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CLINICAL PRESENTATION AND TREATMENT OPTIONS OF CALCINOSIS CUTIS IN AUTOIMMUNE CONNECTIVE TISSUE DISEASES

PhD thesis

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List of Abbreviations

ACTD	autoimmune connective tissue disease
cm	centimeter
CT	computed tomography
dcSSc	diffuse cutaneous systemic sclerosis
DM	(adult) dermatomyositis
e.g.	for example
ELISA	enzyme-linked immunosorbent assay
ENA	extractable nuclear antibody
ESWL	extracorporeal shock wave lithotripsy
FDA	Food and Drug Administration
FGF23	fibroblast growth factor-23 coding gene
g	gram
g/d	gram daily
g/kg	gram per bodyweight kilogram
g/kg/month	gram per bodyweight kilogram monthly
g/m^2	gram per square meter of body surface
Gla	γ-carboxyglutamic acid
GALNT3	polypeptide N-acetylgalactosaminyltransferase-3 coding gene
HS	hydrogen sulfide
JAK	Janus kinase

JDM	juvenile dermatomyositis
KLOTHO	Klotho coding gene
lcSSc	limited cutaneous systemic sclerosis
MCTD	mixed connective tissue disorder
mg	milligram
mg/d	milligram daily
mg/kg/d	milligram per bodyweight kilogram daily
mg/m^2	milligram per square meter of body surface
mg/ml	milligram per milliliter
ml	milliliter
MRI	magnetic resonance imaging
n	number of sample elements
NFκB	nuclear factor κ-light-chain-enhancer of activated B cells
pH	potential of hydrogen
POEMS	polyneuropathy-organomegaly-endocrinopathy-monoclonal plasma cell disorder-skin changes syndrome
SAMD9	sterile α-motif domain containing-9 coding gene
SLE	systemic lupus erythematosus
SSA	anti-Sjögren's syndrome-related antigen
SSc	systemic sclerosis
STAT	signal transducer and activator of transcription
STS	sodium thiosulfate

TNFα	tumor necrosis factor-α
UCTD	undifferentiated connective tissue disorder

1. Introduction

1.1. Calcinosis cutis

Calcinosis cutis is characterized by the ectopic deposition of calcium salts in the skin. This disorder, long recognized yet often disregarded, manifests as a complication of various underlying conditions. According to the prevailing factors precipitating calcification, it can be subdivided into four distinct subtypes: dystrophic, metastatic, iatrogenic, and idiopathic.(1) Some authors also classify calciphylaxis (calcifying uremic arteriolopathy) as a subtype of calcinosis.(2) Calcification of tissues may also occur in other organs, primarily in cases of inflammation, e.g. atherosclerosis, periarticular calcification, renal calcification, and brain calcification. Calcified lesions in the skin manifest as firm, whitish-yellowish, or skin-colored papules, nodules, or plaques, and occasionally as large subcutaneous masses. Calcinosis can be asymptomatic; however, it is often accompanied by pain due to inflammation, nerve compression, or bacterial superinfection. Other symptoms include restricted mobility of the adjacent joints and ulceration with drainage of a purulent or liquefied chalky substance.

1.1.1. Clinical forms of calcinosis cutis

1.1.1.1. Dystrophic calcinosis

The most common dystrophic subtype is characterized by the precipitation of calcium salts on damaged or altered collagen or elastic fibers.(3, 4) These soft tissue alterations may result from traumatic injuries (e.g. burn trauma, chronic pressure), inherited connective tissue abnormalities (e.g. pseudoxanthoma elasticum, Ehlers-Danlos syndrome), inflammatory changes (e.g. graft-versus-host disease, chronic venous insufficiency), neoplasms (e.g. basal cell carcinoma, pilomatricoma, cysts), or destructive soft tissue infections and infestations (e.g. onchocerciasis, cysticercosis).(1, 5, 6) The largest group in which calcinosis can be detected is that of autoimmune connective tissue diseases (ACTDs).(7) In theory, each ACTD can develop calcification. However, it is most commonly observed in systemic sclerosis (SSc), particularly in its limited form. The limited cutaneous SSc (lcSSc) is characterized by distal skin sclerosis, significant vasculopathy (telangiectasia and pulmonary arterial hypertension), and less severe

internal organ fibrosis. Conversely, the diffuse type shows severe, widespread skin and internal organ fibrosis, less prominent vascular changes, less frequent calcinosis, and a less favorable prognosis. Another ACTD that is more commonly complicated by calcinosis is dermatomyositis (DM), especially the juvenile-onset form. The presence of calcinosis is an uncommon occurrence in conjunction with other ACTDs, such as systemic lupus erythematosus (SLE). Deposits form at sites of trauma, periarticular areas, and on areas of bone prominence where the subcutaneous adipose tissue is relatively thin (e.g. elbows, knees, fingers).(7) Different clinical presentations of calcinosis cutis are shown in Figures 1 and 2.

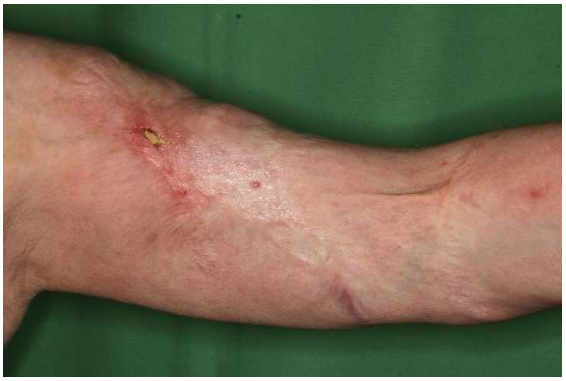

Figure 1 Dystrophic calcinosis cutis nodule developing in a burn keloid on the left arm of a female patient, many years after the burn injury. Modified figure after own publication.(8)

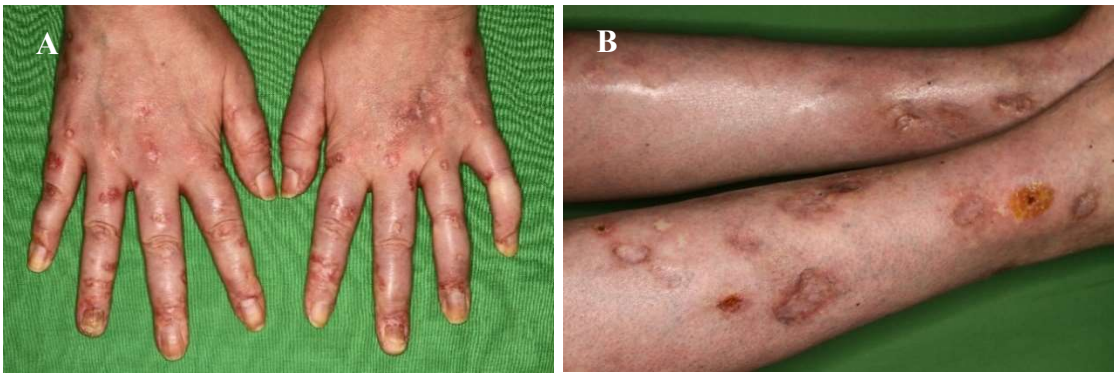

Figure 2 Dystrophic calcinosis presenting as ulcerated nodules on the hands (A) and shins (B) of a female patient with SLE. Modified figure after own publication.(9)

1.1.1.2. Metastatic calcinosis

Metastatic calcification is a consequence of an imbalance in calcium metabolism, resulting in altered levels of calcium or phosphate in the blood serum. Under normal circumstances, a balanced calcium-to-phosphate ratio maintains calcium in solution. Should the ratio change, the precipitation of calcium salts will ensue. Disturbances of this nature may be precipitated by a variety of endocrine and internal disorders, including, but not limited to, chronic renal insufficiency, familial hypocalciuric hypercalcemia, primary, secondary, or tertiary hyperparathyroidism, pseudohyperparathyroidism, granulomatous diseases via ectopic mediators, vitamin D hypervitaminosis, and milk-alkali syndrome.(7, 10) As indicated by the existing literature, the formation of lesions is associated with the degree of hyperphosphatemia. These lesions primarily manifest around joints and, on occasion, in other organs, including vessels, kidneys, lungs, and gastric mucosa.(7) The condition is also called tumoral calcinosis; however, it is essential to note that the terminology on this topic is inconsistent. The term 'tumoral calcinosis' refers to both metastatic and idiopathic cases of genetic origin. In addition, some authors have also classified calciphylaxis as a subtype of metastatic calcinosis, a calcifying vasculopathy that primarily affects the arterioles and is observed in patients with end-stage kidney disease.(7, 11)

1.1.1.3. Iatrogenic calcinosis

Iatrogenic calcinosis is an adverse effect of medical care involving the contact or injection of calcium-containing liquids or gels, which results in the deposition of calcium salts.(7) Electrode gels used for electroencephalography, intravenous calcium chloride, calcium gluconate, subcutaneous nadroparin, calcium supplementation after transplantation, and chemotherapy-induced tumor lysis have all been reported to cause iatrogenic calcinosis.(12-14) As indicated by the findings in studies, direct contact with calcium-containing materials has been demonstrated to result in erythema and swelling. It may progress to the formation of yellowish-white or brown nodules, which can be accompanied by ulceration or necrosis. As localized hypercalcemia is a transient condition, the skin lesions are expected to resolve shortly after the discontinuation of the agent (generally within 2-6 months).(15) If remnant deposits remain, surgical removal is an option.

1.1.1.4. Idiopathic calcinosis

Idiopathic calcinosis is a term used to describe a group of calcifying disorders of genetic or unknown origin. The diseases in this subgroup include subepidermal nodal calcinosis (calculi cutanei), milia-like calcinosis, idiopathic penile calcinosis, scrotal or vulvar calcinosis, and familial tumoral calcinosis.(7, 16-18) Familial tumoral calcinosis is a group of diseases in which genetic alterations in the phosphate excretion pathway lead to an abnormal calcium-to-phosphate ratio. Two forms of the disease have been identified: hyperphosphatemic and normophosphatemic.(19-22) Tumoral calcinosis is characterized by the formation of giant masses of calcified deposits, primarily surrounding joints. The age of onset is typically during childhood or adolescence.

1.1.1.5. Calciphylaxis

Calciphylaxis is a rare disorder that primarily complicates end-stage renal disease and is also known as calcifying uremic arteriolopathy. While some authors have classified it as the fifth subtype of calcinosis, we concur with those who do not consider it a form of calcinosis due to its distinct clinical presentation, pathophysiology, and etiology.(7, 10) Compared with other forms of calcinosis, in which subcutaneous calcified nodules are formed, calciphylaxis is distinguished by the calcification of the arterioles (predominantly hydroxyapatite).(4) Vascular calcification emerges due to secondary or tertiary hyperparathyroidism, arising from hyperphosphatemia in chronic renal insufficiency. There are also rare cases of non-uremic calciphylaxis complicating other diseases such as primary hyperparathyroidism, malignant tumors, connective tissue diseases, diabetes, alcohol-induced liver disease, Crohn's disease, excessive weight loss, vitamin D deficiency, polyneuropathy-organomegaly-endocrinopathy-monoclonal plasma cell disorder-skin changes (POEMS) syndrome, chemotherapy-induced protein C or S deficiency, and calcium nadroparin therapy in osteomalacia.(23)

This process is regarded as an active cellular process, which involves the differentiation of vascular smooth muscle cells to acquire osteogenic properties.(11) The condition has been determined to be vasculopathy rather than a deposition disorder. The precise mechanisms underlying this phenomenon remain to be fully elucidated. It is plausible that the accumulation of toxins in chronic renal insufficiency can induce reactive oxygen species, resulting in oxidative stress and inflammation. These suppress the expression of calcification inhibitors (fetuin-A, matrix γ-carboxyglutamic (Gla) protein) while inducing

osteogenic differentiation genes in vascular smooth muscle cells. An additional mechanism contributing to this process is the imbalance in the calcium and phosphate levels in renal insufficiency, which has been shown to increase parathyroid hormone secretion.(24) These factors activate the nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) cascade, resulting in calcification of the media layer in the vessels, endovascular fibrosis, and intimal hyperplasia. Consequently, the emergence of the Virchow triad (slow circulation, vascular injury, and hypercoagulability) leads to thrombosis and necrosis.(25)

Calciphylaxis is characterized by the development of more than two intensely painful, therapy-resistant ulcers on predominantly areas with more substantial adipose tissue of the trunk (abdomen, buttocks, breasts), the extremities, or penis, accompanied by retiform purpura, livedo racemosa, and blisters. The presence of necrosis (Figure 3) and gangrene with black crusts may be observed. Concomitant conditions are also possible, such as inflammatory myopathy, rhabdomyolysis, muscle atrophy, or joint contractures.(10) The prognosis is generally considered to be poor, with a mortality rate ranging from 45% to 80%.(26) The development of calciphylaxis is associated with several risk factors, including female sex, an imbalance in calcium phosphate metabolism, elevated aluminum and alkaline phosphatase levels in the blood serum, specific comorbidities (obesity, diabetes, liver disease, hypalbuminemia, long-term dialysis), and certain medications (calcium, vitamin D, corticosteroids, calcitriol, warfarin).(26)

Different subtypes of calcinosis are summarized in Table 1.

Figure 3 Typical necrotic calciphylaxis lesion on the right toe of a male patient with end-stage kidney disease

Table 1 Different subtypes of calcinosis cutis

Subtype	Form	Causative mechanism	Associated underlying disorder
Dystrophic		Soft tissue damage	ACTD Trauma Inherited connective tissue abnormalities Graft-versus-host disease Chronic venous insufficiency Neoplasms Skin infections and infestations
Metastatic		Elevated serum calcium-to-phosphate ratio	Chronic renal insufficiency Familial hypocalciuric hypercalcemia Hyperparathyroidism Pseudohyperparathyroidism Granulomatous diseases D hypervitaminosis Milk-alkali syndrome
Iatrogenic		Soft tissue damage with elevated serum/local calcium-to-phosphate ratio	Use of calcium-containing liquids, gels
Idiopathic	Scrotal/vulvar Idiopathic penile Subepidermal nodal Milia-like	Unknown	
	Familial tumoral	Partly elevated serum calcium-to-phosphate ratio	GALNT3, KLOTHO, FGF23, SAMD9 mutation
Calciphylaxis	Uremic	Elevated serum calcium-to-phosphate ratio	Chronic renal insufficiency
	Nonuremic	Unknown	Hyperparathyroidism Malignant tumors Connective tissue diseases Diabetes Alcohol-induced liver disease Crohn's disease Excessive weight loss Vitamin D deficiency POEMS Therapeutics

Abbreviations: ACTD, autoimmune connective tissue disease; FGF23, fibroblast growth factor-23 coding gene; GALNT3, polypeptide N-acetylgalactosaminyltransferase-3 coding gene; KLOTHO, Klotho coding gene; POEMS, polyneuropathy-organomegaly-endocrinopathy-monoclonal plasma cell disorder-skin changes syndrome; SAMD9, sterile α-motif domain containing 9 coding gene

1.1.2. Pathomechanism of calcinosis cutis

The pathomechanism of the various calcinosis subtypes differs from one another. Some forms are associated with an imbalance in calcium and phosphate homeostasis, including the metastatic subtype and some entities in the idiopathic group. The latter group includes hyperphosphatemic familial tumoral calcinoses, which are genetic disorders involving gene mutations that encode different receptors or molecules in the phosphate excretion pathway. The aberrant function of these gene products leads to an accumulation of phosphate in the serum and an abnormal calcium-to-phosphate ratio. The genes involved are GALNT3, KLOTHO, and FGF23.(19-21) A normophosphatemic form is also known, which is caused by an alteration in the SAMD9 gene.(22) Some papers in the literature hypothesize that idiopathic scrotal calcinosis results from dystrophic calcinosis following cyst rupture; however, other research groups have found no evidence to support this theory.(27) The pathophysiology of the idiopathic subtype is essentially unknown. One of the calcinosis subtypes is referred to as iatrogenic; however, in terms of the pathogenesis, this type is mixed dystrophic and metastatic, as it shares the causative roles of tissue damage and local hypercalcemia caused by the therapeutic agent. The present thesis and other research groups concentrate on dystrophic calcinosis; data concerning the other subtypes are scarce.

Dystrophic calcinosis manifests in the context of disrupted collagen or chronic tissue damage.(28) The precise pathomechanism remains to be fully elucidated. The development of this condition is attributed to a complex interplay of factors, including decreased pH levels that suppress calcification inhibitors and phosphate binding to denatured proteins in necrotic cells.(1, 29) The predominant deposits observed are hydroxyapatite, carbonate apatite, and amorphous calcium phosphate.(7, 10) Chronic inflammation may also play an important role.(30) This theory is supported by the presence of elevated levels of inflammatory cytokines (interleukin 1, 1β, 6, and tumor necrosis factor-α (TNFα)) in the calcified fluid, as well as elevated levels of mannose-binding lectin in the sera of patients with calcinosis.(31, 32) Another contributing factor is presumably tissue hypoxia and oxidative stress. This assertion is corroborated by the overexpression of the hypoxia-associated glucose transporter-1 and advanced glycation/lipoperoxidation end-products and hypoxia-induced vascular endothelial growth factor, as well as reduced perfusion of the calcinosis site.(33-37) In SSc patients,

ulnar artery occlusion, hypoxia-associated osteoclast activity, and digital ulceration have also been demonstrated.(36, 38) Recurrent microtrauma may also contribute to the development of calcinosis. This theory is supported by the observation that calcinosis primarily affects the dominant hand in SSc, a disease characterized by an inherently disturbed compensatory angiogenesis with proliferative obliterative vasculopathy.(39-41) As indicated by the findings outlined in the literature, disturbed extracellular matrix mineralization metabolism has been described as a potential contributing factor. This disturbance is associated with increased levels of calcinosis activator osteonectin, osteoprotegerin, and fibroblast growth factor-23 (FGF23). Additionally, research has indicated a relationship between hypoxia-inhibited vitamin K activity, resulting in the inactivation of the vitamin K-dependent calcinosis suppressor matrix Gla protein.(42-47) Decreased levels of other mineralization inhibitors, including fetuin A and inorganic pyrophosphate, were also identified in the sera of patients.(48, 49) An excess of signal transducer and activator of transcription-1 (STAT), a member of the Janus kinase (JAK)-STAT pathway that regulates mitochondrial calcium release, has also been reported in DM.(50, 51) It is hypothesized that ischemia is the primary pathogenic factor in SSc. In contrast, inflammation is the primary pathogenic factor in DM.(52) Environmental factors may also play a role in calcification, especially in the iatrogenic form. Furthermore, any trauma, particularly if recurrent, has been shown to promote calcium salt deposition. The potential impact of dietary factors on calcification has been postulated based on observations indicating that elevated phosphorus intake and diminished magnesium intake in murine models of pseudoxanthoma elasticum result in accelerated calcification.(53, 54)

1.1.3. Diagnosis of calcinosis cutis

There is no universally accepted diagnostic algorithm for suspected calcinosis, even among the ACTDs, where calcinosis poses a particular challenge. Additionally, it is debatable whether searching is necessary in all ACTD cases or only in symptomatic ones. Another question is in which diseases and risk areas it should be examined. A salient question pertains to the potential of measuring calcinosis extent to monitor disease activity or as an indicator of therapeutic efficacy. A seasoned professional may be capable

of diagnosing calcinosis through a physical examination, particularly if the patient has an underlying predisposing condition. Calcinosis manifests as whitish-yellowish, skin-colored, erythematous papules, plaques, or nodules, exhibiting a bony consistency upon palpation.

1.1.3.1. Imaging modalities

Depending on the accessibility of local resources, different imaging modalities or histopathology may be employed to confirm the diagnosis. Some authors posit that plain radiography should be considered the first-line diagnostic modality due to its cost-effectiveness. Calcinosis can manifest in various forms, including nodular, sheet-like, reticular, amorphous, and linear distribution (Figure 4).(55, 56) Radiographic scoring systems have been established to standardize the evaluation of calcinosis burden.(57, 58) Ultrasound may also facilitate the diagnosis with high specificity and sensitivity, providing excellent visualization of the surrounding tissues. However, other findings call into question the value of this method,(37, 58-60) which is much more time-consuming and depends heavily on the examiner's expertise. On the other hand, it mitigates the risk of radiation exposure. Various imaging modalities are employed in experimental settings, including multidetector or dual-energy computed tomography (CT) and magnetic resonance imaging (MRI). These modalities offer a more comprehensive assessment of the extent and relationship to adjacent tissues.(59, 61) CT has been documented to exhibit superior sensitivity.(62) The MRI technique can be enhanced by incorporating gradient-echo imaging. However, when assessing adjacent tissues, MRI is superior.(63) 18F-fluorodeoxyglucose positron emission tomography has been employed in diagnostic procedures. However, it is plausible that inflammation associated with calcinosis is more readily detectable than the calcification itself.(64, 65)

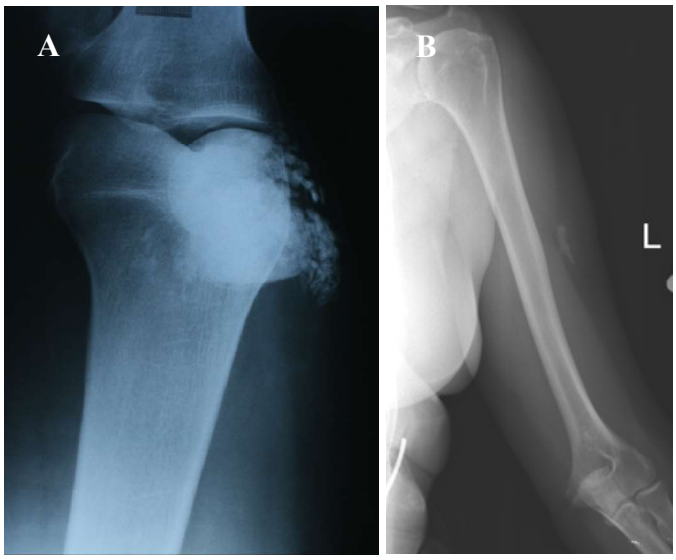

Figure 4 Net-like fine granular dystrophic calcinosis over the patella on plain radiography (A) and dense granular dystrophic calcinosis of the left arm (B) on plain radiography. Modified figure after own publication.(8)

1.1.3.2. Histopathology

An alternative diagnostic approach involves histopathological examination, which necessitates the partial or complete removal of the deposit. Calcium deposits are stained basophilic when subjected to hematoxylin and eosin staining and manifest as black when von Kossa staining is employed (Figure 5).(1) Sparse granular or larger nodular calcinosis can be differentiated (Figure 6). As calcified deposits can be readily identified through radiological imaging and impaired wound healing is linked to calcified lesions, non-invasive confirmation methods are preferable to invasive biopsy procedures. In cases where histopathological confirmation is necessary to ascertain the underlying condition or to remove the deposit due to associated symptoms, this approach is recommended. The differential diagnosis of calcinosis includes ectopic ossification, gout trophies, and cysts.

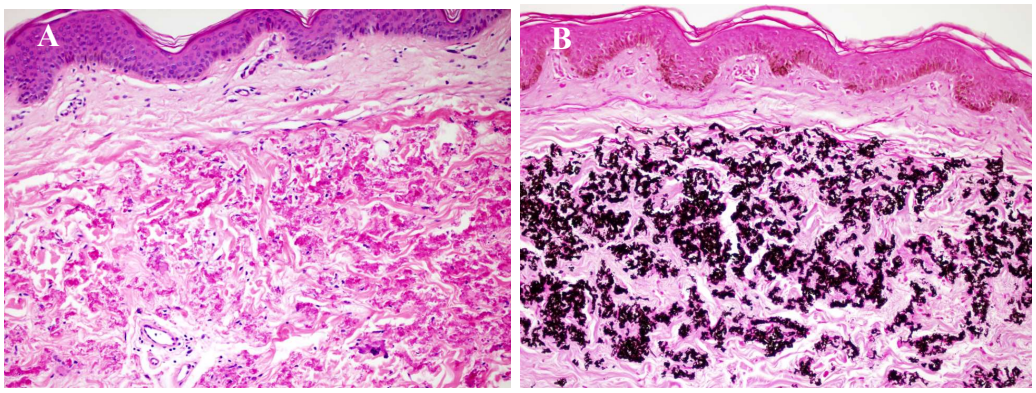

Figure 5 Hematoxylin and eosin (A) and von Kossa staining (B) of fine granular calcification in a skin sample of a pseudoxanthoma elasticum patient (magnification x20). Modified figure after own publication.(2)

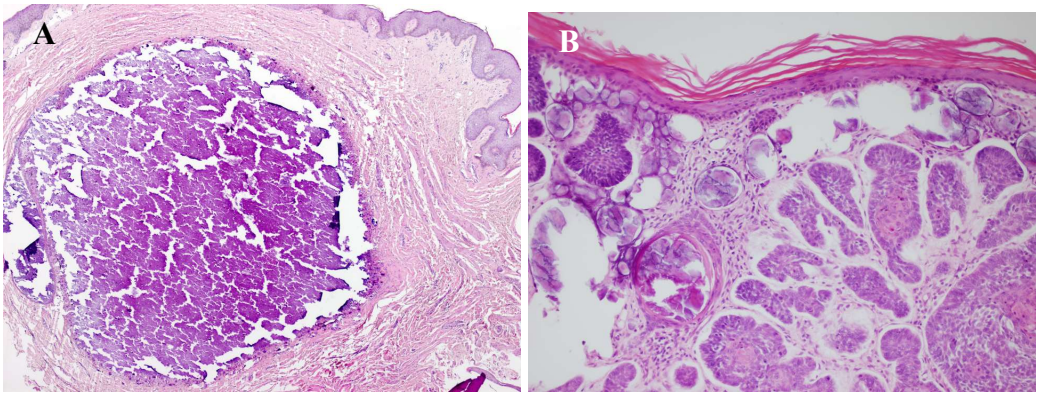

Figure 6 Nodular calcification in scrotal calcinosis (magnification x4) (A) and fine calcification in basal cell carcinoma with hematoxylin and eosin staining on skin samples (magnification x10) (B). Modified figure after own publication.(2)

1.1.4. Treatment of calcinosis cutis

Although calcinosis can be devastating and result in significant impairment to patients' quality of life, there is currently no universially effective treatment available to address this condition. Several pharmacotherapies and procedural therapies have been attempted; however, none have exhibited consistent efficacy. No established therapeutic guidelines exist, and the extant literature on this subject is limited. Due to the relative rarity of the disease and wide background heterogeneity, the implementation of randomized controlled trials is rendered almost unfeasible. The therapeutic recommendations that have been

formulated are, thus, based on case reports, case series, and cohort studies, as well as a very small number of randomized trials. The majority of published reports address the management of dystrophic calcinosis in ACTDs. The selection of therapeutic intervention is contingent upon the extent and location of calcinosis, concomitant symptoms, and comorbidities. In contrast, the underlying ACTD subtype does not appear to be a significant factor in the decision.(52, 66) In some cases, treating the underlying disease is crucial, for example, in cases of inflammation in ACTDs, as DM or vasculopathy in SSc, since ACTD activity is associated with calcinosis burden.(67, 68) Nevertheless, anti-inflammatory therapy for the underlying ACTD is insufficient for managing calcinosis; specific treatment to resolve the mineralization is necessary. It is noteworthy that DM-associated calcinosis has been observed to resolve spontaneously within a few months or years, while SSc-associated calcinosis tends to progress.(69) Calcified nodules frequently result in pain, inflammation, and restricted joint mobility. Ulceration of the nodules is also frequent, often resulting in superinfection and the drainage of liquefied calcium salts. Ulceration represents a particular problem in ACTDs, given the inherently disturbed wound healing and immunosuppressed status (due to disease and therapy) of this population. Another issue to consider is the highly compact nature of the calcified nodules, enabling only modest penetration of the therapeutic agents. In many cases, the blood supply to the surrounding areas is impaired, particularly in ACTDs such as SSc. Another issue that merits attention is the necessity of treatment for asymptomatic micro-calcinosis. In a review, *Elahmar et al.* concluded that pharmacological therapy is not necessary in cases of asymptomatic calcinosis. However, they recommended improving the blood supply and avoiding trauma to maintain the status quo.(70) Conversely, any calcified deposit, even the smallest one, can act as a nucleus for the further enlargement of the deposits. This can endanger ulceration of the overlying skin and lead to associated complications. In the case of symptomatic calcinosis, removal is unquestionable.

There are several pharmacological options to treat calcinosis, targeting different processes. Given the lack of universal efficacy among pharmaceuticals, combined or sequential approaches with multiple pharmaceuticals or procedural treatments are occasionally necessary.(70) The present work does not address the therapy of subtypes other than dystrophic calcinosis, such as metastatic and hyperphosphatemic tumoral calcinosis, which are endocrinology or internal medicine issues, nor iatrogenic or

idiopathic calcinosis, where the primary treatment is surgical removal. The present thesis delves into the treatment of dystrophic calcinosis, focusing on cases stemming from ACTD.

1.1.4.1. Procedural treatments

The efficacy of topical and minimally invasive procedural therapies has been demonstrated for superficial calcified deposits, albeit with variable outcomes. The underlying principle of procedural treatments is the removal or destruction of calcified deposits, to enhance clearance by the phagocyte system or facilitate the penetration of pharmacotherapies.

Laser therapy utilizes an intense beam of light to vaporize calcified deposits. This method offers several advantages, including minimal damage to the surrounding tissues, excellent visualization of the operative site, rapid hemostasis, and satisfactory cosmetic outcomes.(71-75) To ensure patient comfort and prevent pain associated with the therapy, topical or intradermal anesthesia is typically administered. The efficacy of the carbon dioxide laser has been demonstrated in treating digital lesions associated with SSc.(71, 72) A single session is sufficient for lesions measuring less than 2 cms with no reported recurrences.(76) A systematic review of 21 patients treated with different lasers revealed that 71% exhibited a favorable response (57% complete, 14% partial) with concomitant pain relief or cessation of pain.(77) The lasers utilized in the study included carbon dioxide (18 patients), erbium-doped yttrium aluminum garnet (one patient), picosecond with carbon dioxide (one patient), and diode (one patient). The adverse effects observed included infection (11%), recurrence, hypopigmentation, irritation, and, most commonly, scarring or hyperkeratosis (56%).(71, 77-79)

Extracorporeal shock wave lithotripsy (ESWL) is a procedure that utilizes acoustic energy to fragment ureteral stones and is also applied in calcified tendinitis. A low risk of adverse effects characterizes this treatment modality. It was reported to be efficacious for cases of calcinosis in multiple SSc patients, with three sessions, administered at three-week intervals.(80, 81) Ulcerated or small deposits exhibited a high probability of positive response. At the same time, larger lesions have shown minimal or no response.(80) However, the pulverization of calcified masses can potentially enhance the efficacy of the other therapeutic modalities, such as surgery or intralesional sodium

thiosulfate (STS), when utilized as an adjuvant.(80, 82) The decompression of the tissues resulting from the procedure has been demonstrated to alleviate pain.(80, 83) Furthermore, a systematic review of 11 patients revealed a 64% response rate, with reduced pain or cessation of pain in 7/10 patients following ESWL. This corresponded to a median pain relief of three points on the visual analogue scale. The intensity of pain relief increased to eight points in a patient who received intralesional STS after the ESWL.(77) The only adverse effect observed was transient fever in one case.(83) A prospective study of three patients yielded similar results.(81)

Surgical removal is a potential treatment option for calcified deposits. However, calcinosis lesions demonstrate suboptimal healing, and surgical intervention carries inherent risks of infection and impaired range of motion. Consequently, its application should be reserved for symptomatic lesions.(20) Additionally, recurrence has been documented.(73, 84, 85) Indications for surgical intervention include abscessing, localized, large, symptomatic lesions. In light of our observations and the extant literature, we posit that surgical removal constitutes the primary treatment modality for lesions, in which the causative factor can be eradicated with minimal risk of recurrence, for instance, iatrogenic calcinosis, calcifying neoplasms, or trauma-induced calcinosis.(2, 8) A retrospective study of 11 patients found complete response in eight patients and partial response in the other three patients.(66) Another retrospective study of seven patients reported uneventful recovery after complete excision (no safety margin and closure with local flap) with no complications during a 1-year follow-up period.(86) Further case reports support the beneficial effect. Excision has been demonstrated to alleviate pain and result in functional improvement.(66, 73) Superior outcomes and fewer side effects have been observed with high-speed dental bur.(87) In cases where complete removal is not feasible, the drainage or curettage of the inflamed and painful lesions may prove beneficial.(88-90) Minimally invasive debulking can be viable in sensitive areas such as the fingertips.

Acetic acid iontophoresis, followed by ultrasound, disperses acetate, which iontophoresis replaces with carbonate ions in the calcified deposit, forming more soluble calcium acetate. This approach was documented in three patients with SSc-associated

calcinosis. Despite radiological improvement, patients reported no change in their complaints.(91)

Hematopoietic stem cell transplantation has emerged as a potential treatment for recalcitrant severe calcinosis. This therapeutic modality involves restoring immune function by augmenting regulatory T cells and inhibiting auto-aggressive lymphocytes. Experience with this treatment is minimal; however, the results have been encouraging in a small number of SSc patients.(92, 93)

1.1.4.2. Pharmacotherapies

Systemic therapy is considered the optimal approach in multiple, widespread calcinosis cases. The mechanisms of anti-calcinotic pharmacotherapies can be categorized into three distinct approaches: chelating calcium, blocking the cellular calcium channels, and inhibiting calcium mobilization from bones. Calcinosis-related pain, inflammation, and ulceration may also be a therapeutic target. The most commonly utilized agents are calcium channel blockers, primarily diltiazem. However, it should be noted that the pharmacotherapeutic possibilities in question have low evidence (IV) and recommendation grade (C).

In recent years, there has been an increasing interest in STS. This agent exhibits vasodilatory, anti-inflammatory, and chelator properties, yet the underlying mechanism by which it dissolves calcification remains to be elucidated. Its efficacy in treating calciphylaxis, another calcifying disease, has been well-documented, supporting its potential use in calcinosis. However, the extant body of research on this topic remains relatively limited, with only a few cases having been published to date. The discussion section is intended to provide a comprehensive overview of the subject matter of STS therapy, given its close association with this thesis.

Calcium channel blockers, principally *diltiazem,* are the most prevalent treatment of calcinosis, particularly in cases associated with juvenile DM (JDM) and DM.(66, 94-98) The inhibition of calcium uptake into cells may impede the formation of calcified deposits. Its administration is also recommended for severe, extensive calcinosis.(90) The dosage administered ranged from 60 to 480 mg/d. The results are somewhat contradictory, but higher doses seem to have a beneficial effect of partial response, which is expected in

approximately half of the patients.(2, 66, 99-101) We also observed this dose dependence in our practice.(2) Other studies reported no improvement.(102, 103) Overall, no complete response was reported. Potential side effects include nausea, headache, hypotension, peripheral edema, and drug interactions. A combination of prednisolone, pamidronate, and aluminum hydroxide was also reported.(90)

Bisphosphonates inhibit bone resorption, thereby reducing calcium release and inflammatory cytokine production by macrophages.(31, 104) Consequently, these drugs have been shown to alleviate pain and enhance mobility. Authors reported efficacy of bisphosphonates (alendronate, disodium etidronate) in addressing calcinosis in patients with DM and SSc with 10 mg/kg/d orally or 1 mg/kg/d intravenously.(31, 105-107) Other doses administered included etidronate 800 mg/d, alendronate 10 mg/d, and intravenous pamidronate 70-75 mg for three consecutive days in three monthly intervals.(90) A retrospective study with seven patients revealed that intravenous pamidronate achieved radiological improvement in half of the cases. The vast majority of the patients also reported alleviation of associated symptoms.(108) However, other reports found no improvement.(66, 109) The possible side effects include fever and mandibular osteonecrosis.(90)

Colchicine exerts its therapeutic effect by impeding microtubule polymerization, disrupting chemotaxis, and phagocytosis. This mechanism contributes to its anti-inflammatory effect. In some cases of DM and SSc, the administration of 1 mg/d reduced calcinosis-associated inflammation, although the calcified deposits did not regress.(66, 110) A dosage of 0.6 mg twice daily was also administered. Potential adverse effects include gastrointestinal symptoms.(90)

Minocycline, a calcium-chelating agent, has been shown to alleviate inflammation by inhibiting matrix metalloproteinases. Consequently, it may be a viable treatment for calcinosis-associated inflammation and ulceration prevention.(104, 111) However, a subsequent study reported no significant impact on calcinosis.(66) The recommended dosage is 100 mg/d. Adverse effects may include dizziness, nausea, and dark discoloration of calcified lesions.(90)

Ceftriaxone was administered intravenously at a dosage of 2 g/d for a duration of 20 days to a patient diagnosed with morphea-associated calcinosis. Ceftriaxone is an antibiotic with anti-inflammatory properties and the capacity to bind calcium.(112) The available data on this treatment method is currently quite limited.

Rituximab, a B-cell depleting agent that inhibits CD20, was also evaluated. The authors reported a significant improvement in calcinosis after 7-12 months in SSc.(113, 114) However, no improvement with rituximab was also published.(115, 116) The doses administered ranged from 375-1000 mg/$m^{2,}$ two or four times. Potential adverse effects include infusion reactions, infections, and neutropenia.(117, 118)

Infliximab*,* a TNFα inhibitor, was administered based on the rationale that calcinosis and the TNFα-308 A promoter polymorphism may be associated.(119) The efficacy of infliximab in JDM has been demonstrated.(120, 121) Adverse effects include infection and tumor propagation.(90)

Tofacitinib JAK inhibitor therapy has emerged as a potential therapeutic option, given the established role of the JAK-STAT pathway in the cutaneous manifestations of DM, involving the expression of proinflammatory cytokines.(51, 122) Two DM patients with calcinosis showed notable improvement after 28 weeks of tofacitinib 5 mg twice daily. The only reported adverse effects were weight gain and transient hypercalcemia.(90, 122)

Intravenous immunoglobulin is an anti-inflammatory therapy in doses of 2 g/kg/month that has been proposed as a treatment for calcinosis with positive results in SSc and mixed results in DM.(35, 123, 124)

Intralesional corticosteroids inhibit the proliferation of fibroblasts and the progression of inflammation. Consequently, these medications may potentially reduce the occurrence of calcinosis. Triamcinolone acetonide 20 mg/ml or triamcinolone diacetate 25 mg/ml monthly was utilized in SSc.(125) Adverse effects include excess hair growth at the treatment site and injection-related discomfort.

Warfarin is a vitamin K antagonist. It has been posited as a potential therapeutic agent in calcinosis; however, its effect is contradictory. It has been demonstrated to inhibit the vitamin K-dependent carboxylation of glutamic acid, which has been linked to a decrease

in matrix Gla protein, which is thought to inhibit calcification.(104, 126) However, the literature on the subject is inconclusive, with some studies suggesting that it may either alleviate or promote calcification.(127, 128) There is a body of research that supports the efficacy of warfarin, as well as research that contradicts this claim.(66, 129-132) The dose administered was 1 mg/d. It is essential to acknowledge that the available studies have a limited sample size, which restricts the overall strength of the evidence. Consequently, the utilization of warfarin in the management of calcinosis may not be recommended.(133)

Probenecid promotes phosphate excretion by the kidneys, thereby inhibiting calcification in doses ranging from 250 to 1500 mg/d.(28, 134) It has been observed to be efficacious in calcinosis associated with JDM.(134-137)

Aluminum hydroxide sequesters phosphorus and inhibits its gastrointestinal absorption, reducing the formation of hydroxyapatite and calcium phosphate, thereby reducing the calcinosis burden.(138) Publications have reported on the tolerability and effectiveness of aluminum hydroxide in treating calcinosis in JDM.(139-141) The recommended dosage range is 1.8 to 2.4 g/d. It is generally well tolerated; however, potential side effects include hypomagnesemia, hypophosphatemia, constipation, anemia, and dementia or cognitive decline with chronic use.(90)

Treprostinil, a prostacyclin analog, is administered intravenously in 0.125 mg three times daily doses, with escalation of 0.125 mg every 3-4 days, up to the maximum tolerated dose. It is employed in treating SSc to address its role as a vasodilator, aiming to improve pulmonary hypertension and digital ulcers. Calcinosis in SSc is associated with digital ulcers; hence, treprostinil's potential as a preventative measure against calcinosis enlargement is noteworthy. However, it is essential to note that the tolerability of this therapy is suboptimal, with frequent adverse effects, including headaches and gastrointestinal complaints.(142)

Neem oil with* Hypericum *plant extract, a mixture of neem oil (extract from *Azadirachta indica*) and *Hypericum perforatum*, was applied for calcinosis-associated ulceration in 33 SSc patients. This mixture is widely used in wound care. The study's findings revealed

that 45% of the subjects experienced complete healing, with a significant improvement for the remaining lesions. Notably, no recurrence was observed.(143)

In a review, *Lau et al.* recommended treatment of the underlying ACTD to prevent calcinosis formation or progression. A synopsis of the treatment options reveals the following recommendations: for cases of diffuse, inflamed, ulcerated calcinosis, administration of colchicine or minocycline is advised, while in the absence of inflammation, diltiazem is recommended. For limited lesions, the treatment of choice is topical STS for smaller (less than 0.2 cm) and intradermal STS for larger (less than 2.0 cm) lesions (which are discussed in more detail in the discussion section), or surgery in case of associated complaints such as pain.(90) Surgical removal should also be carried out for those lesions where the causative factor can be removed with a low risk of recurrence, and topical therapy can be chosen for idiopathic or iatrogenic calcinosis, where the trigger factor is no longer present.(77)

1.2. Sodium thiosulfate therapy

1.2.1. Sodium thiosulfate in general

STS (pentahydrate) is a Food and Drug Administration (FDA)-approved, inorganic, water-soluble agent with anti-inflammatory, chelator, vasodilator, and antihypertensive properties.(144, 145) It also exhibits antioxidant properties by donating electrons, scavenging mitochondrial reactive oxygen species, and forming glutathione in a reaction with superoxide.(146) In addition, it has been demonstrated that *in vivo*, it is the main oxidation product of the endogenous gasotransmitter hydrogen sulfide (HS).(147) HS regulates several physiological cellular functions, including apoptosis, inflammation, proliferation, angiogenesis, oxygen sensing, and metabolism.(148-154) It has been demonstrated to relax vascular smooth muscle, and it functions as an antioxidant by scavenging free radicals, inhibiting mitochondrial reactive oxygen species production, and increasing glutathione production.(155-161) As reported by *Kimura et al.*, HS generates more efficient polysulfides.(162, 163) It is assumed that reactive sulfur species (thiyl radicals, disulfides, sulfenic acids, and disulfide-S-oxides, etc.) play a pivotal role in the mechanism of sulfide action.(164, 165) These elements are produced in the sulfide or sulfate oxidation pathway and alter the activity of redox-sensitive proteins and

enzymes by oxidation and reduction.(166) Physiological storage is HS, acid-labile sulfide and sulfane sulfurs (glutathione, persulfides, polysulfides, polythionates, thiosulfate, and thiosulfonates).(165-167) A reduced level of HS has been reported in animal models of hypertension, atherosclerosis, ischemia-reperfusion injury, Parkinson's disease, etc.(168-171) There was a statistically significant decrease in plasma levels of acid-labile sulfide and sulfane sulfur in patients diagnosed with cardiovascular disease.(167, 172) In the mitochondria, thiosulfate (and its sodium salt) is produced from HS by quinone oxidoreductase, sulfur dioxygenase, rhodanese (thiosulfate sulfurtransferase), and 3-mercaptopyruvate sulfurtransferase enzymes.(173, 174) STS may also be generated from methemoglobin via flavin oxidoreductase.(175) The reverse reaction is catalyzed by thiosulfate reductase and sulfite oxidase in a glutathione-dependent manner, except in the brain, heart, intestine, testes, and colon, which lack these enzymes; thus, STS is the primary metabolite in these tissues.(174, 176-178) *Olson et al*. posited that in normoxia, HS is oxidized to STS, and hypoxemia or a reducing environment leads to the release of HS from STS. Therefore, it can be inferred that STS also plays a role in oxygen sensing.(154) STS transportation across the cell membrane is conducted by a sodium-sulfate co-transporter.(178) Thiosulfate is an unstable sulfane sulfur, which easily oxidizes and reduces with thiols.(179, 180) The scientific community has yet to reach consensus on whether the release of HS is the key action in the effect of STS or if STS itself is the executive signaling molecule, and the exact mechanism remains to be fully elucidated.(144) *Sen et al*. observed that oral thiosulfate increased depleted HS levels and restored endothelial function in a murine model of heart failure. This observation led to further publications on the successful administration of STS in cardiovascular disease models.(144, 181) As *Tokuda et al*. reported, inhalation of HS led to increased plasma thiosulfate levels and rhodanese activity, which prevented lipopolysaccharide-induced inflammation in mice in a dose-dependent manner.(182) *Shirozu et al.* marked STS as the potential cytoprotective intermediate.(151) In addition, sodium sulfide increased intracellular levels of STS, which was also achieved by exogenous STS. The latter increased intracellular levels of STS but not sulfide and persulfides. The protective effect of sodium sulfide diminished with the removal of STS, and was restored when STS was added.(178) This suggests that thiosulfate is the signaling molecule in cellular regulatory processes, not HS.(183)

1.2.2. Medical use of sodium thiosulfate

In recent years, the therapeutic effects of STS have garnered researchers' interest, resulting in an increased number of publications on the subject. Beyond its medical applications, STS finds use in food preservation, water dichlorination, bleaching, and photographic processing. The initial medical application of STS was in the treatment of acute cyanide poisoning with sodium nitrite. In this treatment, STS donates sulfur to the rhodanese enzyme, converting the toxic cyanide into the nontoxic thiocyanate.(184-186) Furthermore, STS functions as an optional antidote for carbon-monoxide toxicity, aniline poisoning, nitrobenzene poisoning, and acute sulfide poisoning.(187-190) STS also plays a protective role in cisplatin chemotherapy, preserving renal, auditory, neurological, and hematological functions.(191-195) The capacity in question has been hypothetically linked to platinum binding and total clearance of metabolites.(196) It is a well-known and effective therapy for calciphylaxis, delaying the progression of arterial calcification.(197-199) Hypothetically, it may also play a role in treating neuronal ischemia, heart failure, hypertension, acute lung injury, pneumonia, obesity, and diabetes.(144, 200)

Moreover, the protective antioxidant effect of STS was reported in experimental rat and murine models of angiotensin II-induced renovascular hypertension, congestive heart failure, renal injury, and ischemia-reperfusion injury.(181, 201-205) Recent studies have indicated that STS has the potential to supplant the University of Wisconsin cold storage solution in the preservation of harvested donor kidneys before transplantation. The rationale for this substitution is that STS has been demonstrated to yield enhanced survival and improved function in stored and perfused kidneys.(206) The efficacy of STS has also been demonstrated in managing recurrent urinary stones in rat and insect models, lung injury, and neurodegenerative diseases.(178, 207-210)

1.2.3. Safety issues of sodium thiosulfate

STS has been demonstrated to be a safe therapeutic agent with low toxicity, as evidenced by various studies, with a lethal dose of 2.5 g/kg intravenously in rats. The side effects of this treatment are generally tolerable and manageable compared to those observed with other sulfide donors. The most common adverse effects reported are gastrointestinal symptoms (nausea, vomiting, diarrhea), hypotension, and hypernatremia.(144) *Neuwelt et al*. reported no significant adverse effects with doses of 16–20 g/m^2 intravenously in

humans in carboplatin ototoxicity.(211) As demonstrated in the study of *de Koning et al*., a 15 g intravenous dose was also well tolerated and safe in patients with acute coronary syndrome.(212) Further studies reported an 8% to 15% frequency of metabolic acidosis with doses of 10–25 g/d in dialyzed patients.(198) It is also approved for children to treat cisplatin-induced ototoxicity.

1.2.4. Sodium thiosulfate therapy in calcifying disorders

Intravenous STS is among the first-line treatment options for calciphylaxis, a calcifying disorder characterized by calcification of the medial layer of the arterioles, resulting in subcutaneous tissue necrosis.(213, 214) This condition is associated with an increased risk of cardiovascular death.(215) The oral route has also been documented in a few patients, with doses ranging from 1.8 to 3.6 g/d.(216) Intralesional STS (250 mg/ml) has demonstrated encouraging outcomes in active areas of the disease. Improvement could be expected after a couple of weeks with no recurrence.(197) This is attributable to its vasodilatory, antioxidant, and calcium-chelating properties, resulting in dialyzable calcium thiosulfate.(145, 198) The promising results support its utilization in other calcifying disorders, primarily calcinosis cutis.

2. Objectives

Calcinosis cutis is a long-known yet scarcely investigated disorder. Given the relative rarity of the disease and the broad background heterogeneity, investigation of this condition is challenging. Consequently, retrospective observations are of paramount importance. It manifests as a complication of various entities, most commonly ACTDs. Although it is referred to as a rare complication, it impacts a significant number of ACTD patients. However, the paucity of data regarding its precise prevalence remains a significant limitation. The issue of treatment remains unresolved. Ulceration and the associated pain have been shown to have a substantial impact on patients' daily lives. Consequently, the development of effective therapeutic interventions is imperative. In recent years, attention has been drawn to STS, which is used as a first-line agent in treating uremic calciphylaxis, a calcifying disorder, where the efficacy of systemic administration has been demonstrated. Additionally, there is encouraging data on using STS for smaller calcinosis lesions via topical or intradermal administration. While these positive results support the beneficial effects of oral or intravenous administration in calcinosis, there is still minimal evidence in the literature (a few case reports), and much remains to be uncovered.

The present research sought to investigate the epidemiology and treatment of calcinosis cutis in ACTDs, with a focus on the following questions:

1. Study I: To evaluate the prevalence of calcinosis cutis in ACTDs at our Department of Dermatology between 2003 and 2023.
   - *1.1.* Are there any discrepancies in the frequency of calcinosis formation among the various ACTD subsets?
   - *1.2.* Are there any differences in the mean age of onset of calcinosis and the mean time interval from diagnosis of the ACTD, location, and ulceration frequency between different ACTD subsets?
   - *1.3.* Are there any autoantibodies that exhibit a significant correlation to calcinosis?

2. Study II: To evaluate the efficacy of STS therapy, administered orally and intravenously, in the management of dystrophic calcinosis cutis.
   2.1. What is the dosage and administration frequency of the therapy?
   2.2. What is the response to therapy, and are there any subpopulations that would derive greater benefit from the treatment?
   2.3. What are the adverse effects and the tolerability of the therapy?

3. Methods

The Regional Institutional Scientific and Research Committee of Semmelweis University, Budapest, Hungary, waived ethical approval for both studies, license numbers: 257/2023 (date of approval 30/11/2023) and 5/2024 (date of approval: 29/01/2024). All patients included in the studies were treated at the Department of Dermatology, Venereology, and Dermatooncology, Faculty of Medicine, Semmelweis University, Budapest, Hungary, a regional dermatological disease center. All patient data (medical reports, laboratory results, immunserology results, and imaging reports) were collected and retrieved from our department's dedicated medical reporting and data retrieval system (eMedSolution, T-Systems Hungary Ltd., Budapest, Hungary, Version: 2024/Q1/1). All patients have provided informed consent for utilizing and publishing their clinical photographs captured during medical visits.

3.1. Methods of study I

A retrospective cross-sectional study was conducted in our department to obtain information on the prevalence of calcinosis in ACTDs. For identifying patients with an ACTD, annual code statistics of the International Classification of Diseases 10th Revision codes for ACTDs were carried out from 1st January 2003 to 1st January 2024 in the eMedSolution medical reporting and data retrieval system. The search codes are outlined in Table 2. These diagnostic codes cover the following diseases: diffuse cutaneous SSc (dcSSc), lcSSc, DM, JDM, SLE, mixed connective tissue disease (MCTD), undifferentiated connective tissue disease (UCTD), and autoimmune connective tissue overlap syndromes.

Table 2 International Classification of Diseases, 10th Revision codes searched for in our retrospective calcinosis prevalence study

Code	Encoded disorder
M3210	Systemic lupus erythematosus with organ and organ system involvement
M3280	Other forms of systemic lupus erythematosus
M3290	Systemic lupus erythematosus not otherwise specified
M3300	Juvenile dermatomyositis
M3310	Other dermatomyositis
M3400	Progressive systemic sclerosis
M3410	CR(E)ST syndrome
M3480	Other forms of systemic sclerosis
M3490	Systemic sclerosis not otherwise specified
M3510	Other overlap syndromes
M3580	Other specified systemic disease of connective tissue
M3590	Systemic involvement of the connective tissue
L9420	Calcinosis cutis

Abbreviations: CR(E)ST syndrome calcinosis-Raynaud sign-esophagus dysmotility-sclerodactyly-telangiectasia syndrome (limited cutaneous systemic sclerosis)

This method yielded a total of 1,576 cases. The cases were manually assessed by reading the available medical reports in the data retrieval system to determine whether an expert dermatologist, rheumatologist, or immunologist had confirmed the ACTD diagnosis. Duplicate cases that were encoded for multiple ACTDs were counted once. Due to the absence of comprehensive documentation of all symptoms and alterations, retrospective reclassification of the subtypes was impossible. Consequently, reliance was placed on expert opinion, as documented in the medical reports. Cases in which the diagnosis was either incorrect or lacked confirmation were excluded from the analysis. After excluding cases that were incorrectly classified, 839 ACTD patients were identified. The electronic medical records of these patients were manually reviewed for the presence of calcinosis. Cases in which calcinosis was not confirmed by radiological imaging, histopathology, or an expert dermatologist, rheumatologist, or immunologist were excluded. The following information was extracted: sex, underlying ACTD, age at diagnosis of ACTD, age at

diagnosis of calcinosis, time interval between the diagnosis of the ACTD and onset of calcinosis, location of calcinosis, ulceration at calcinosis sites, detectable autoantibodies in calcinosis patients' blood sera, histopathological reports, and reports on confirmatory imaging techniques (plain radiography, ultrasonography, MRI, and CT). Histopathological examinations were conducted on skin samples stained with hematoxylin and eosin. Immunserology examinations of patients' sera were carried out to detect commonly associated autoantibodies using immunofluorescence technique on Hep-2 cells, enzyme-linked immunosorbent assay (ELISA) technique, and immunoblotting. A variety of commercially available kits have been utilized over the years.

3.2. Methods of study II

A retrospective case series study was conducted in our department to evaluate the effectiveness of systemic (oral and intravenous) STS therapy for calcinosis. Initially, patients diagnosed with calcinosis were identified using annual code statistics of the International Classification of Diseases 10th Revision codes, obtained from the eMedSolution data retrieval system. The search code was for calcinosis cutis, L9420. The research period covered the time interval between 1st January 2003 and 1st January 2023. A manual review of cases coded as calcinosis was conducted with special regard to confirming calcinosis and utilization of STS therapy. Cases in which only topical or intralesional STS had been administered were excluded from the study. Seven cases were identified that had been treated with systemic STS for dystrophic calcinosis. A comprehensive evaluation of the medical reports from the attending physician, photographic records of the calcinosis lesions, laboratory reports, and radiological reports was conducted. The following data were collected: sex, underlying disease, activity of the underlying ACTD, prior and concomitant immunosuppressive therapy, prior and concomitant therapy against calcinosis, location of calcinosis, extent of calcification, presence of ulceration, age at onset of calcinosis, age at the start of STS therapy, duration of calcinosis at start of STS therapy, dosage and frequency of intravenous and oral STS therapy, treatment duration, response, method of evaluation of the response, serum

calcium and phosphate levels before, during, and after treatment, side effects, patient complaints, patient compliance, and reason for discontinuing STS therapy.

The therapeutic agent was prepared at the University Pharmacy of Semmelweis University. For oral treatment, enteric-soluble STS capsules containing 1–2 g of STS pentahydrate (*Merck KGaA*, Darmstadt, Germany) with no excipients were prepared. The intravenous solution was composed of 392.3 g of STS pentahydrate (*Merck KGaA*, Darmstadt, Germany), 5.22 g of disodium phosphate dehydrate, and sodium hydroxide for pH adjustment (up to a maximum of 0.001 g). Water for injection was added to yield a total volume of one liter of solution. One or two ampoules of the solution (each containing 12.5 g of STS) were infused over 30 minutes, diluted in 500 ml of saline. The duration of the therapy and the total dosage were contingent upon the specific clinical scenario and the patient's tolerance for adverse effects. The response to treatment was evaluated based on reports from the attending physician and photographic and radiological reports. A complete response indicated total healing, partial response referred to regression in size, improvement in associated symptoms and complications, or stabilization followed by progression after discontinuation, and no response indicated no change or progression.

A comprehensive literature review was also conducted using the *PubMed* online database and the keywords 'sodium thiosulfate', 'calcification', and 'calcinosis cutis' to identify studies on orally or intravenously administered STS therapy in calcinosis. Cases with insufficient or inadequate data regarding STS dosage were excluded from the analysis. This literature review identified ten patients. The data concerning these cases' epidemiology and STS therapy were subsequently subjected to a data analysis.

4. Results

4.1. Results of study I

4.1.1. Calcinosis prevalence

A total of 839 patients had a confirmed ACTD, with a male-to-female ratio of 159:680, of whom 6.67% (56/839) exhibited calcinosis (male-to-female ratio = 11:45). The presence of calcinosis was confirmed by plain radiography, ultrasonography, MRI, histopathology, or two different modalities in 22/56, 8/56, 3/56, 9/56, and 9/56 cases, respectively. SLE was the most frequently diagnosed ACTD (n = 464, with a male-to-female ratio = 44:420), calcinosis was observed in 1.94% (9/464) of the patients. This was followed by DM, which was diagnosed in 175 patients (male-to-female ratio = 58:117). 156/175 were diagnosed with the adult form, while 19/175 patients were diagnosed with the juvenile form. 19/175 patients exhibited calcinosis, with a prevalence of 8.33% (13/156 patients) in DM and 31.58% (6/19 patients) in JDM. SSc was diagnosed in 155 patients (male-to-female ratio = 28:127), of whom 118/155 had dcSSc and 37/155 had lcSSc. Calcinosis was detected in 14.19% (22/155) of SSc patients, with a prevalence of 11.02% in diffuse type (13/22) and 24.32% (9/22) in the limited type. A total of 25 overlap connective tissue syndromes were identified (male-to-female ratio = 3:22). 12% (3/25) exhibited calcinosis, exclusively female patients. The underlying conditions were classified as SLE/rheumatoid arthritis, DM/SLE, and DM/SSc overlap. The study population included 15 patients diagnosed with UCTD (male-to-female ratio = 2:13), none of whom exhibited calcinosis. The study identified five female patients with MCTD, calcinosis was observed in 3/5 patients. The prevalence of calcinosis in different ACTDs in our population is shown in Figure 7.(217)

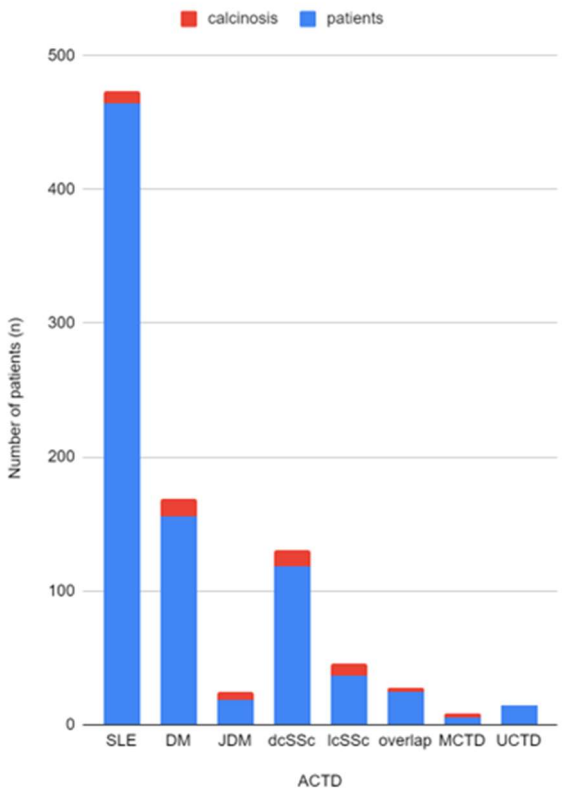

Figure 7 Prevalence of calcinosis in different ACTDs in our study population(217)

4.1.2. Characteristics of calcinosis in different autoimmune connective tissue diseases

In the calcinosis population, the mean age at the diagnosis of the underlying ACTD was 41.16 ±19.47 years (range 4–75 years). The mean age at onset of calcinosis was 47.49 ±20.91 years (range 4–75 years), and the mean time to the onset of calcinosis was 5.96 ±8.62 years (range −11 to 33 years). SSc was the most prevalent ACTD complicated by calcinosis (22/155 patients). The extremities were most commonly affected in 30/56 patients. This was followed by the trunk, and more than one site in 10/56 and 14/56 cases, respectively. Ulceration was observed in 39.28% of the cases.(217)

In SSc complicated by calcinosis, in the diffuse type, locations were observed in the lower extremities, upper extremities, trunk, and more than one area in 8/13, 5/13, 1/13, and 2/3 cases, respectively. In the limited type, the upper extremities, lower extremities, and more than one area were involved in 6/9, 4/9, and 1/9 cases, respectively. Ulceration complicated approximately half of the cases (7/13 in dcSSc and 4/9 patients of lcSSc). Among patients with calcinosis and DM, the distribution of locations across the upper

extremities, trunk, lower extremities, and more than one area was observed in 8/13, 6/13, 4/13, and 4/13 patients, respectively. In JDM, the distribution of locations was as follows: the upper extremities, lower extremities, trunk, and more than one area in 4/6, 2/6, 1/6, and 1/6 cases, respectively. Ulceration occurred in only one case in both the DM and JDM groups.(217) In SLE complicated by calcinosis, the affected area was the upper and lower extremities, trunk, and more than one area in 6/9, 6/9, 1/9, and 3/9 patients, respectively. Ulceration was observed in 6/9 patients. Among the patient population with overlap connective tissue syndromes and calcinosis, the location of the lesions was observed to be in the lower extremities in 1/3, and in more than one area in 2/3 patients. Ulcerations were noted in 2/3 patients. In MCTD, calcinosis occurred involving the lower and upper extremities in 2/3 and 1/3 patients, respectively. Ulceration was reported in one patient. The characteristics of our calcinosis population are presented in Table 3.(217)

Table 3 Characteristics of our calcinosis population. Modified table after own publication.(217)

ACTD	Calcinosis (n)	Male (n)	Female (n)	Mean age at diagnosis (y) (± SD)	Mean age at calcinosis onset (y) (± SD)	Time interval to calcinosis (y) (± SD)
DM	13	4	9	52.23 (± 14.70)	55.08 (± 15.63)	2.85 (± 4.69)
JDM	6	5	1	7 (± 2.37)	9.17 (± 3.31)	2.17 (± 1.17)
dcSSc	13	1	12	39.85 (± 16.32)	45.85 (± 17.45)	6.31 (± 6.75)
lcSSc	9	0	9	53.89 (± 17.13)	60.75 (± 21.82)	2.25 (± 4)
SLE	9	1	8	35 (± 15.84)	50.22 (± 16.38)	15.22 (± 11.97)
Overlap	3	0	3	45 (± 4)	55.67 (± 15.57)	10.67 (± 14.36)
MCTD	3	0	3	43.67 (± 9.02)	46.67 (± 19.65)	3 (± 13.53)
UCTD	0	0	0	-	-	-

Abbreviations: ACTD, autoimmune connective tissue disease; dcSSc, diffuse cutaneous systemic sclerosis; DM, dermatomyositis; JDM, juvenile dermatomyositis; lcSSc, limited cutaneous systemic sclerosis; MCTD, mixed connective tissue disease; n, number of patients; SD, standard deviation; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease; y, years

4.1.3. Associated autoantibodies

Anti-nuclear antibodies were detected in the patients' sera with the immunofluorescence technique in 89.28% (50/56) of the patients. A minimum titer of 1:160 was observed in 76.78% of the cases. The immunofluorescence technique revealed positivity for anti-chromatin and anti-cytoplasm antibodies in 42.85% and 17.85% of the calcinosis patients, respectively. Extractable nuclear antibody (ENA) and anti-SSA were detected using ELISA technique in 33.92% and 25% of the patients, respectively. SSc patients with calcinosis demonstrated positivity for anti-Pm/Scl, anti-centromere, and anti-Scl-70 antibodies in 7/22, 6/22, and 4/22 cases, respectively. Patients with DM demonstrated positivity for anti-cytoplasm, anti-Mi2α, anti-Pl-12, anti-Pl-7, anti-NXP2, and anti-Tif1γ in 3/19, 3/19, 2/19, 1/19, 1/19, and 1/19 patients, respectively. SLE patients with calcinosis demonstrated positivity for anti-nucleosome, anti-dsDNA, SSA, anti-RNP/Sm, and anti-β2GPI in 6/9, 5/9, 5/9, 4/9, and 3/9 cases, respectively. Due to its high prevalence in autoimmune diseases, ANA was not considered a relevant associated autoantibody. Consequently, no specific antibodies were identified as being associated with calcinosis.(217)

4.2. Results of study II

4.2.1. Characteristics of sodium thiosulfate therapy

The study group comprised six female patients with ACTD and one male patient with localized scleroderma (morphea). The mean age at the onset of calcinosis was 41.1 ±15.6 years (range 25-70 years). The duration of calcinosis before initiating STS therapy was an average of 3.8 ±4 years (range 0-11 years). Notably, except for one patient with progressing SSc, all other patients were in an inactive stage of their ACTD during the STS therapy. Except for one patient with secondary hyperparathyroidism, which manifested years after the onset of calcinosis, the study population exhibited no relevant comorbidities. Hyperparathyroidism maintains an environment conducive to calcification, potentially contributing to calcinosis development. The vast majority of patients have previously undergone immunosuppressive therapy (systemic corticosteroids, methotrexate, azathioprine, cyclosporine, mycophenolate mofetil, cyclophosphamide, rituximab, and chloroquine) for their ACTD. However, these therapies did not improve calcinosis. Therapeutic interventions for calcinosis (diltiazem, colchicine, doxycycline, and ESWL) demonstrated no efficacy in 3/4 of patients. Most patients exhibited multiple smaller calcified lesions in the upper extremities (elbows, wrists, and fingers), involvement of the lower extremities (predominantly shins), trunk, and widespread was seen in 3/7, 2/7, 1/7, and 1/7 patients, respectively. The following related complaints were reported: ulceration with liquefied calcium drainage, pain, restricted joint mobility, and recurrent inflammation in 5/7, 4/7, 3/7, and 3/7, respectively.(9)

The therapeutic approach encompassed three distinct schemes: sequential intravenous and oral administration, simultaneous intravenous and oral delivery, and exclusive oral STS in 5/7, 1/7, and 1/7 patients, respectively. The dosage of intravenous STS ranged from 12.5 to 25 g, administered two or three times per week for one week per month, resulting in a total monthly dose of 50-125 g. The mean treatment duration was 4.5 ±3.9 months (range 1-12 months). The dosage of oral STS ranged from 1 to 8 g per day, with an average duration of 25.7 ±38.3 months (range 7-112 months). The decision to transition from intravenous to oral administration was predicated on patient convenience and enhanced tolerance of adverse effects. An analysis of the data about STS dosage and administration frequency is summarized in Table 4.(9)

Table 4 Data of systemic sodium thiosulfate therapy for dystrophic calcinosis cutis in our department between 2003 and 2024. Modified table after own publication.(9)

Disease	Age (calcinosis duration) at start (y)	Intra-venous STS (g)	Frequency	Duration (months)	Oral STS (g/d)	Duration (months)	Response
SSc	38 (6)	12.5-25	2-3x/w/m	2	2-6	9	NR
DM	54 (1)	12.5-25	3x/w/m	4	8	19+	PR
MCTD	36 (11)	25	3x/w/m	5	2-4	7	NR
SLE	37 (1)	25	4-5x/m	12	1-8	112+	PR**
SSc	44 (2)	25	3x/w/m	1	2-8	11	PR
SLE	70 (0)	25	2x/w/m	3	3-4	14	NR
deep morphea	36 (6)	-	-	-	2-6	8	PR

Abbreviations: DM, dermatomyositis; g, gram; g/d, grams daily; MCTD, mixed connective tissue disease; NR, no response; PR, partial response; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; STS, sodium thiosulfate; x/m, times monthly; x/w/m, times per week monthly once; +, ongoing at the time of publication; y, years; **: concomitant diltiazem therapy

4.2.2. Outcome and efficacy of sodium thiosulfate therapy

The majority of patients exhibited adequate compliance, with only 2/7 patients demonstrating non-compliance. The therapeutic endpoints included continuous administration without adverse effects, discontinuation due to inefficacy, discontinuation for other reasons, and loss of follow-up in 2/7, 2/7, 2/7, and 1/7 of the patients, respectively. Complete, partial, and no response were observed in 0/7, 4/7, and 3/7 patients, respectively. Despite the absence of complete responses, 57.1% of the patients exhibited improvement with STS therapy, particularly those who could tolerate higher STS doses. Lesions that were smaller, inflamed, or ulcerated demonstrated a greater probability of positive response (Figures 8-10). The response was defined as follows: radiographic improvement, a reduced number and/or size of the lesions, softening of the nodules, healing of the ulcers, cessation of pain, decreased frequency of inflammation, and/or improved mobility of adjacent joints.(9)

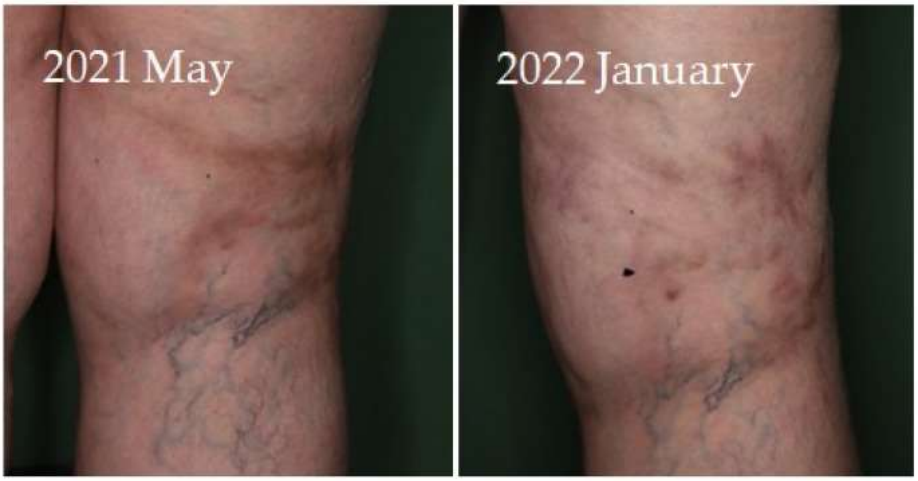

Figure 8 Improvement of inflamed calcified lesions on the thigh of a female DM patient receiving STS therapy, photographed at initiation and at 6 months of treatment. Patient reported cessation of pain after the first three STS infusions.(9)

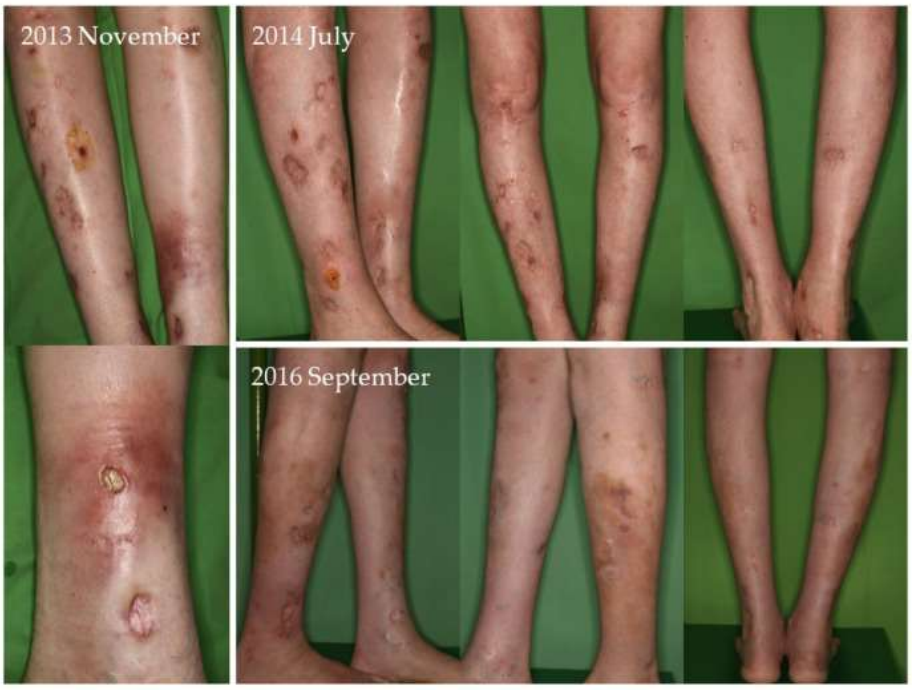

Figure 9 Ulcerated calcinosis lesions of the lower extremities in a female SLE patient showing improvement with significantly less frequent infections during systemic STS therapy, photographed at initiation, 8 months, and 3 years of treatment.(9)

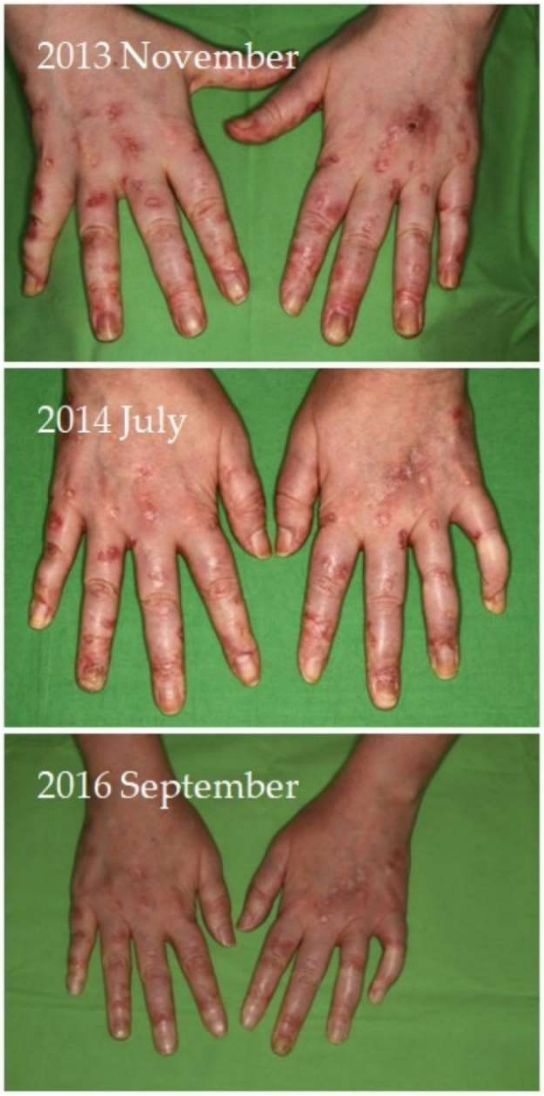

Figure 10 Calcinosis of the hands of a female SLE patient showing continuous regression during systemic STS therapy, photographed at initiation, 8 months, and 3 years of treatment.(9)

4.2.3. Adverse effects and tolerability of sodium thiosulfate therapy

The observed adverse effects included tolerable gastrointestinal complaints (nausea and dose-dependent diarrhea) in 5/7 patients and minor fatigue. No severe or persistent adverse effects were reported. Serum calcium and phosphate levels remained within normal ranges before and after treatment. In one case, despite prior calcium supplementation for osteoporosis, STS (a chelating agent) demonstrated a significant effect without any progression of osteoporosis or adverse side effects after a 112-month follow-up period.(9)

5. Discussion

5.1. Calcinosis in autoimmune connective tissue diseases

Systemic sclerosis may be complicated by calcinosis. It has been postulated that this may be the initial symptom of the disease, particularly in the limited form, although it typically manifests as a long-term consequence of the disease, developing after more than ten years.(28, 66) Data from various research groups indicate a significant prevalence of calcinosis in SSc, ranging from 18% to 49% of the patients.(102, 217-221) As indicated by the findings of an international multicenter cohort study of 7,056 SSc patients, the prevalence of calcinosis is around 22%.(222) A study revealed a 24.7% occurrence of calcinosis in cases of dcSSc and a 24.8% occurrence in cases of lcSSc. Scleroderma-associated calcinosis is characterized by the presence of small lesions; however, in rare instances, it may extend to large areas.(223) Imaging techniques have facilitated the identification of silent calcinosis, with a prevalence of 50% observed in early scleroderma.(224) The formation of calcinosis is precipitated by areas subject to recurrent microtrauma.(1) In SSc, the most commonly affected areas are the hands (65-83%), the proximal upper extremities, and the proximal lower extremities in 27% and 22%, respectively.(28, 56, 219) Calcinosis has been observed to impair quality of life and result in significant functional impairment. However, the risk factors for calcinosis development in SSc remain to be elucidated. Some papers have reported a strong association between calcinosis, digital ulcers, and osteoporosis. Other associated factors include older age, disease duration, limited form, fingertip scars, acroosteolysis, arthritis, facial telangiectasia, cardiac, pulmonary, and gastrointestinal involvement.(42, 48, 58, 68, 102, 218-221, 224-227) Autoantibodies more commonly detected in patients' sera included anti-centromere, anti-PM/Scl, and anti-cardiolipin antibodies.(228, 229) In contrast, other authors have reported a correlation with the diffuse subtype, anti-scl-70, and ANA positivity.(220, 230) Myositis was observed less frequently in patients with SSc-associated calcinosis.(231)

Dermatomyositis is the second most common ACTD complicated by calcinosis, particularly its juvenile form. In this form, calcinosis develops in up to 70% of the cases after a mean disease duration of 2 to 3 years. Conversely, a lower frequency has been

documented in adult DM and polymyositis, with a later onset (after an average disease duration of 8 years), manifesting as 20-37% in DM and 2.2% in polymyositis.(1, 66, 232-236) Although calcinosis generally presents as fine granular lesions on the extremities and trunk, it can also manifest as an extensive form (exoskeleton).(1, 66) According to the extant literature, the risk factors for developing calcinosis may include uncontrolled disease, delayed diagnosis, prolonged disease duration, and fingertip ulcers.(237, 238) The beneficial effect of early aggressive therapy of DM has been reported; however, no association with disease flare has been found.(67, 232) Other reported risk factors are dysphagia and Gottron's sign. The presence of associated autoantibodies, such as anti-NXP2/MJ and PM/Scl, has been identified in relevant studies.(235, 238, 239) Notably, calcinosis in DM may serve as a paraneoplastic indicator of hematological or solid malignancies.

Systemic lupus erythematosus rarely presents with calcinosis; if associated, it is typically an incidental radiographic finding.(66, 240) There is a paucity of data on calcinosis in SLE. In a retrospective study, *Tiao et al*. found an incidence of 6.76% (10/148 patients, all female and had a mean age of 53 ±17.4 years).(241) Calcinosis can develop as a late complication of SLE, with a mean duration of 21.5 years.(66) It primarily affects the lower extremities.(241) Other typical locations include the interphalangeal joints, forearms, elbows, buttocks, and the peri-auricular area.(242) *Tiao et al*. found an association with Raynaud's phenomenon and a higher titer of ANA autoantibodies (minimum 1:160), anti-RNP, anti-Smith, anti-Ro, and anti-dsDNA antibodies, but not with disease flare.(241)

Mixed connective tissue disorder, undifferentiated connective tissue disorder, and overlap syndromes are rarely associated with calcinosis formation.(66, 243) Involvement of the extremities, hands, and feet has been reported after a mean disease duration of 2.7 years in UCTD and 6.3 years in MCTD.(66)

5.2. Sodium thiosulfate therapy in calcinosis

Even though calcinosis affects a significant number of patients with different ACTDs, it remains an unmet therapeutic need because our understanding of this neglected disorder

is incomplete. As our knowledge deepens, novel therapeutic options are being developed. STS therapy has garnered attention within the scientific community due to its long-standing utilization and proven efficacy in calciphylaxis. Recent studies have demonstrated the effectiveness of this approach in addressing symptoms related to calcinosis, suggesting its potential to facilitate wound healing as well. The focal point of interest is the targeted administration directly to the lesions, with encouraging outcomes. These positive results also support the systemic administration of STS in treating larger calcinosis lesions where topical or intradermal administration would be impractical or not feasible. The precise mechanism through which STS dissolves calcification remains to be elucidated; however, its therapeutic efficacy in calcinosis cutis is believed to be linked to its anti-inflammatory (HS production), vasodilative (nitric oxide synthase regeneration), antioxidant (free radical reduction), and chelating properties (calcium solubility increase by up to 100,000-fold).(144, 146, 244) The latter prompts the question of its potential impact on bone mineralization, particularly in children. A systematic review and meta-analysis by *Wen et al*. found no effect on bone density in 503 hemodialyzed patients receiving STS for vascular calcification.(245) In our practice, we have seen no negative impact on pre-existing osteoporosis in a patient with SLE-associated calcinosis.(9) The administration route is either intravenous, oral, intralesional, or topical, each with advantages and disadvantages.

Topical application, with or without occlusion, has been established to be a safe therapy. However, given its limited penetration, its efficacy is diminished for larger or deeper-seated calcinosis lesions. In a retrospective study by *Howard et al*., STS was applied twice daily, compounded at a ratio of 50:50 with petrolatum or *Eucerin* ointment (*Beiersdorf AG*, Hamburg, Germany) until the lesions cleared up. Complete resolution was observed in lesions measuring less than 0.2 cm. The resolution rates were 78% and 20% for lesions measuring 0.2-0.3 cm and 0.3-0.5 cm, respectively.(246) A 68% response rate was observed with the topical application of 10-25% STS in zinc oxide twice daily.(90, 247-249) A review of 48 patients revealed that complete, partial, and no responses occurred in 19%, 63%, and 6% of the cases, respectively.(77) Topical sodium metabisulfite at a concentration of 25% may serve as an alternative.(250, 251) *Ricardo et al*. found that STS and sodium metabisulfite were efficacious in 78% of the cases, exhibiting an excellent safety profile.(248) However, long-term therapy and patient self-application are required,

which poses a challenge in compliance.(252) It may be administered for smaller lesions or in areas that are difficult to treat. Although the treatment may not reduce the size of calcified deposits, patients have reported a cessation of pain and functional improvement.(244, 248, 253) However, it is essential to note that skin irritation or pruritus may complicate the therapy. The efficacy of the treatment can be enhanced through its combination with picosecond laser therapy.(77) In our clinical practice, no significant impact on calcified lesions was observed, although most lesions were larger than 0.2 cm (unpublished data).

Intralesional administration is a more efficacious modality, as it ensures medication delivery directly to the target calcified deposits. However, it should be noted that the procedure is invasive, with the potential for infection at the injection site. Furthermore, it necessitates frequent patient visits. To prevent pain associated with the procedure, it is imperative to utilize either topical or intradermal anesthesia. A 0.1-1 ml dose of undiluted 12,500 mg (50 ml, 250 mg/ml) STS was administered every three weeks until clearance was achieved. This treatment resulted in the complete resolution of lesions measuring less than 2.0 cm. Larger deposits demonstrated no response to this therapy.(246) A systematic review involving 53 patients reported complete, partial, and no responses in 36%, 38%, and 25% of the patients, respectively. Despite the absence of radiological improvement in all patients, some patients reported an improvement in pain. After administration, no laboratory alterations were detected, and it is anticipated that improvement will be observed exclusively in the treated lesions, where the agent attains a higher concentration. The following side effects have been documented: therapy-associated pain (11%) and injection-site infection (9%).(77) However, some papers contest the efficacy of intralesional STS, it should be noted that low doses and long injection intervals may diminish the efficacy of the treatment in these studies.(254) Intralesional STS can be used with ESWL therapy to enhance the dissolution of calcified lesions.(77) In our practice, significant improvement was observed in a patient with deep morphea-associated multiple, progressing calcinosis, treated with intralesional STS administered at an interval of three weeks (0.1-0.2 ml per lesion). This improvement includes a reduction in lesion size and number and a decrease in inflammation. These observations were made following the failure of several other therapies. Some of the patient's lesions were also treated with a single ESWL session after a couple of months during which intralesional

STS was administered. These lesions demonstrated complete clearance. ESWL was rendered unfeasible for the residual deposits due to the unrecognizably small (with plain radiography) lesion size following intralesional STS therapy (unpublished data).

Oral administration is a convenient method that does not require hospital admission. Despite its generally recognized safety, diarrhea is notable and is manageable through dose adjustment. A study reported a suboptimal STS absorption from the gastrointestinal system, even when an oral solution was administered.(255) Oral STS has been demonstrated to alleviate calcinosis burden, predominantly regarding associated symptoms and lesion size. Nevertheless, the available data on this subject is quite limited. It can be posited that a more gradual release from capsules would be preferable. A review of the extant literature reveals only three cases in which oral STS was utilized, albeit exclusively in conjunction with intravenous STS. The oral STS doses ranged from 1.5 to 3 g/d for 1 to 6 months. Of the two cases in which the administration was conducted for six months, one demonstrated a partial response. Gastrointestinal side effects complicated two cases, and two more were complicated by catheter infection.(256) In our practice, we administered 1-8 g of oral STS, primarily in combination with intravenous STS. This resulted in a partial response in half of the patients, with only mild gastrointestinal side effects. Although STS therapy is generally well-tolerated, given the gastrointestinal sclerosis present in over 90% of SSc patients, it may not be well absorbed from the intestines when given orally in this population. Additionally, a recognized adverse effect of oral administration is the occurrence of dose-dependent diarrhea, a risk that should be avoided in SSc patients who already contend with malabsorption. Consequently, oral STS is not a suitable treatment option for patients with SSc. However, in cases of ACTDs that are not complicated by gastrointestinal involvement (e.g., DM and SLE), oral STS appears to be a promising therapeutic approach.

Intravenous administration of STS is more efficacious than oral administration due to its vasodilator effect, which enhances blood supply. This could be particularly beneficial for SSc patients with disease-associated vasculopathy. A notable disadvantage of intravenous administration is the necessity of a hospital background, although the conventional SSc therapy protocol also incorporates vasodilator infusion therapy with prostacyclin and other rheologicals. Due to its beneficial effects, STS could also play a role in managing

other ACTD-associated calcinosis cases, particularly in patients hospitalized for different reasons or requiring frequent inpatient admission. Intravenous administration is characterized by its inconvenience; side effects, including headache, vomiting, hypotension, and metabolic acidosis may also complicate it.(256-259) Only ten cases in the literature have been published of the administration of intravenous STS for calcinosis.(9) Doses ranged from 9 to 25 g one to three times a week or five times a week for one week of the month. Treatment duration ranged from one week to eight months.(256-258, 260, 261) A partial response was observed in 5/10 patients, and 5/10 demonstrated no response. Notably, the nonresponsive cases involved the lowest frequency and shortest duration of intravenous STS administration. The side effects observed included gastrointestinal complaints, fatigue, catheter infection, and metabolic acidosis in 6/10, 4/10, 2/10, and 1/10 patients, respectively. 3/10 patients exhibited no adverse effects.(9) A comprehensive overview of the various STS administration routes is provided in Table 5.

Table 5 Characteristics of different forms of STS therapy

Route	Preparation	Frequency	Increase in effectiveness	Indication	Side effects
Topical	50% in petrolatum/ Eucerin or 10-25% in zinc oxide	2 x/d	Occlusion, laser	<0.2 cm, superficial	Irritation, Pruritus
Intra-lesional	0.1-1 ml of 250 mg/ml solution	3 weekly	ESWL	<2.0 cm	Infection Pain
Oral	1-8 g	d	Dose increase, combination	Multifocal, inflamed, ulcerated	Dose-dependent diarrhea
Intra-venous	12.5-25 g of 250 mg/ml solution	1-3 x/w or 2-5 x/w/m	Combination with oral STS	Multifocal, inflamed, ulcerated	Headache vomiting hypotension hypernatremia metabolic acidosis

Abbreviations: cm, centimeter; d, daily; g, gram; mg/ml, milligrams per milliliter; ml, milliliter; STS, sodium thiosulfate; x/d, times daily; x/w, times per week, x/w/m times per week for one week of the month

6. Conclusion

6.1. Study I.: To evaluate the prevalence of calcinosis cutis in autoimmune connective tissue diseases at our Department of Dermatology between 2003 and 2023

• In this study, a retrospective analysis was conducted to ascertain the prevalence of calcinosis cutis in ACTDs.

•Calcinosis cutis has been demonstrated to substantially impact the lives of patients suffering from ACTD.

• A significant frequency was observed in SSc and DM compared to SLE, showing a rare association with calcinosis.

• A comparison of the clinical manifestations revealed similarities and disparities among the various ACTD subgroups complicated by calcinosis.

• Ulceration was observed with a higher frequency in SSc and SLE but a lower frequency in DM. No associated autoantibodies have been identified.

6.2. Study II.: To evaluate the efficacy of oral and intravenous sodium thiosulfate therapy in the management of dystrophic calcinosis

• A retrospective investigation was conducted to assess the systemic administration of STS in patients with calcinosis. This is the first study to report a higher dosage.

• It appears that STS treatment has a positive effect on the inflammation associated with calcinosis and promotes wound healing in ulcerated calcinosis lesions; however, complete resolution was not achieved.

• STS therapy has been demonstrated to be generally well-tolerated and safe.

The collective results of the two studies suggest that calcinosis is a significant issue in ACTDs, with ulceration being the primary complication, requiring effective treatment. Systemic STS therapy appears to play a role in the therapy, particularly in cases of inflamed and/or ulcerated calcinosis.

7. Summary

Calcinosis cutis is an under-researched disorder that manifests as a complication of various conditions, most commonly ACTDs. The complexity of the pathogenesis remains a subject of investigation. While it is regarded as rare, there is a paucity of data in the literature regarding the exact prevalence. In our study, a retrospective assessment of the prevalence of calcinosis in ACTDs was conducted over a 20-year period. Calcinosis developed in 11.02% and 24.32% in dcSSc and lcSSc after a mean time interval of 6.31 and 2.25 years from the ACTD diagnosis. A frequency of 31.58% and 8.33% was detected in JDM and DM after a mean time interval of 2.17 and 2.85 years. Calcinosis developed in 1.94% of SLE patients after a mean time interval of 15.22 years.

To date, no effective treatment has been identified for managing calcinosis. Besides the variable efficacy of the therapeutic options, many of them exhibit unacceptable adverse effects. The predominant concerns pertain to pain, ulceration, and restricted joint mobility, which are associated with significant morbidity and have the potential to compromise quality of life. Asymptomatic calcinosis also carries the potential for enlargement and subsequent ulceration of the overlying skin. As our understanding of this condition deepens, novel therapeutic modalities for this frequently therapy-resistant condition are being developed. Recently, there has been an increased focus on STS therapy, a vasodilator and chelating agent with antioxidant properties, the first-line systemic treatment for another calcifying disorder, calciphylaxis. The effectiveness of the substance has also been shown in the treatment of small calcinosis lesions when administered topically or intralesionally. These positive results support the systemic administration for treating larger calcinosis lesions, where topical or intradermal administration would be impractical or not feasible. STS therapy has generally been found to be well tolerated and safe; however, the scope of clinical experience with this therapy remains limited. In our retrospective study, the efficacy of systemic STS was evaluated in a small population, with regard to the literature cases. A partial response was observed, primarily in inflamed, ulcerated calcinosis, with the administration of higher dosages compared to those reported in the literature. Further studies are necessary to explore this specific treatment in greater detail, as well as to investigate additional treatment options for calcinosis.

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9. Bibliography of the candidate's publications

9.1. Publications directly related to this thesis

Róbert L, Bánvölgyi A, Lőrincz K, Holló P, Hidvégi B. Systemic Sodium Thiosulfate as an Adjunct Treatment in Calcinosis: A Retrospective Study. *J Clin Med*. 2023;12(24):7741. doi: 10.3390/jcm12247741. **IF: 3,0**

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9.2. Publications not directly related to this thesis

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9.3. Conference presentations

Clinical presentation and therapeutic options of calcinosis cutis 2024.11.21. Hungarian Dermatological Society 97. Annual Meeting, Budapest

Scleroderma spectrum. 2024.10.10. Continuing Education in Dermatology, Budapest

Extramammary Paget-disease. 2023.11.17. Hungarian Dermatological Society 96. Annual Meeting, Siófok

Extragenital primary chancre. 2023.11.16. Hungarian Dermatological Society 96. Annual Meeting, Siófok

Clinical presentation of calciphylaxis. 2022.01.29. Hungarian Dermatological Society 94. Annual Meeting, online

Clinical presentation of calcinosis. 2019.04.06. SkinAcademy, Budapest

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