Calcinosis in poly-dermatomyositis: clinical and laboratory predictors and treatment options

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Abstract

Objective
We aimed to identify the possible clinical and laboratory predictors of calcinosis in a cohort of patients with a diagnosis of polymyositis (PM) and dermatomyositis (DM).

Methods
We carried out a retrospective analysis of a cohort of myositis patients attending our clinic between January 2013 and May 2014.

Results
74 patients (58 females, 16 males) with PM (30 cases), DM (30 cases), overlap syndrome (13 cases) and inclusion body myositis (1 case) were enrolled. Sixteen patients (21.6%) had calcinosis that occurred a mean of 43.7 months after diagnosis of PDM. At multivariate analysis, patients with calcinosis experienced longer follow-up duration ($p=0.006$), anti-PM/Scl ($p=0.033$) and anti-NXP2 ($p=0.024$) positivity compared to patients without calcinosis. Furthermore, anti-NXP-2 positive C+ showed a diffuse form of calcinosis from the beginning and lower frequency of respiratory tract involvement. No single drug or associations of drugs was found effective in the treatment of calcinosis.

Conclusion
A longer follow-up period of time, DM diagnosis and positivity for PM/Scl and NXP-2 could all be considered risk factors which foresee the development of calcinosis. Moreover, the positivity for antibodies to NXP-2 depicts a distinct phenotype of calcinosis with an early onset and quick widespread dissemination.

Key words
calcinosis, myositis, autoantibodies, anti-NXP-2, anti-PM/Scl

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Introduction

Calcinosis represents a severe complication of poly-dermatomyositis (PDM): it could develop in about 44–70% of children with juvenile dermatomyositis (JDM), especially when a delay of diagnosis or a poor control of the disease occurs (1). Calcinosis can also arise in adult cases of polymyositis (PM) and dermatomyositis (DM) respectively in 5 and 10%, and recent data reported up to 20% prevalence of calcinosis in adult DM (2). Calcinosis is defined as a clinical condition characterised by the deposition of insoluble salts of calcium in skin, subcutaneous tissues and muscle, which may be responsible for pain and functional disability.

In JDM, cutaneous calcinosis typically occurs between 2–3 years after the onset of the disease, much earlier than in other connective tissue diseases and in adult PDM, in which it appears about 8 years after diagnosis. Calcinosis is usually localised in damaged tissues (due to local trauma or inflammatory process), without significant changes of calcium and phosphate serum levels (3). Several pathogenetic hypotheses are proposed, including inflammatory infiltrates in calcific deposits (4, 5); a local vascular ischaemia (6); a dysregulation of mechanisms controlling the deposit/solubility of calcium and phosphate (7, 8) and mitochondrial damage of muscle cells during DM (9).

Predictive parameters of the development of calcinosis in JDM were identified in early onset of DM, long disease duration, poor response to conventional drugs (1, 10-12). In patients with systemic sclerosis (SSc) an association of calcinosis with arthritis and digital ulcers was noticed (6).

Recently a new myositis-specific antibody, named anti-MJ/NXP-2, has been reported in association with cutaneous calcinosis in JDM (13) as well as in adult DM (14, 15). So far, in adult patients, a clinical profile of risk factors for calcinosis has not been defined. In addition, although many therapeutic aids have been proposed, calcinosis of the adult seems particularly refractory and currently there are no known treatments of proven efficacy (2).

The aim of this study is to identify possible clinical and/or immunological prognostic factors and treatment options in a monocentric cohort of patients with a diagnosis of PDM and calcinosis.

Materials and methods
Patients
Patients affected by PM, DM (16), inclusion body myositis (IBM) (17) and overlap syndromes (OS) with myositis such as systemic lupus erythematosus (SLE) (18), SSc (19), rheumatoid arthritis (RA) (20) and Sjögren syndrome (SSj) (21) attending our outpatient clinic between January 2013 and May 2014 were enrolled in the study: all cases with calcinosis (C+) and, as a control group, patients without calcinosis (C-) were considered. Clinical, serological and treatment data were retrospectively collected from clinical charts.

Calcinosis was defined as the presence of calcium deposition in the skin, subcutaneous tissues or muscle on physical examination and with plain radiographs in every patient. The therapy effect was assessed at the end of treatment and defined as "yes" when calcinosis improved, "partial" when stable and "no" when calcinosis worsened based on subjective reporting by a patient and on clinical evaluation by trained rheumatologists according to Galimberti *et al.* (22).

Methods
Antinuclear antibodies (ANA) were tested by indirect immunofluorescence (IIF) on HEp-2 cells and considered positive at titre ≥1:160 (BioRad, Hercules, CA, USA). Myositis-specific and associated autoantibodies (MSA and MAA) were detected by counterimmunoelectrophoresis (23), immunoprecipitation (IP) using 35-S-methionine-labeled K562 cell extract, RNA components analysis of immunoprecipitates by silver staining and anti-Jo-1 ELISA as previously described (24).

Ethics
The study was approved by the Institutional Review Board of the Hospital. The patients' written consent was obtained according to the Declaration of Helsinki, and the study was conducted

in compliance with the standards currently applied in our country.

Statistical analysis
Comparison between patients with and without calcinosis was performed with student's 2-tailed *t*-test for continuous variables and Chi-square or Fisher's exact tests for categorical variables. A multivariate analysis was conducted by a logistical regression model (Statview); *p*-values less than 0.05 were considered significant. Odds ratio (OR) and 95% confidence interval (CI 95%) were also calculated.

Results
Demographic and clinical data
Seventy-four patients (58 females, 16 males) with PM (30 cases), DM (30 cases), OS (13 cases) and inclusion body myositis (1 case) were enrolled. The majority of them were Caucasian (72; 97.3%), one African and one Asian. Mean age at disease onset was 43±17.4 years, and the average follow-up was 56 months (range 1 to 288 months). Calcinosis was found in 16 out of 74 patients (21.6%): 11/16 with DM (68.7%), 4/16 OS (25%) and 1/16 PM (6.2%). The prevalence of calcinosis was 36.7% in DM (11/30) 30.8% among the OS (4/13) and 3.3% (1/30) in PM cases. Calcinosis occurred a mean of 43.7 months (± 71) after the diagnosis; in 4 cases the onset was concomitant and for 1 patient it occurred before the diagnosis.

At onset 69% of calcinosis was isolated, while after one year more than 60% of our patients had multiple sites involved. Pelvic girdle, hands and extremities were the most frequently affected sites (50%, 37.5% and 31% respectively). About a quarter of cases showed calcinosis at root of upper limb; elbow, trunk and face were more rarely involved. Pain was associated in 50% of cases, superinfection in 38%, ulceration in 25%. About a third of cases were asymptomatic.

Comparison between patients with (C⁺) and without calcinosis (C⁻)
Clinical and serological features of patients with (C⁺) and without calcinosis (C-) are listed in Table I. C⁺ patients showed a quite longer follow-up

Table I. Clinical and laboratory data of 16 patients with calcinosis (C⁺) and 58 patients without calcinosis (C⁻) at the univariate analysis.

	C⁺, n. 16 (%)	C⁻, n. 58 (%)	*p*-value	OR (CI 95%)
Follow-up, months; mean ± SD	201.8 ± 118.7	111.9 ± 87.4	0.001	
DM	11 (68.7)	19 (32.7)	0.021	4.5 (1.2-17.7)
PM	1 (6.2)	30 (51.7)	0.003	0.06 (0.003-0.54)
Overlap syndrome	4 (25)	9 (15.5)	NS	
IBM	0 (0)	1 (2)	NS	
F:M ratio	13/3	45/13	NS	
Fever	7 (44)	18 (31)	NS	
Fatigue	15 (94)	55 (95)	NS	
Facial erythema	9 (56)	17 (29)	NS	
Heliotrope rash	9 (56)	17 (29)	NS	
Gottron's papules	8 (59)	11 (19)	0.02	4.27 (1.14-16.4)
Raynaud's phenomenon	10 (63)	29 (50)	NS	
Arthritis	5 (31)	27 (47)	NS	
Myositis	15 (94)	51 (88)	NS	
Fingertip ulcers	3 (18.75)	2 (6.7)	NS	
CK elevation	8/15 (53)	48/56 (86)	0.012	0.19 (0.04-0.79)
Dyspnea	12 (75)	48 (83)	NS	
Dysphagia	10 (63)	33 (57)	NS	
Heart involvement	1 (6)	7 (12)	NS	
ILD	8 (50)	20 (34)	NS	
Alveolitis	2 (13)	7 (12)	NS	
Respiratory muscle defect	5 (31)	20 (34)	NS	
DLCO reduction (< 70% predicted)	6/14 (43)	29/55 (53)	NS	
FVC reduction (<70% predicted)	0/14 (0)	10/55 (18)	NS	
Altered capillaroscopy	9/10 (90)	31/38 (82)	NS	
Scleroderma pattern	7/10 (70)	15/38 (39)	NS	
Electromyography alterations	13/13 (100)	43/55 (78)	NS	
Muscle biopsy alteration	6/6 (100)	31/36 (86)	NS	
Skin biopsy alteration	5/5 (100)	12/13 (92)	NS	

(201.8 *vs*. 111.9 months, *p*=0.001), a higher percentage of DM (*p*=0.026, OR 4.5, CI 95%=1.16–19.38) and less prevalence of PM (*p*=0.001, OR 0.067, CI 95%=0.03–0.54). OS was more frequently detected in C⁺ group, without statistical significance. Concerning clinical data, C⁺ cases more frequently showed Gottron's lesions (*p*=0.021, OR 4.27, CI 95%=1.14–16.4), a low frequency of high CK elevation (*p*=0.012, OR 0.19, CI 95%=0.04–0.79), but no other statistical differences were found between the two groups. Fingertip ulcers were detected only in a few patients, more frequently in C⁺ than in C- without statistical difference. Two out of the three patients with calcinosis and fingertips ulcers were affected by scleromyositis with antibodies to PM/Scl. Only one out of the 6 patients with calcinosis of the hands concomitantly had fingertips ulcers. Furthermore, no differences were found in the frequency of other systemic sclerosis features. Regarding cancer occurrence, no differences between the two groups were recorded.

Autoantibody analysis
ANA were globally found in 52 patients (70.3%), with 5 cytoplasmic pattern; anti-ENA in 72.9% of cases. Anti-ENA were more frequently detected in C⁺ group (94% *vs*. 67%), with a nearly significant *p*-value (0.053), mostly represented by anti-Jo1 (23%), anti-Ro/SSA (23%), anti-NXP-2 (10.8%) and anti-Ku (9.4%). No differences between C⁺ and C- groups were reported, except for anti-PM/Scl which were found more frequently in C⁺ cases (*p*=0.0068, OR 19, CI 95%=1.7–490), probably due to a higher number of overlap cases in these patients. Anti-NXP-2 antibodies seem to be more frequent in C⁺ cases, although the difference is not statistically significant (Table II).

Anti-NXP-2 + patients with calcinosis
Four patients with calcinosis showed anti-NXP-2 antibodies: all of them showed DM and 3 out of 4 NXP-2 positive patients presented multiple site involvement since the onset of the disease, representing 60% of patients with early diffuse calcinosis (three out

Table II. Autoantibodies distribution in 16 patients with calcinosis (C^+) and 58 patients without calcinosis (C^-).

	C^+ n. 16 (%)	C- n. 58 (%)	*p*-value	OR (95% CI)
ANA	14 (88)	38 (66)	NS	
ENA positivity	15 (94)	39 (67)	0.053	
MSA	7 (43.7)	24 (41.4)	NS	
MAA	9 (56.2)	26 (44.8)	NS	
Jo-1	3 (19)	14 (24)	NS	
Ku	0 (0)	7 (12)	NS	
U1RNP	0 (0)	3 (5)	NS	
PM/Scl	4 (25)	1 (2)	0.0068	19 (1.7-490)
NXP-2	4 (25)	4 (7)	0.061	
Mi2	0 (0)	2 (3)	NS	
Ro/SSA (60 and/or 52 KD)	3 (19)	14 (24)	NS	
Ro/SSA+La/SSB	2 (13)	1 (2)	NS	
MDA-5	2 (13)	3 (5)	NS	
SRP	0 (0)	1 (2)	NS	
SMN	0 (0)	1 (2)	NS	

Table III. Treatment options used in 16 patients with calcinosis.

	n. (%)	Mean duration, months (SD)	Treatment effect
Calcium-channel blockers (CCHB)	2 (13)	36 (17)	no
Diltiazem	7 (43.7)	32.2 (14.5)	no
Bisphosphonates	11 (69)	61 (39.8)	no
Warfarin	2 (13)	77 (77)	no
Intravenous immunoglobulin (IVIg) 2gr/kg every 4 weeks	7 (44)	6 months	Yes in 1 case
Colchicine	9 (56)	35.7 (37.8)	Yes in 1 case
Rituximab	2 (13)	1.5 cycle	Yes in 1 case
Infliximab 5mg/kg every 4 weeks	2 (13)	8.5 (6.3)	no
Sodium thiosulphate ointment	5 (31)	7.6 (6)	Partial in 1 case
Surgery	6 (38)	-	Yes in 3

of five). By comparing 4 anti-NXP-2+ with calcinosis with other 12 patients with calcinosis, we recorded a lower frequency of dyspnea (*p*=0.027;OR: 0.03; CI 95%=0.0–0.9), and higher values of DLCO (mean 92%±17.4% *vs.* 64%±15.3%; *p*=0.02).

Overlap syndromes
An OS of PDM and other systemic connective tissue disease was diagnosed in 13 of our patients (17.6%), respectively 4 cases among C^+ and 9 among the C^- groups. Overlap SSc-myositis was the most common OS (7 cases, 53.8%), followed by the overlap of PDM-SLE (4 cases, 30.8%), PDM-SSj (2 cases, 15.4%) and PDM-RA (1 case, 7.7%). By comparing the antibody profile of patients with the OS to the PDM alone we reported a higher frequency of anti-PM/Scl (*p*=0.016, OR18, CI 95%=1.14-500) and anti-Ku antibodies (*p*=0.16, OR8.59, CI 95%=1.31–60.8) in patients with the OS. Moreover,

antibodies to PM/Scl were more frequent in scleromyositis with calcinosis (*p*=0.029, OR infinite, CI 95% 0.8-infinite) *versus* those without calcinosis.

Multivariate analysis
MSA define clusters of patients with distinctive clinical features, therefore it is possible that a number of clinical and laboratory features might not be independently associated with calcinosis. For the multivariate analysis we included the features associated to the univariate analysis plus the anti-NXP-2 positivity, because during the first analysis this antibody almost reached the significant threshold. By the multivariate analysis, most of the previous associations persisted: calcinosis resulted independently associated with longer follow-up duration (*p*=0.006, OR 0.006, CI 95%=1.004–1.022), anti-PM/Scl (*p*=0.33, OR 20.9, CI 95%=1.28–340) and inversely associated with CK elevation at disease onset (*p*=0.05, OR

0.97, CI 95%=0.009–1.003). Consequently our hypothesis concerning anti-NXP-2 antibody has been confirmed, and this antibody (*p*=0.024, OR 21.9, CI 95%=1.5–319) could be considered as an independent predictive risk factor for calcinosis development.

Treatment
All patients received oral steroid upon diagnosis, and in both groups the most common dosage prescribed was at 0.5–1 mg/kg/day without any statistical differences (56% in C^+ and 60% in C^- patients respectively); during follow-up prednisone was mostly given at 5–10 mg/daily. Methotrexate and azathioprine were the immunosuppressant most frequently recommended, followed by cyclosporine-A, mycophenolate mofetil, cyclophosphamide and hydroxychloroquine. By comparing the use of immunosuppressant in patients C^+ and C^-, no differences were noted regarding the number of immunosuppressant used or concerning the doses of steroids except for the higher use of azathioprine among patients with calcinosis (69% *vs* .17%, *p*<0.001 OR 10.56, CI 95%=2.6–45.8). Different calcinosis-specific treatments were settled: calcium-channel blockers (*i.e.* diltiazem 90 to 240 mg/day or nifedipine 30 mg/day), bisphosphonates (oral alendronic acid in 8 cases, oral risedronic acid in 2, *i.m.* clodronate in 1 case), warfarin (anticoagulant dose), intravenous Ig (2 g/kg/month for 6 months), colchicine, rituximab (500 mg/week for 4 consecutive weeks), infliximab (3 mg/kg/6 weeks), sodium thiosulphate ointment (3% then 10% concentration) (Table III). None of these treatments allowed the reduction of calcinosis or the prevention of new sites involved, however for few cases there was a subjective improvement in associated symptoms (*i.e.* pain reduction).
Surgical removal was performed in 6 cases: in 3 patients a relapse of calcinosis occurred, while 3 cases showed a total remission. Two patients presented infective complication, locally in one case and evolved in sepsis in the other case.

Discussion
In our study the prevalence of calcinosis in DM reached 36.7% while in OS

it stretched to 30.8%. These figures are higher if compared to most recent paper (14) but are in line with other previous papers (25, 26). A possible explanation for these discrepancies could be that our study considered every consecutive patient seen in our outpatient clinic, satisfying the classification criteria for idiopathic myopathies (16) and therefore a number of patients diagnosed in our Institution and presently in remission were not included in the analysed cohort.

In the present study, clinical predictors of development of calcinosis were identified in DM diagnosis, Gottron's papules and a longer follow-up. The duration of follow-up could be considered as a surrogate marker of the disease duration, it can subsist due to the persistence of active clinical problem, as unresolved calcinosis, while the longer follow-up in C^+ patients is consistent with what reported in previous studies (14). However, despite a follow-up of more than 15 years, in 5 out of 16 (30%) of our cases, calcinosis was detected at the diagnosis of DM or before. In the remaining 70% of cases calcinosis appears relatively early during the follow-up, with a mean of 4 years since the onset of PDM. In addition, while at the onset only a few patients presented diffuse calcinosis, after one year more than 60% reported multiple sites involved. The rapid onset after diagnosis and the rapid spread of locations makes it more likely that the onset of calcinosis represents a specific pattern of disease rather than the consequence of a chronically active disease (27). No other clinical manifestations seem to differ between C^+ and C^- cases and our results confirm what has been recently published by Valenzuela (14), except for the distribution of calcinosis that in 37.5% of our patients involved the hands with the absence of fingertip ulcers that are instead prominent in Valenzuela's experience (14). Furthermore, in previous researches an between fingertip ulcers and antibodies to MDA-5 was reported (14, 28), while in our cohort the association was not confirmed, both in our present and past studies (24). This lack of association could exist due to the low number of cases considered.

It is well known that calcinosis is more related to DM than PM, as in JDM, and this epidemiological association has been confirmed in our population. Conversely, CK levels were significantly higher in C^- compared to C^+ at the onset of disease.

Even though not statistically significant, a trend to more ANA and anti-ENA positivity was recorded in C+ group. Laboratory predictors of onset of calcinosis in this study could be found in anti-NXP-2/MJ and PM/Scl. The association with anti-PM/Scl is justified by the inclusion of OS in our study in which this antibody is over-represented ($p<0.0152$) and associated with calcinosis ($p<0.003$) as already reported in the literature (26, 29). On the contrary, anti-Ku associated with OS ($p=0.0178$) seems to protect from the appearance of calcinosis, even though this result is not statistically significant. Concerning MSA, anti-NXP-2/MJ antibodies were significantly associated to C^+ by multivariate analysis and therefore they represent the main serological marker of calcinosis in DM, in adults as well as in JDM. Furthermore, since the beginning of the study, anti-NXP2$^+$ C^+ subgroup showed a particular phenotype characterised by lower prevalence of respiratory muscle involvement, and diffuse distribution of calcinosis, representing a marker of more severe calcinosis in adults (30), as well as in JDM (13). Anti-NXP-2 antibody has been recently reported to associate with cancer in IIM patients (31, 32). However, this is a controversial topic since in none of our previous experiences, as well in that of others (33), neither in previously 10 anti-NXP-2 positive patients (15), nor in the present study we have detected cancer-associated myositis despite the long follow-up (mean 117.6 months).

In the past, calcinosis was attributed to a persistently active disease (27) measured by a number of prescribed immunosuppressant drugs. No difference between the number of immunosuppressants and steroid dose used in each group of patients was observed in our series. Nevertheless, azathioprine was more frequently used in C^+ cases, probably in order to obtain a better control of calcinosis deposition because of

the ineffectiveness of the previously prescribed drugs. We also prescribed many drugs commonly and specifically devoted to contrast the onset and/or the diffusion of calcinosis (34). Unfortunately, no single drug or association of drugs was found effective in the treatment of calcinosis. Even the use of infliximab, a biologic agent blocking the TNF-α, that is considered a possible inducer of calcium deposition, was ineffective in our experience as well as in the others (35), while it has been reported to be effective in JDM (36). Surgical removal seems to be effective in some patients: unfortunately 3 out of 6 patients (50%) who underwent excision of calcinosis developed serious infective complications.

Our study has some limitations. The most important one regards the small number of patients enrolled in the study. Nevertheless, a recent paper (14) with a similar number of patients has been published showing similar results especially regarding the length of follow-up and the relevance of anti-NXP-2/MJ antibody as marker of calcinosis. Another issue can be identified in the empirical evaluation of calcinosis in response to treatment which is based only on subjective clinical reporting. However, as far as we know, there are currently no validated outcome measures to assess calcinosis in myositis.

In conclusion, in this study, predictors of development of calcinosis could be identified in a longer follow-up, DM diagnosis and positivity for PM/Scl and NXP-2. Moreover, the positivity for antibodies to NXP-2 depicts a distinct phenotype of calcinosis with an early onset and quick widespread dissemination and lower prevalence of muscle respiratory tract involvement. Persistence of calcinosis resistant to any pharmacological treatment can justify further research on calcinosis in PDM patients comparing C^+ *versus* C- with myositis.

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