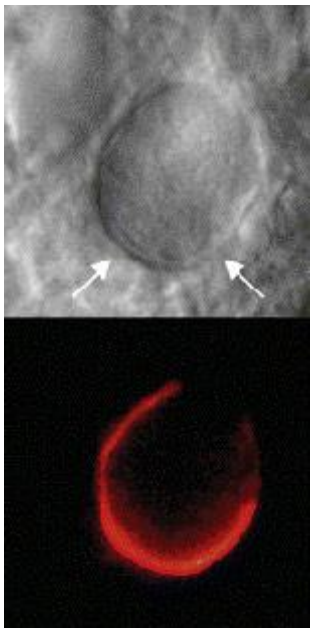
Line Art



- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.

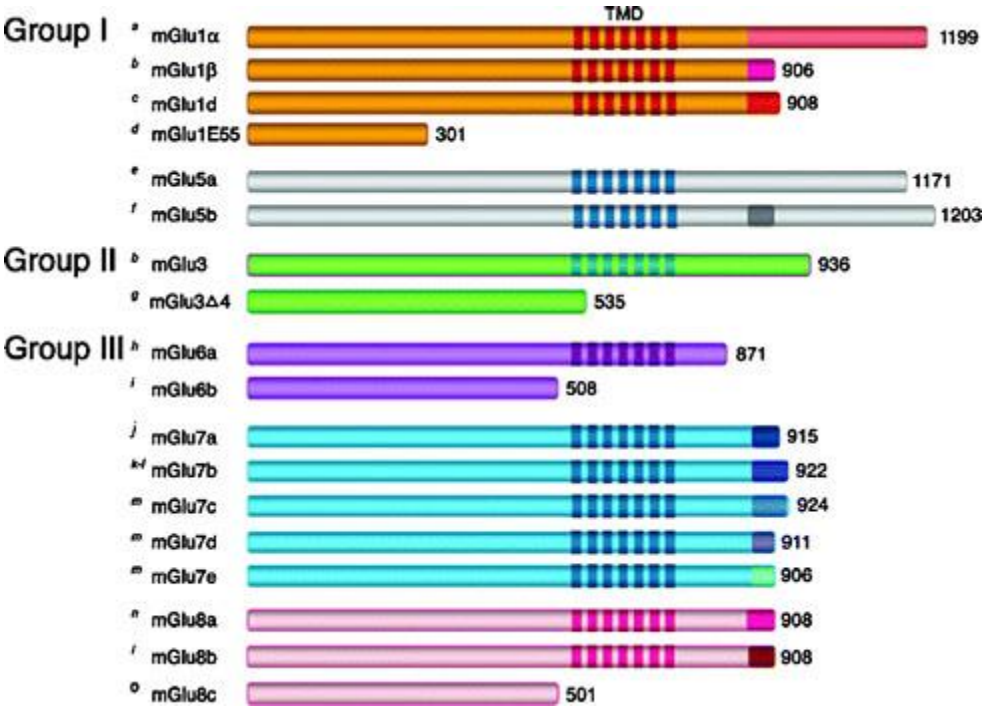
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,

  "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- When preparing your figures, size figures to fit in the column width.
- For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
- For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)
- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)
- Any figure lettering has a contrast ratio of at least 4.5:1

Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Submission

- Supply all supplementary material in standard file formats.
- Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.
- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

- Always use MPEG-1 (.mpg) format.

Text and Presentations

- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be converted to PDF if no interaction with the data is intended.
- If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.
Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation can be achieved by following the rules of good scientific practice, which include:

- The manuscript has not been submitted to more than one journal for simultaneous consideration.
- The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the re-use of material to avoid the hint of text-recycling ("self-plagiarism")).
- A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. "salami-publishing").
- No data have been fabricated or manipulated (including images) to support your conclusions
- No data, text, or theories by others are presented as if they were the author's own ("plagiarism"). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted.

  Important note: the journal may use software to screen for plagiarism.

- Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities - tacitly or explicitly - at the institute/organization where the work has been carried out, **before** the work is submitted.
- Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

In addition:

- Changes of authorship or in the order of authors are not accepted **after** acceptance of a manuscript.
- Requesting to add or delete authors at revision stage, proof stage, or after publication is a serious matter and may be considered when justifiably warranted. Justification for changes in authorship must be compelling and may be considered only after receipt of written approval from all authors and a convincing, detailed explanation about the role/deletion of the new/deleted author. In case of changes at revision stage, a letter must accompany the revised manuscript. In case of changes after acceptance or publication, the request and documentation must be sent via the Publisher to the Editor-in-Chief. In all cases, further documentation may be required to support your request. The decision on accepting the change rests with the Editor-in-Chief of the journal and may be

turned down. Therefore authors are strongly advised to ensure the correct author group, corresponding author, and order of authors at submission.
- Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results. This could be in the form of raw data, samples, records, etc.

If there is a suspicion of misconduct, the journal will carry out an investigation following the COPE guidelines. If, after investigation, the allegation seems to raise valid concerns, the accused author will be contacted and given an opportunity to address the issue. If misconduct has been established beyond reasonable doubt, this may result in the Editor-in-Chief's implementation of the following measures, including, but not limited to:

- If the article is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note.
- The author's institution may be informed.

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.
Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" before the References when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. double blind peer review) as well as per journal subject discipline. Before submitting your article check the Instructions for Authors carefully.
The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.
The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Disclosure of potential conflicts of interest

Authors must disclose all relationships or interests that could influence or bias the work. Although an author may not feel there are conflicts, disclosure of relationships and interests affords a more transparent process, leading to an accurate and objective

assessment of the work. Awareness of real or perceived conflicts of interests is a perspective to which the readers are entitled and is not meant to imply that a financial relationship with an organization that sponsored the research or compensation for consultancy work is inappropriate. Examples of potential conflicts of interests **that are directly or indirectly related to the research** may include but are not limited to the following:

- Research grants from funding agencies (please give the research funder and the grant number)
- Honoraria for speaking at symposia
- Financial support for attending symposia
- Financial support for educational programs
- Employment or consultation
- Support from a project sponsor
- Position on advisory board or board of directors or other type of management relationships
- Multiple affiliations
- Financial relationships, for example equity ownership or investment interest
- Intellectual property rights (e.g. patents, copyrights and royalties from such rights)
- Holdings of spouse and/or children that may have financial interest in the work

In addition, interests that go beyond financial interests and compensation (non-financial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.
The corresponding author collects the conflict of interest disclosure forms from all authors. In author collaborations where formal agreements for representation allow it, it is sufficient for the corresponding author to sign the disclosure form on behalf of all authors. Examples of forms can be found

- here:

The corresponding author will include a summary statement **on the title page that is separate from their manuscript,** that reflects what is recorded in the potential conflict of interest disclosure form(s).
See below examples of disclosures:
Funding: This study was funded by X (grant number X).
Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committee Z.
If no conflict exists, the authors should state:
Conflict of Interest: The authors declare that they have no conflict of interest.

Research involving human participants and/or animals

1) Statement of human rights

When reporting studies that involve human participants, authors should include a statement that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.
If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that the independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study.
The following statements should be included in the text before the References section:
Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."
For retrospective studies, please add the following sentence:
"For this type of study formal consent is not required."

2) Statement on the welfare of animals

The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed, and that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted (where such a committee exists).
For studies with animals, the following statement should be included in the text before the References section:
Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."
If applicable (where such a committee exists): "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted."
If articles do not contain studies with human participants or animals by any of the authors, please select one of the following statements:
"This article does not contain any studies with human participants performed by any of the authors."
"This article does not contain any studies with animals performed by any of the authors."
"This article does not contain any studies with human participants or animals performed by any of the authors."

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or

parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.
The following statement should be included:
Informed consent: "Informed consent was obtained from all individual participants included in the study."
If identifying information about participants is available in the article, the following statement should be included:
"Additional informed consent was obtained from all individual participants for whom identifying information is included in this article."

After Acceptance

Upon acceptance of your article you will receive a link to the special Author Query Application at Springer's web page where you can sign the Copyright Transfer Statement online and indicate whether you wish to order OpenChoice and offprints. Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Open Choice

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription), Springer now provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription-based article, but in addition is made available publicly through Springer's online platform SpringerLink.

- Springer Open Choice

Copyright transfer

Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.
Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative Commons Attribution License..

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Publication of color illustrations is free of charge.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.
After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Editorial procedure

Double-blind peer review

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit:

- A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should be avoided.
- A separate title page, containing title, all author names, affiliations, and the contact information of the corresponding author. Any acknowledgements, disclosures, or funding information should also be included on this page.