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Effectiveness and safety of rituximab for the treatment of refractory systemic sclerosis associated calcinosis: A case series and systematic review of the literature

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ABSTRACT

Objective: To analyze the effectiveness and safety of rituximab (RTX) for the treatment of refractory systemic sclerosis (SSc)–associated calcinosis.

Methods: We undertook an observational study of patients with this complication treated with 1 or more cycles of RTX (1 g × 2 weeks) and evaluated for at least 12 months after RTX treatment in a single center. The primary outcome measures of the study were the improvement of calcinosis symptoms (pain, signs of local inflammation, and new episodes of skin ulceration) and the radiologic evolution of the calcification(s).

Results: We treated 8 patients with refractory SSc-related calcinosis with RTX (off-label use). The main indications for RTX were complicated calcinosis unresponsive to previous therapies with concomitant arthritis in 2 patients and refractory arthritis or interstitial lung fibrosing disease in the remaining 6 patients.

The mean number of RTX cycles administered was 3.12 ± 2.1 (range, 1-7), the median duration of RTX treatment was 9 months (interquartile range [IQR], 7.5-36 months), and the median follow-up after the first infusion of RTX dose was 19 months (IQR, http://catsalut.gencat.cat/web/.content/minisite/catsalut/proveidors_professionals/medicaments_farmacia/phf_mhda/informes_camse/esclerosi_sistemica/Dictamen-CAMS_-ES_-web.pdf (n.d.) 5-45 months). Four patients (50%) had a significant improvement in clinical symptoms (sustained improvement in the visual analog scale for pain of at least 50% and no new episodes of local inflammation or skin ulceration). Two of these patients (25%) also had a complete resolution or significant reduction in the size of the calcification(s) on X-ray, according with the radiographical scoring system for calcinosis developed by the Scleroderma Clinical Trials Consortium. In the remaining 4 patients (50%), RTX did not provide any significant clinical or radiologic benefit for calcinosis.

The frequency of adverse effects was low, occurring in only 1 patient (12.5%), who developed upper respiratory tract infections not requiring hospitalization.

Conclusion: Our preliminary data suggest that RTX may be helpful as a rescue therapy in selected cases of severe and refractory SSc-related calcinosis.

1. Introduction

Calcinosis, the deposition of calcified material in the skin and subcutaneous tissues, is a frequent and debilitating complication in patients with systemic sclerosis (SSc), affecting as many as 25% of patients [1].

SSc-associated calcinosis is a form of the so-called dystrophic calcification, a term used to describe the deposition of calcified material in damaged tissues in the presence of normal calcium/phosphorus metabolism [2,3]. These deposits can be solid or liquid or, more often, can fluctuate between both states. The calcinosis lesions are commonly

Abbreviations: ACA, *anti-centromere antibodies*; *ANA*, *anti-nuclear antibodies*; *DM*, *dermatomyositis*; *EULAR*, *European League Against Rheumatism*; *IQR*, *interquartile range*; *PRISMA*, *preferred reporting items for systematic reviews and meta-analyses*; *RTX*, *Rituximab*; *Scl-70*, *anti-topoisomerase I antibodies*; *SCTC*, *Scleroderma Clinical Trials Consortium scoring system*; *SSc*, *systemic sclerosis.*

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located in the fingertips, but also of the forearms, elbows, and knees. They have variable sizes and shapes, ranging from a tiny speck, pea-sized, to larger tumorous (> 1 cm), often amorphous, deposits imprecisely named pseudotumoral calcinosis. They do not solely represent bothersome lumps causing cosmetic issues but also are frequently associated with significant morbidity, including pain, recurrent episodes of local inflammation, and skin ulceration (which sometimes can be complicated by infection), causing significant functional disability and impaired quality of life.

The etiology of calcinosis in SSc is not well understood. One hypothesis suggests that tissue ischemia may contribute to the development of calcinosis, which is supported by recent studies reporting an association between calcinosis and ischemic manifestations of SSc, such as digital ulcers, acro-osteolysis, and digital amputation [1,4-9]. Chronic inflammation has also been thought to play a role in the tissue damage that serves as a nidus for dystrophic calcification; however the exact mechanisms remain unclear [4].

To date, there are no accepted treatment options for SSc-associated calcinosis; several drugs and non-pharmacologic interventions have been tried with disappointing or modest results [10]. Surgical excision of calcium deposits remains the mainstay of treatment for cases unresponsive to previous therapies, but this is an invasive procedure with potential risks, and frequent recurrence [10]. New therapeutic options with greater efficacy are clearly needed to manage these patients' illness properly.

There is new evidence that B-cell depletion with rituximab (RTX) could be an effective intervention in patients with SSc. Observational case-control study data from the European League Against Rheumatism (EULAR) Scleroderma Trials and Research group has suggested that RTX therapy may reduce the progression of skin thickening and lung fibrosis, especially in a subgroup with early diffuse SSc, but there was no report of efficacy in other manifestations of the disease, including calcinosis [11]. In recent years, some case reports have suggested that RTX could be an attractive therapeutic option in cases of calcinosis, some of them refractory to standard therapies [12,13].

In this preliminary study, we assessed the effectiveness and safety of RTX in a case series of patients with SSc-related calcinosis, and perform a systematic review of the literature to analyze the current evidence of the therapeutic use of RTX in this complex situation.

2. Methods

2.1. Selection of patients

The sample included 8 patients with refractory SSc-related calcinosis, all fulfilling the EULAR/ACR 2013 criteria for SSc [14], treated in a compassionate use with 1 or more cycles of RTX (1 g × 2 weeks) at Bellvitge University Hospital, and evaluated for at least 12 months after RTX treatment. The main indication for RTX was complicated calcinosis unresponsive to previous therapies with concomitant arthritis in 2 patients and refractory arthritis or interstitial lung fibrosing disease in the remaining 6 patients.

In all cases, written informed consent was obtained from the patients and the off-label use of RTX was approved by our local health authorities [15]. Clinical records of patients were anonymized before analysis. The study was approved by our institutional ethics committee (Clinical Research Ethics Committee of Bellvitge University Hospital) and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference for Harmonization.

2.2. Treatment protocol

All patients received a first cycle of therapy consisting of 100 mg of methylprednisolone and 1000 mg of RTX given intravenously on days 1 and 14. Further cycles consisting of the same regimen were repeated on clinical relapse. Ongoing therapy with diltiazem (in 100% of patients) and colchicine (50%) at stable doses for at least 3 months prior to RTX treatment, remained unchanged in all cases.

2.3. Clinical data

A retrospective analysis of prospectively collected data recorded according to a specifically designed protocol was performed. Baseline data collected at the time of RTX prescription included age, gender, disease duration, SSc cutaneous subset, location of calcinosis, presence or history of vascular manifestations (including Raynaud's phenomenon, digital ulcers and pulmonary hypertension), presence of acro-osteolysis, visceral organ involvement (interstitial lung disease, arthritis, gastrointestinal, cardiac or renal involvement), serological status for anti-nuclear antibodies (ANA), anti-centromere (ACA) and anti-topoisomerase I (Scl-70) antibodies, presence of osteoporosis and details of the past and current therapies for calcinosis, as well as for the remaining SSc manifestations.

Information about RTX therapy included the number of cycles received, dose administered, duration of treatment from the first dose, time and reason for discontinuation of the treatment (if any), tolerability and side effects. The end point of patient follow-up was the date of the last clinic visit.

All patients had X-rays at baseline pre-RTX and follow-up every 12 months.

2.4. Outcomes

The efficacy of RTX was evaluated taking into account 2 outcome measures: 1) the improvement of the symptoms of calcinosis and 2) the radiological evolution of the calcification(s).

In terms of assessed symptoms related to calcinosis, we included pain secondary to calcinosis (evaluated with a patient visual analogue scale [VAS] for pain ranging from 0 to 100 mm), signs of local inflammation (categorized as yes/no) and new episodes of skin ulceration (yes/no) as assessed by the clinician. Clinical response was defined as a sustained improvement in the VAS of ≥50% and no new episodes of local inflammation or skin ulceration.

The radiological calcinosis burden was measured using 2 scoring systems: the simple scoring system proposed by Johnstone et al. [7] and, in patients with calcinosis involving the hands, the new radiographical scoring system for calcinosis developed by the Scleroderma Clinical Trials Consortium (SCTC) [16].

According to the classification proposed by Johnstone et al. [7], calcinosis was subdivided into mild, moderate and severe depending on the density of calcinosis and the number of separate sites of calcinosis using the following definitions: 1) mild: a single site of low-density calcinosis; 2) moderate: medium-density calcinosis at 1 or more sites or a single site of high-density calcinosis; and 3) severe: > 1 site of high-density or mixed-density calcinosis. We used the following definitions to categorise the density of calcinosis: low density = less dense than trabecular bone; medium = similar to trabecular bone; and high = similar to or more dense than bone cortex [16].

One of the authors (JN) reviewed all the XRs using the simple scoring system and the SCTC scoring system for calcinosis affecting the hands. In all cases X-rays were scored twice, with an interval of 1 week between the 2 interpretations, to ensure intra-obsever reliability.

In those patients with calcinosis involving the hands and apparent radiological improvement, this was verified by another author (AV), who independently checked the calcinosis burden using the SCTC scoring system after receiving the de-identified hand radiographs, presented in a randomized and blinded fashion. In cases of inter-observer difference, a consensus was achieved for the final score.

Radiological response was defined as the complete resolution of the calcification(s) on the X-ray or as a significant reduction in calcification size, without appearance of new lesions.

2.5. Literature search strategy and selection criteria

In addition to our case series, a systemic review of reports on SSc-related calcinosis treated with RTX published in international journals was also performed in accordance with the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) for Individual Patient Data systematic reviews (PRISMA-IPD) statement [17]. Searches were conducted in the PubMed database (i.e., including MEDLINE, National Library of Medicine, and PubMed Central) for the period between January 2000 and August 2018 by using the strategies recommended by the Cochrane handbook. Search terms included "*rituximab*," "*calcinosis*," and "*systemic sclerosis*" or "*scleroderma*". Only reports in English were selected for review. All potentially relevant abstracts were retrieved and reviewed. The references of the studies obtained were then examined to identify additional reports.

The MEDLINE search resulted in 8 articles [12,13,18–23]. After evaluation of the full text, two of these articles were excluded because they described two patients with SSc treated with RTX due to hematologic malignancies (1 case of lymphoma and 1 case with solitary extramedullary plasmacytoma) [22,23]. Therefore, 6 articles were finally selected for review [12,13,18,21], identifying 11 well-documented cases of patients with SSc-related calcinosis who were treated with RTX. We used a standard form to recover the demographic, clinical, laboratory and hemodynamic features, treatment modalities, and outcomes.

2.6. Statistical analysis

Continuous data are described as the mean ± standard deviation (SD) and range or median with interquartile range (IQR), whereas categorical variables are presented as the number of cases with percentages. Continuous variables were compared using the Student *t*-test or median test. Categorical variables were analyzed using the Chi-squared test or Fisher exact test when the expected values were < 5, and by calculating the 95% confidence intervals (CIs) for the differences between proportions using Newcomb method. Statistical significance was defined as $p < .05$. Assessment of intraobsever reliability was made with the kappa statistics.

3. Results

3.1. Original study

3.1.1. Baseline characteristics

Thus far, we have treated 8 SSc patients with calcinosis with RTX (off-label use). Their baseline characteristics and outcomes of treatment are summarized in Table 1.

Patients were predominantly female (89%), with a mean age of 53 ± 17 years (mean ± standard deviation; range 26–73) and a median disease duration of 6.5 years (IQR 3.25–13.75). Four patients (50%) had limited cutaneous skin involvement. Regarding their autoantibody profile, 50% (4/8) of the patients tested positive for anti-Scl-70 antibodies, whereas ACA antibodies were positive in 37.5% (3/8); the remaining patient was positive for ANA with non-centromeric pattern (ANA Nucleolar Pattern).

All patients had previous or concomitant vascular and visceral organ involvement. The most frequent symptoms were gastrointestinal involvement, mainly gastroesophageal disease (100%), arthritis (87.5%), interstitial lung disease (62.5%), digital ulcers (62.5%), and myopathy (25%). Pulmonary artery hypertension, primary cardiac involvement and renal involvement were documented in 12.5% of the patients. Previous or ongoing therapies for these manifestations included calcium-channel blockers (87.5%), antiplatelet agents (100%), topical nitroglycerin (75%), proton pump inhibitors (87.5%), prokinetic agents (62.5%), pentoxifylline (12.5%), endothelin receptor antagonist drugs (50%), phosphodiesterase 5 inhibitors (50%), prostacyclin analogues (37.5%), low-dose corticosteroids (100%), cyclophosphamide (75%), azathioprine (12.5%), mycophenolate (75%), methotrexate (25%), and hydroxychloroquine (12.5%).

Regarding the location of calcinosis lesions, hands were affected in 87.5% of the cases, limbs (forearms, elbows, and/or knees) in 50%, and feet in 12.5% (patients could have lesions of calcinosis in more than one location). Before receiving RTX treatment, diltiazem (100%), colchicine (87.5%), non-steroidal anti-inflammatory drugs (62.5%), bisphosphonates (50%), and surgery (12.5%) had been used unsuccessfully in these patients (mean number of previous treatments received: 3 ± 0.86; range 2-4). Using the simple scoring system, 6 (75%) patients had severe calcinosis and 2 (25%) moderate calcinosis on X-ray at first RTX infusion. The intraobsever agreement was good (kappa = 0.82).

3.1.2. Effectiveness and safety of RTX

The number of RTX cycles administered was 3.12 ± 2.1 (range, 1-7), the median duration of RTX treatment was 9 months (IQR: 7.5-36 months), and the median follow-up after the first infusion of RTX dose was 19 (IQR: 15-45 months).

Four patients (50%) had a clinical response. A radiological response was achieved only in 2 of these patients (25%) as assessed by the SCTC scoring system. One patient had severe calcinosis in both hands, many of them with a periarticular location (Fig. 1A) and lesions of pseudotumoral calcinosis in her left forearm at baseline (Fig. 1B and C) along with severe polyarthritis (DAS28: 6.12). After 46 months of therapy (Fig. 1D and E), she had a complete resolution of most calcinosis lesions of the hands (pre-RTX SCTC scores: left hand 336, right hand 341.375 and total 677.376; post-RTX scores: left hand 17.875, right hand 13.375 and total 31.25) and a clear reduction in the size of the calcinosis lesions in the forearm. Despite clinical improvement (complete disappearance of pain for long periods and no new episodes of local inflammation or skin ulceration), she had a progression of the deformity of the second and fourth fingers of the right hand (mean DAS28 during follow-up of 2.75). The other patient had a moderate calcinosis in the fat pad of the left third fingertip at first RTX infusion (Fig. 2). In this case, the pre-RTX and post-RTX scores measured using the SCTC scoring system were 2.75 and 1, respectively. Neither of them had drainage or surgical removal of these deposits that could explain the improvement of symptoms.

In the remaining 4 patients (50%), RTX did not provide any significant clinical or radiological benefit for calcinosis. In 1 of these patients, RTX was discontinued due to inefficacy; in the other three, treatment was maintained due to its beneficial effect in arthritis and interstitial lung disease.

The frequency of adverse events was low, occurring in only 1 patient (12.5%), who developed upper-respiratory tract infections not requiring hospitalization. No cases of severe infusion reactions or haematological abnormalities were reported.

3.2. Literature review

Table 2 summarizes the main clinical characteristics and outcomes of the 11 patients found in the literature [12,13,18,21]. Due to the heterogeneous definitions of treatment response, it was difficult to obtain consistent results. However, considering the response as the disappearance or significant clinical improvement of the calcinosis, a complete or partial response was reported in 45.5% (5/11) of cases.

No clinical predictor of response could be identified, because in the comparative analysis between responders versus non-responders, no significant differences were found in efficacy with respect to the 2 posologies of RTX (lymphoma versus rheumatoid arthritis regimen), or the number of cycles of RTX administered.

One of these patients died 10 months after starting RTX due to aspiration pneumonia and multiple digital ulcer infection [17]. In the remaining cases, no serious adverse events were reported.

Table 1
Main clinical characteristics and outcomes of our 8 patients.

Patient N°	1	2	3	4	5	6	7	8
Age (yrs)/Gender	71/Women	62/Women	58/Women	73/Women	32/Women	26/Women	46/Men	56/Women
SSc duration (yrs)	16	4	14	13	6	3	7	3
SSc cutaneous subset	Diffuse	Limited	Diffuse	Limited	Limited	Diffuse	Limited	Diffuse
Clinical manifestations	DU, GI, ILD	GI, arthritis, myopathy	DU, GI, ILD, arthritis	GI, arthritis, myopathy	DU, GI, PAH, renal,cardiac disease, arthritis	DU, GI, ILD, arthritis	GI, ILD, arthritis	DU, GI, ILD, arthritis
Autoantibodies	Scl-70 (+)	ACA (+)	Scl-70 (+)	ACA (+)	ACA (+)	Scl-70 (+)	ACA (−) Scl70 (−)	Scl-70 (+)
Location of calcinosis	Hands	Hands and forearms	Hands	Hands and forearms	Limbs	Hands	Hands	Hands, limbs, and feet
Burden of calcinosis according with the SSS*	Moderate	Severe	Severe	Severe	Severe	Severe	Moderate	Severe
Previous treatment for calcinosis	Diltiazem Colchicine	Diltiazem Colchicine NSAID, BF Surgery	Diltiazem BF, NSAID	Diltiazem Colchicine NSAID, BF	Diltiazem Colchicine NSAID	Diltiazem Colchicine	Diltiazem Colchicine	Diltiazem Colchicine BF, NSAID
Previous/concomitant treatment for the remaining SSc manifestations	Cs, CYC, AZA, MMF, AAS, CCB, topical NTG, PA, ERAS, PDE-5 inhibitors, PPI, prokinetic agents	Cs, AAS, CCB, topical NTG, PPI, prokinetic agents, HCQ, MTX	Cs, CYC, MMF, AAS, CCB, topical NTG, Pentoxifylline ERAS, PDE-5 inhibitors, PA, PPI, prokinetic agents	Cs, AAS, CCB, PPI, MTX	Cs, CYC, MMF, HCQ, AAS, CCB, Topical NTG, ERAS, PDE-5 inhibitors, PA, PPI	Cs, CYC, MMF, AAS, CCB, ERAS, PDE-5 inhibitors, PPI, prokinetic agents, topical NTG	Cs, CYC, MMF, AAS, CCB, PPI prokinetic agents, topical NTG	Cs, CYC, MMF, AAS, CCB, PPI topical NTG
Osteoporosis confirmed by DXA	Yes	Yes	Yes	Yes	No	No	No	No
Acro-osteolysis	Yes	No	Yes	No	No	Yes	No	No
Indication to RTX	ILD	Calcinosis + Arthritis	ILD + Arthritis	Calcinosis +Arthritis	ILD	ILD	ILD + Arthritis	ILD + Arthritis
Duration of RTX treatment (mo)	15	6	30	38	42	15	6	12
N° of RTX cycles	2	1	4	7	6	3	1	2
Follow-up after first dose of RTX (mo)	15	12	54	46	42	21	12	16
Ongoing therapy for calcinosis	Diltiazem Colchicine	Diltiazem Colchicine	Diltiazem	Diltiazem	Diltiazem Colchicine	Diltiazem Colchicine	Diltiazem	Diltiazem
Ongoing therapy for Raynaud's phenomenon or PAH	Diltiazem,AAS topical NTG, ERAS, PDE-5 inhibitors	Diltiazem, AAS topical NTG	Diltiazem,AAS topical NTG, ERAS, PDE-5 inhibitors	Diltiazem, AAS	Diltiazem, AAS, topical NTG, ERAS, PDE-5 inhibitor	Diltiazem, AAS, topical NTG, ERAS, PDE-5 inhibitor	Diltiazem,AAS topical NTG	Diltiazem, AAS, topical NTG
Clinical response of calcinosis to RTX therapy	No	No	No	Yes	No	Yes	Yes	Yes
Radiologic response	No	No	No	Yes	No	Yes	No	No
Adverse events	No	No	No	No	No	Upper respiratory tract infections	No	No

All patients received the same RTX posology (1 g 2 wk. apart).
All patients had Raynaud's disease.
Abbreviations: ACA: anti-centromere antibodies; AAS: acetylsalicylic acid; AZA: azathioprine; BF: bisphosphonates; CCB: calcium channel blockers; Cs: low-dose corticosteroid; CYC: cyclophosphamide; DU: digital ulcers; ERAS: endothelin receptors antagonists, GI: gastrointestinal involvement; HCQ: hydroxychloroquine; ILD: interstitial lung disease; MMF: mycophenolate mofetil; MTX: methotrexate; NSAID: non-steroidal anti-inflammatory drugs; NTG: nitroglycerin; PA: prostacyclin analogues; PAH: pulmonary arterial hypertension; PDE: phosphodiesterase; PPI: Proton pump inhibitors; RTX: rituximab.
* SSS: simple scoring system proposed by Johnstone et al. [14]

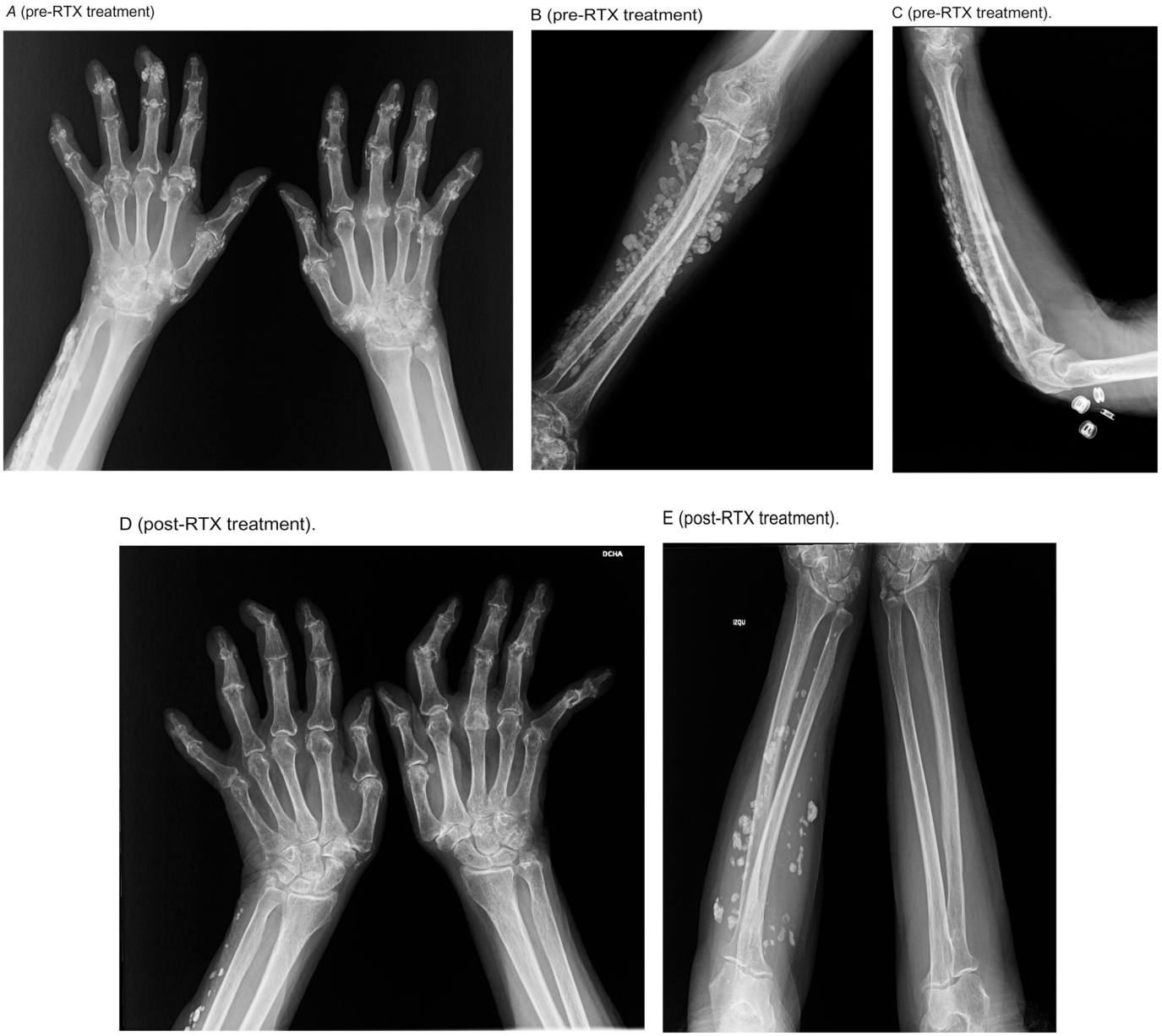

Fig. 1. Case 4. A 73-year-old woman with limited SSc and lesions of pseudotumoral calcinosis in her left forearm as well as in both hands, many of which had a periarticular location. The radiologic images prior to treatment with rituximab (Fig. 1A, B and C) and after 46 months of therapy (Fig. 1D and E) show a complete resolution of most calcinosis lesions of the hands and a clear reduction in the size of the calcinosis lesions in the forearm.

4. Discussion

Calcinosis remains a major clinical problem in patients with systemic sclerosis (SSc). The detailed pathophysiology of this complication is poorly understood, which has difficulted the detection of effective therapies. New therapeutic options with greater efficacy are clearly needed to properly manage these patients.

The role of inflammation in the development of calcinosis is unclear, but has some supporting evidence published in the literature in recent years. For example, in patients with dermatomyositis (DM) and calcinosis, elevated serum interleukin-1 levels have been found. Similarly, the presence of IL-6, IL-1B, and tumour necrosis factor–alpha in the milk of calcium (calcium-laden fluid collections) of juvenile patients with DM and calcinosis supports a role for activated macrophages and inflammation in at least a subset of these patients [24]. In addition, one case-control study demonstrated that mannose-binding lectin levels (one receptor of the lectin pathway of complement) were significantly elevated in SSc patients with calcinosis in comparison with SSc patients without this complication, as well as in the subset of patients with digital ulcers and pitting scars [25].

Because the pathogenesis of calcinosis is not well understood, its treatment is still empirical and remains a major clinical challenge. Different therapies such as antiplatelet agents, warfarin, vasodilators (including calcium channel blockers, pentoxifylline, and cilostazol), colchicine, bisphosphonates, minocycline, sodium thiosulfate, intravenous immunoglobulins, as well as a few non-pharmacologic treatments (carbon dioxide laser-tissue vaporization and extracorporeal shock wave lithotripsy) have been tried with disappointing or modest results. New therapeutic options with greater efficacy are clearly needed to properly manage these patients. In this clinical scenario, RTX appears as an attractive therapeutic option, in view of recent evidence that this therapy may be also effective in the underlying disease. Its safety in patients with SSc seems to be reasonable and similar to data available in other autoimmune diseases [11,26]. As a consequence, RTX

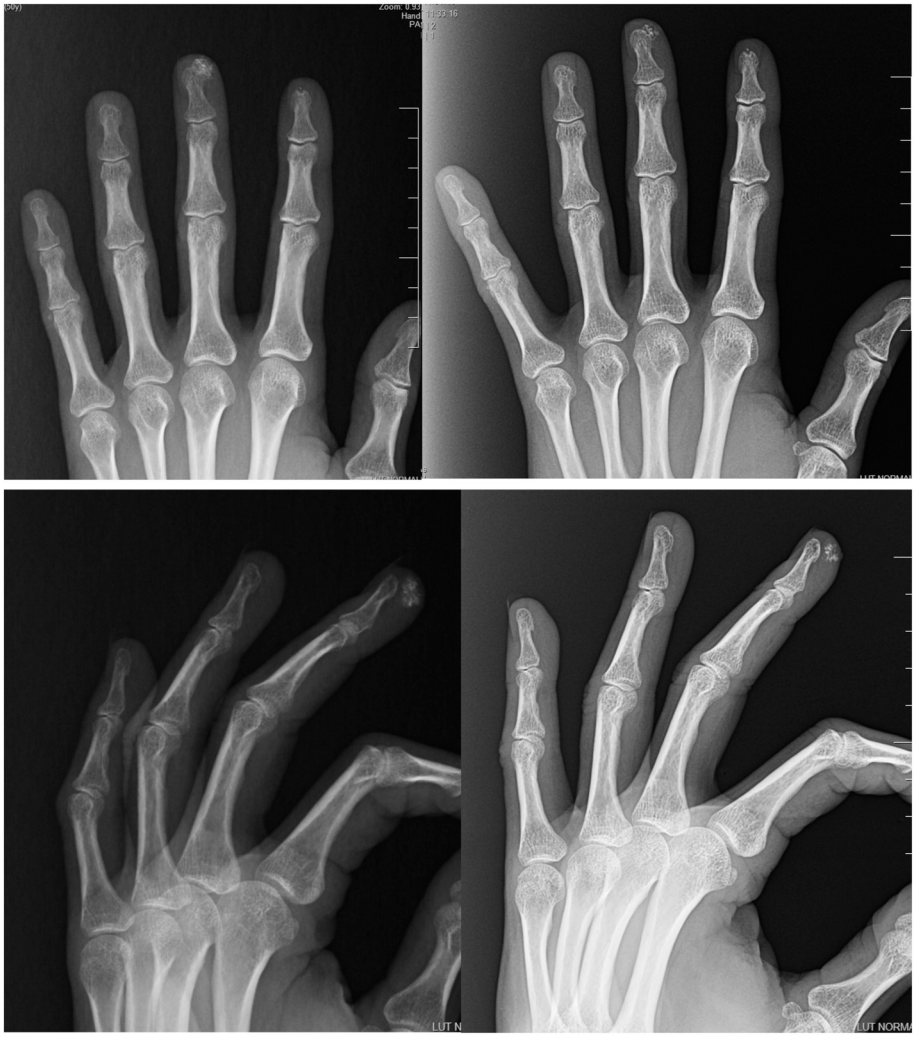

Fig. 2. Case 6. A 26 year-old woman with diffuse SSc and calcinosis lesion in the fat pad of the left third fingertip. Radiologic evolution of the lesion prior to treatment with rituximab and after 21 months of therapy.

is increasingly being used off-label to treat some SSc manifestations, mainly interstitial lung fibrosing disease, severe cutaneous involvement, or arthritis.

According to our data, the effectiveness of RTX in refractory SSc-associated calcinosis seems modest but not negligible, achieving after several cycles a significant clinical improvement in 50% of patients, with a concomitant radiological improvement of the calcification(s) in 25% of these cases, with an acceptable rate of adverse events (non-severe infections in 12.5% of the cases).

Previously published experience on the use of RTX in SSc-associated calcinosis is scarce and comes from a small number of case reports, with a total of 11 well-documented cases in the literature [12,13,18,21]. The overall analysis of these cases shows an improvement of calcinosis in 45.5% of cases, which is consistent with that observed in our series. Another important data is the fact that, if we consider our patients and those published in the literature, in 47% (9/19) of the cases in which RTX was instituted for calcinosis (as the only cause or 1 of the most important), the treatment was maintained at the end of the follow-up period, an indirect sign that the clinician was satisfied with the benefit it provided. Interestingly, we observed a dramatic clinical and radiological response in the patient with the most severe calcinosis, raising the possiblilty that the burden of calcinosis is associated with inflammation, and RTX might be an appropriate choice in this setting.

It is true that the definition of response to RTX in published cases is variable and vague and that a clinical improvement does not imply or confirm an improvement of calcinosis burden. There have been efforts to develop reliable patient reported outcomes specifically for calcinosis in SSc, however these have not yet been validated nor sistematically used to assess the clinical response to treatment in patients with SSc-related calcinosis [27]. However, with dealing with one of the most unresponsive complications of SSc, a substantial clinical relief, especially in severe or refractory cases, is encouraging. This is similar to the way in which we accept the efficacy of RTX in reducing the progression of interstitial lung fibrosing disease or severe cutaneous involvement, without it being proven to give a complete resolution of cutaneous or pulmonary fibrotic changes.

The reason why RTX is useful in the treatment of calcinosis in some patients and not in others is unknown, since the published evidence did not allow us to identify any predictive factor of response. Of note, in 1 of the published reports, 1 patient died 10 months after starting RTX due to aspiration pneumonia and multiple digital ulcer infection [19]. No therapeutic agent comes without its pitfalls, and RTX is well known in the rheumatoid arthritis setting to be associated with an increased risk of severe infections. For this reason, its use should be considered only in selected cases of severe refractory SS-related calcinosis.

When interpreting the results of our study, one needs to consider the

Table 2
Clinical characteristics and outcomes of 11 reported patients with SSc-associated calcinosis treated with rituximab.

Author (Reference)	Daoussis [13] *N* = 1	De Paula [12] N = 1	Dubos [18] *N* = 1	Poormoghim [19] N = 1	Giuggioli [20] N = 1	Giuggioli [20] N = 1	Giuggioli [20] N = 1	Giuggioli [20] N = 1
Age (yrs)/Sex	53 / Women	54/Women	38/Men	54/Women	68/Women	80/Women	74/Women	24/Women
SSc duration (yrs)	12	[15]	13	23	25	20	9	3
SSc cutaneous subset	Limited	Limited	Scleromyositis	Limited	Limited	Limited	Limited	Diffuse
Clinical manifestations	GI, ILD	Pericarditis, ILD,	Myositis, skin involvement	GI,DU	ILD, GI	ILD, arthritis, DU,GI	ILD, arthritis, DU	Arthritis, DU,GI
Autoantibodies	NA	ANA antinucleolar pattern	Anti PM/Scl	Scl 70	ACA	ACA	ACA	ANA antinucleolar pattern
Location of calcinosis	Limbs (right knee and elbow) and hand (right thumb	Right hand (first and fourth fingers)	Right hand and forearms	Extensive calcinosis cutis (hands, limbs and feet)	NA	NA	NA	NA
Previous/concomitant treatments	Cs, PPI	Cs,CYC,CCB, ARA II	MTX,BF, Sodium thiosulfate, thalidomide	Cs,CCB,PPI,warfarin, colchicine	PA,CCB,Cs	PA,CCB,Cs	PA, LEF,CCB, Cs	ERAS,PA,MMF,Cs,CCB
Indication to RTX	ILD + calcinosis	ILD + artritis	Refractoy calcinosis	Refractory extensive calcinosis cutis	ILD + calcinosis	ILD + arthritis + calcinosis	ILD + arthritis + calcinosis	Arthritis + calcinosis + skin involvement
Dose	4 weekly 375/m^2 each	4 weekly 375/m^2 each	4 weekly 375/m^2 each	1 g × 2 separated by [15] days	4 weekly 375/m^2 each	4 weekly 375/m^2 each	4 weekly 375/m^2 each	4 weekly 375/m^2 each
N° of RTX cycles	2	2	1	2	2	2	3	1
Follow-up after first dose of RTX (mo)	41	7	7	10	30	18	30	18
Response of calcinosis to RTX therapy	Calcinosis significantly improved and pain disappeared	Complete remission of calcinosis un both fingers	New flare of calcinosis with new locations (progresion of calcinosis)	Significant improvement of pain but increased number/sizes of calcified lesions on X-Ray	Unchanged	Unchanged	Unchanged	Improved
Adverse events	No	No	No	She died at 10 mo. after starting RTX due to aspiration pneumonia and multiple digital ulcer infection.	NS*	NS*	NS*	NS*

	Giuggioli [20] N = 1	Giuggioli [20] N = 1	Hurabielle [21] *N* = 1
Age (yrs)/Sex	29/Men	41/Women	61/Women
SSc duration (yrs)	12	4	4
SSc cutaneous subset	Diffuse	Diffuse	Diffuse
Clinical manifestations	ILD, DU	ILD, arthritis,DU,GI	DU,ILD,arthritis
Autoantibodies	Scl70	Scl70	Scl70
Location of calcinosis	NA	NA	Right hand
Previous/concomitant treatments	ERAS,PA,MMF, CCB, Cs	ERAS,PA,MMF, CCB,Cs	NA
Indication to RTX	ILD + calcinosis +skin involvment	ILD + Arthritis +skin involvement	ILD + arthritis
Dose	4 weekly 375/m^2 each	4 weekly 375/m^2 each	1 g × 2 separated by 15 days
N° of RTX cycles	5	4	2
Follow-up after first dose of RTX (mo)	48	36	NA
Response of calcinosis to RTX therapy	Improved	Improved	New flare of calcinosis with new locations (progresion of calcinosis)
Adverse events	NS*	NS*	No

Abbreviations: ACA: anti-centromere antibodies; ARA II: angiotensin II receptor antagonist; AZA: azathioprine; BF: bisphosphonates; CCB: calcium channel blockers; Cs: low-dose corticosteroid; CYC: cyclophosphamide; DU: digital ulcers; ERAS: endothelin receptors antagonists, GI: gastrointestinal involvement; LEF: leflunomide; MMF: mycophenolate mofetil; MTX: methotrexate; NA: data not available; NS: Not specified; PA: prostacyclin analogues; PPI: Proton pump inhibitors; RTX: rituximab.

* Giuggioli et al. [20] presents a series of 10 SSc patients treated with RTX. With regard to the safety of RTX, only few and generally mild side-effects were recorder after treatment. In particular, infusion-related reactions were observed in 2/10 patients. Only one patient developed bacterial infection of the urinary tract that needed the hospitalization and intravenous antibiotics, with RTX cycle discontinuation.

limitations derived from its observational nature, the small sample size, the use of concomitant treatment with dilitiazem (100% of cases) and colchicine (50%) and the lack of a control group. The last 2 issues difficult the interpretation of the effect of RTX on the natural course of SSc-associated calcinosis. However, although the effect on calcinosis progression cannot be attributed to RTX alone, to account for this we included in the study only patients with refractory SSc-related calcinosis despite previous treatment with diltiazem (100%), colchicine (87.5%), non-steroidal anti-inflammatory drugs (62.5%) and/or bisphosphonates (50%) for at least 3 months before starting RTX. In addition, patients were used as their own controls, by maintaining unchanged the concomitant ongoing therapy for calcinosis at stable doses during the follow-up period. In addition, the rate of efficacy observed in the literature review may be influenced by the fact that most reports include cases with a favourable response, whereas cases without such a response are often not reported.

5. Conclusion

In summary, there is no definitive effective treatment for SSc-associated calcinosis to date. Our results, and those previously reported, suggest that RTX may be helpful in some patients with SSc-related calcinosis. These positive data remain preliminary and need to be interpreted with caution, restricting for the moment the off-label use of RTX as a rescue therapy to selected cases of severe and refractory SSc-related calcinosis. Further randomized clinical studies with large sample sizes are clearly needed to confirm these preliminary open-label data and to establish the correct dose, therapy length and appropriate use of concomitant medications as well as to identify predictors of response to this treatment.

Competing interests

The authors have declared that no competing interests exist regarding with this manuscript.

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None. This study is not a part of corporate sponsored research.

Ethics approval

Informed consent was obtained from both patients, and their clinical records were anonymized before analysis. The confidential information of the patients was protected in accordance with national laws. The study was approved by our institutional ethics committee (Clinical Research Ethics Committee of Bellvitge University Hospital) and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference for Harmonization.

Author's contributors

All authors had access to the data and meets the Uniform Requirements for Manuscripts Submitted to Biomedical journals criteria for authorship.

Data availability

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are included within the paper.

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