Age-dependent inhibition of ectopic calcification: a possible role for fetuin-A and osteopontin in patients with juvenile dermatomyositis with calcinosis

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Objectives. To assess if age and/or age-dependent variations in the levels of two major calcification regulatory proteins, fetuin-A and osteopontin, could be associated with an increased risk of calcinosis in children with juvenile dermatomyositis (JDM).

Methods. The frequency of calcinosis was derived from a national UK database of 212 cases of JDM. Serum fetuin-A and plasma osteopontin levels were determined using ELISA in 15 JDM patients with calcinosis and 15 JDM patients without calcinosis. Healthy controls were 19 age-matched children, 24 adolescents and 13 adults. Sixteen patients with juvenile idiopathic arthritis (JIA) were additional paediatric disease controls.

Results. Of the 212 JDM cases 10% had calcinosis. Calcinosis patients had younger age of disease onset than those without calcinosis (mean age of 5.3 yrs vs 7.1 yrs, respectively, P = 0.016). No significant difference in fetuin-A or osteopontin could be detected between the two JDM groups. Fetuin-A levels in all groups of children and the adolescent group were much lower than described previously in adults, and there was a significant positive correlation between age and fetuin-A level, and also between osteopontin levels in plasma and serum fetuin-A.

Conclusions. Children who develop JDM at an younger age may have increased risk of developing calcinosis. Physiologically low levels of fetuin-A in young children combined with an additional negative acute-phase effect on fetuin-A due to chronic inflammation could explain in part the propensity to develop ectopic calcification observed in JDM patients, and why calcinosis is less frequent in adults with dermatomyositis.

Key words: Juvenile dermatomyositis, Calcinosis, Fetuin-A, Osteopontin.

Introduction

Juvenile dermatomyositis (JDM) is a rare systemic autoimmune disease with a reported incidence of 1.9 to 4.1 per million children [1, 2]. The disease is characterized by progressive weakness of proximal muscles and cutaneous manifestations [3, 4], and extramuscular involvement contributes significantly to morbidity [5]. The prognosis expressed both as mortality and long-term disability seems to have improved significantly over the last 5–10 yrs, but the disease is still life-threatening in some patients and can have a major impact on well-being, function, growth and development in affected children [6, 7].

Ectopic calcification in skin and muscles is one of the common complications of JDM and has been reported as frequently as in 34% [6]. Children with prolonged untreated active symptoms of JDM are at greater risk of this complication [8]. Oedema of the skin, subcutaneous tissue and fascia demonstrated with MRI may precede the development of ectopic calcification [9], as does chronic cutaneous inflammation [10]. The relatively lower incidence of calcinosis observed in more recent case series could suggest that earlier diagnosis and more aggressive treatment with corticosteroids and immunosuppressant drugs may change the outcome of this particular debilitating complication of the disease [4, 8], although this latter point remains controversial.

Notably, calcinosis is seen much less frequently in adults with dermatomyositis raising the possibility that age-dependent factors may influence the risk of developing ectopic calcification in response to inflammatory muscle and skin injury [11].

Ectopic calcification, currently most extensively studied in cardiovascular disease [12] and chronic renal failure [13], is a highly regulated process involving both inductive and inhibitory mechanisms. Under normal physiological conditions the calcium–phosphate (Ca × P) product in extracellular fluid is at the limit of spontaneous calcium–phosphate deposition. It is thus accepted that changes unfavourably affecting the plasma and/or tissue Ca × P product can drive the calcification process. Additionally, it is now clear that certain pathological states including renal failure favour ectopic calcification [14].

Several inhibitors of ectopic calcification have now been identified and characterized and include matrix Gla protein (MGP), fetuin-A and osteopontin. The contribution of these molecules to the regulation of ectopic calcification in human disease states is becoming increasingly recognized [15], particularly in the development of vascular calcification in patients with chronic renal failure [16].

Fetuin-A (α2-Heremans-Schmid glycoprotein) is a major systemic inhibitor of calcification and is regarded as accounting for as much as 50% of the calcification inhibitory capacity of human plasma [14, 16]. Fetuin-A is produced in human liver but is also expressed in many organs during embryogenesis [14]. Low levels of fetuin-A are associated with higher CRP levels and increased cardiovascular mortality in adults on haemodialysis [16, 17]. It is suggested that chronic low serum fetuin-A contributed to ectopic aortic ring calcification observed in some of the patients in that study. Since fetuin-A has previously been reported to be a negative acute-phase reactant [17–19] it is an attractive protein to study in the context of the calcinosis associated with JDM. Surprisingly, however, serum levels of fetuin-A in healthy children have only
recently been reported by our group (R.S. et al. [19]), and little or no data exist for children with chronic inflammatory diseases. Osteopontin is a phosphorylated acidic glycoprotein that binds strongly to calcium phosphate crystals and inhibits crystal growth, and bone resorption and remodelling is also substantially impaired in the absence of osteopontin [20]. The relationship of osteopontin to chronic inflammation is more complex than fetuin-A since osteopontin has dual properties acting both as a pro-inflammatory and anti-inflammatory cytokine, with the potential ability to limit fibrosis and calcinosis [20].

Since calcinosis commonly occurs in JDM, but is rarely seen in adults with dermatomyositis [11], the hypotheses that we explored in the present paediatric study was first that younger JDM patients would be more at risk of calcinosis; and second, that age-related differences in serum or fetuin-A and/or plasma osteopontin may contribute, in the context of inflammation, to the pathological ectopic calcification observed in children with JDM. To address these hypotheses the aims of this study were to examine the relationship between age of disease onset and presence or absence of clinical calcinosis; and to measure serum fetuin-A and plasma osteopontin in children with JDM (with and without calcinosis); age-matched healthy controls; an older group of healthy adolescents and chronic inflammatory disease controls.

Patients and methods

Selection of JDM patients and controls

Twenty-one patients with JDM and calcinosis were identified from the JDM National Registry and Repository (UK and Ireland) [4] of 212 JDM patients providing an estimate of frequency of calcinosis of 10.0% in this cohort. The presence or absence of calcinosis was verified using the patient’s clinical records and also by blindly reviewing all X-rays and magnetic resonance images. Clinical and laboratory parameters and data regarding treatment at the time of sampling were collected from clinical files. X-ray and ultrasound documentation of subcutaneous calcinosis was noted where available, as well as MRI of muscle, lumbar spine bone densitometry, muscle biopsy, childhood myositis activity score [using Childhood Myositis Assessment Scale (CMAS)] and blood pressure.

Serum and plasma samples were available from 15 of the patients with calcinosis. Fifteen patients without calcinosis were selected from the same database based on the presence of serum and plasma samples available for study and also to closely match the disease duration prior to blood sampling of the calcinosis group, since disease duration has previously been shown to be an important risk factor for calcinosis [8].

The study had local ethical approval and additional approval from the UK JDM Repository Steering Committee, and all samples in this repository are obtained with informed consent for research.

Healthy controls

Samples from healthy children were a gift from the Medical Research Council (MRC) Childhood Nutrition Research Centre (based at the ICH, London), with ethical approval. Two sets of control plasma and serum samples were analysed. The first set of 24 serum samples was collected from healthy adolescents of median age 14.5 yrs (range 14.0–17.0 yrs). A second set of serum samples for fetuin-A analysis was collected from 19 healthy children age matched to the JDM calcinosis group. These controls were significantly younger (P < 0.001) than the adolescents, with median age 9.3 yrs, (range 5–12.8 yrs). In addition, osteopontin was analysed in plasma from 15 healthy adolescents and 13 healthy adults (this latter group included as an additional internal control for comparison with the quoted manufacture reference range for healthy adults); although due to lack of sample availability osteopontin was not measured in healthy child controls.

JIA controls

To examine the potential effect of another chronic inflammatory stimulus on fetuin-A and osteopontin levels, samples from JIA patients were obtained from part of a study of disease mechanisms in JIA, with ethical approval. Serum and plasma samples were collected from patients with JIA and matched one by one with JDM patients without calcinosis based on ESR (±1 mm/h, except for two samples) as a laboratory indicator of comparable inflammatory activity.

Fetuin-A assay

Serum fetuin-A was measured by ELISA using a commercially available kit from Epitope Diagnostics, Inc. (San Diego, CA, USA). All serum samples were separated immediately and frozen in liquid nitrogen until used. The kit was used according to the protocol provided by the manufacturer. In brief, microtitre wells coated with a high affinity polyclonal goat anti-human fetuin-A antibody were incubated with serum samples from each of the study groups diluted 1:10 000; and commercial fetuin-A standards supplied with the kit. After incubation for 2 h at room temperature, peroxidase-conjugated polyclonal anti-human fetuin-A antibody and substrate was added. A standard curve was generated by plotting logarithmic absorbance vs logarithmic human fetuin-A concentration and the best fit line was determined by regression analysis. The intra-assay and inter-assay coefficient of variation were <5.5 and <6.8%, respectively. The reference range for healthy adults quoted by the manufacturer was 0.5–1.0 g/l, and the minimum sensitivity of the assay was 5.0 ng/ml.

Osteopontin assay

Plasma osteopontin was measured by sandwich ELISA using a commercially available kit from R & D Systems, Europe Ltd. (Abingdon, UK) as per manufacturer recommendations. In brief, microtitre wells coated with a polyclonal mouse monoclonal antibody (892817) against the functional region of human osteopontin were incubated with plasma from patient samples (again, separated immediately after venepuncture and frozen in liquid nitrogen until used) diluted 1:25; and standards provided with the kit of known osteopontin concentrations and controls for 2 h at room temperature, followed by peroxidase-conjugated anti-human osteopontin and substrate. The intra-assay and inter-assay coefficient of variation were <4 and <7%, respectively. Quoted manufacturer reference values for healthy adults for osteopontin in EDTA and heparin plasma were 49.2–175 and 53.2–195 ng/ml, respectively. The sensitivity of the assay was 0.011 ng/ml.

All assays (fetuin-A and osteopontin) were performed by a single investigator (V.S.) blinded to the patient group sample allocation.

Statistical analyses

SPSS® version 14 for Windows® (SPSS Inc., Chicago, IL, USA) was used for analyses. Median and range values for patient group continuous variables are presented, unless otherwise stated. Student’s t-test for independent samples was used to compare the mean age of disease onset of the calcinosis patients compared with the rest of the JDM cohort having first confirmed normality of the data. The Kruskall–Wallis and Mann–Whitney U-tests were used to compare continuous variables between the smaller study groups. Correlations between continuous variables were analysed using Spearman’s rank correlation coefficient. For all statistical tests significance was set at P < 0.05.
Results

JDM patient characteristics

Characteristics of the JDM patients at the date of obtaining serum and plasma samples for fetuin-A and osteopontin analyses are summarized in Table 1. Overall, there was no statistically significant difference in any of the characteristics between the two JDM patient groups, although arthritis was observed in 9/15 of the JDM patients with calcinosis and 3/15 of the non-calcinosis group. All samples were taken after treatment had been started, although two patients were off treatment at the time of sampling. The medication received by the patients prior to the date of the blood sample is documented in Table 1.

The 16 JIA disease control patients were matched for ESR with the JDM patients with calcinosis. The median bone densitometry at time of blood sample is 1.65 (range 0.71–7.1 yrs, respectively, P = 0.016). Five out of the 15 patients in the calcinosis group subsequently developed tightness of the skin of the hands at follow-up, which was not observed at initial presentation, reminiscent of scleroderma overlap but with no other features of scleroderma or SSc. Only one of these patients was positive for the autoantibody Scl-70, and in this patient Scl-70 was only detected transiently. The characteristics of the 30 JDM patients at the date of blood sampling for fetuin-A and osteopontin analyses are described subsequently, and summarized in Table 1.

General clinical features and relationship to calcinosis

There was no significant difference in disease duration between the calcinosis and non-calcinosis groups. Inflammatory markers (ESR and CRP) and muscle enzymes (CK and LDH) at the time of serum sampling were in general normal and without differences between the two JDM groups. At the time of sampling all patients had well-controlled disease activity based on inflammatory markers, muscle enzymes and muscle strength. A significant number in each group still had rash, however (see subsequently). Arthritis was observed more frequently in JDM patients with calcinosis (9 out of 15 patients) than those without calcinosis (3 out of 15 patients). Of the 15 calcinosis patients, 5 had lipatrophy, compared with none in the group without calcinosis. There was no difference in median CMAS between the two JDM groups. Of the 15 patients in the calcinosis group, 9 had joint contractures due to previous arthritis compared with none in the group without calcinosis. Lumbar spine bone densitometry, expressed as Z-scores derived from a healthy population of age-matched paediatric controls, was documented close to blood sampling from the JDM patients. The median bone densitometry Z-score in the patients with calcinosis vs non-calcinosis group was –1.65 vs –1.05, respectively (P = 0.3; Table 1).

Cutaneous features of the calcinosis and non-calcinosis patients

Five out of the 15 patients in the calcinosis group subsequently developed tightness of the skin of the hands at follow-up, which was not observed at initial presentation, reminiscent of scleroderma overlap but with no other features of scleroderma or SSc. Only one of these patients was positive for the autoantibody Scl-70, and in this patient Scl-70 was only detected transiently. In contrast, development of skin tightness affecting the hands was observed in only one patient in the non-calcinosis group. All patients in the non-calcinosis group were Scl-70 negative. Apart from this observation there were no other differences in cutaneous features in the calcinosis vs non-calcinosis JDM patients (Table 1).

Treatment received by JDM patients

All patients received standard treatment with MTX and prednisolone, except for one patient without calcinosis who needed only prednisolone. Additional treatment with AZA or ciclosporin had been given to approximately the same number of patients in
each group. Six of the patients in the calcinosis group had previously received cyclophosphamide based on signs of ongoing inflammation, whilst this was the case for three patients in the group without calcinosis. The number of patients who were treated with infliximab was 6/15 and 1/15, respectively in the calcinosis and non-calcinosis group. Two of the patients in the calcinosis group, and two in the non-calcinosis group were off all treatment at the time of blood sampling.

**Serum fetuin-A levels**

There was no significant difference in fetuin-A levels in serum between the JDM patients with and without calcinosis, and healthy age-matched controls (Fig. 1; Table 2). JDM patients with calcinosis had lower fetuin-A levels than those without, although this observation did not reach statistical significance (Fig. 1). There was also no significant difference in fetuin-A levels in the JIA group compared with the JDM patients (with or without calcinosis). Fetuin-A levels in the JIA group were significantly lower than the healthy control children and the healthy adolescents; however, the JIA group was significantly younger than both these control groups (Table 2).

Overall, there was no correlation between ESR levels and fetuin-A levels at the time of sampling. Correlation analysis between age and serum fetuin-A levels (Fig. 2) in patients and controls also indicated that the differences found between the groups in this study were primarily due to age-dependant differences in fetuin-A concentration in serum. This was also confirmed by analysing the correlation between age and fetuin-A levels in healthy controls only (R = 0.479, P = 0.001). However, the levels found in all groups of children were strikingly lower than what has been reported in adults. We were unable to detect any obvious relationship between clinical severity of calcinosis and fetuin-A levels in this study (data not shown).

**Osteopontin analyses**

There was no statistical difference between osteopontin levels in plasma between any of the JDM groups, JIA patients or healthy adolescents. Plasma samples from children age matched to the JDM patients were not available for osteopontin analysis. Both the JDM patient groups, JIA patients and the group of healthy adolescents had significantly higher osteopontin levels than a group of 13 healthy adult controls (P < 0.001, Fig. 3, Table 2). The median osteopontin level in this latter group was 157.5 ng/ml (range 90–187.5 ng/ml), which was similar to the quoted

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**TABLE 2. Age, fetuin-A and osteopontin levels in patients with JDM with and without calcinosis, in JIA patients and normal controls**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (in months)</th>
<th>Serum fetuin-A (g/l) (median/range)</th>
<th>Plasma osteopontin (ng/ml) (median/range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JDM patients with calcinosis</td>
<td>100 (63–209)</td>
<td>0.24 (0.04–0.50)</td>
<td>381.25 (190–752.5)</td>
</tr>
<tr>
<td>JDM patients without calcinosis</td>
<td>131 (53–159)</td>
<td>0.30 (0.17–0.45)</td>
<td>400 (247–610)</td>
</tr>
<tr>
<td>JIA patients</td>
<td>62.5 (22–150)</td>
<td>0.23 (0.10–0.25)</td>
<td>375 (110–765)</td>
</tr>
<tr>
<td>Normal children</td>
<td>112 (60–153)</td>
<td>0.30 (0.21–0.52)</td>
<td>ND</td>
</tr>
<tr>
<td>Normal adolescents</td>
<td>174 (168–204)</td>
<td>0.39 (0.37–0.78)</td>
<td>372.5 (265–717.3)</td>
</tr>
<tr>
<td>Normal adults</td>
<td>ND</td>
<td>ND</td>
<td>157.5 (90–187.5)</td>
</tr>
</tbody>
</table>

**Group comparisons**

<table>
<thead>
<tr>
<th>Group</th>
<th>P-value</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>JDM patients with calcinosis</td>
<td>0.085</td>
<td>0.967</td>
<td>0.747</td>
</tr>
<tr>
<td>All JDM patients (age 119 months) vs JIA patients (age 62.5 months)</td>
<td>&lt;0.001</td>
<td>0.14</td>
<td>0.539</td>
</tr>
<tr>
<td>JIA patients (age 62.5 months) vs normal children (age 112 months)</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>0.870</td>
</tr>
<tr>
<td>Normal children (age 112 months) vs normal adolescents (age 174 months)</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a n=15; median age in months. ND: not done.*
manufacturer reference range healthy adults for EDTA plasma of 49.2–175 ng/ml.

There was a modest but highly significant positive correlation ($R = 0.39$, $P < 0.008$) between fetuin-A levels and osteopontin levels when the three paediatric patient groups (JDM with and without calcinosis, and JIA patients; $n = 44$, two missing data points: one missing osteopontin value from the JDM group and one from the JIA group) were analysed together indicating that when fetuin-A level in serum is low, plasma osteopontin tends to be low as well (Fig. 4).

Discussion

Currently it is not understood why calcinosis occurs in some patients with JDM and not in others, and once it occurs it is difficult to treat. An understanding of the mechanisms regulating ectopic calcification could help explain the link that exists between age, chronic inflammation and calcinosis in JDM, and could ultimately lead to novel therapeutic approaches.

In the present study, we observed that those who developed calcinosis were significantly younger at disease onset compared with those without calcinosis. The JDM patients with calcinosis in the present study were also younger at disease onset (5.3 yrs) than the median age of onset of 6.75 yrs previously reported by our group for the total cohort of JDM patients in the National JDM Registry [4]. This observation that differs from one previously reported series of JDM patients, where no relationship was found between calcinosis and age at diagnosis, albeit in a smaller cohort of patients [6], although is in agreement with a recent observation by Pachman et al. [10].

Ectopic calcification is a complication more often associated with dermatomyositis in childhood than in adults [11] suggesting that age-related factors are relevant. Calcinosis in JDM has previously been reported to be associated with duration of untreated illness [7, 8] and long disease duration [7]. Genetic predisposition may be influential, for example, there may be increased risk in the presence of the TNFRα-308A allele [21].

The mineral observed in biopsies from calcified tissue in children with JDM is hydroxyapatite with crystallite sizes comparable to those found in normal bone [22, 23]. Fetuin-A and osteopontin are important regulatory proteins involved in the control of calcification and deposition of calcium–phosphate crystals as hydroxyapatite [24, 25]. It is also known that fetuin-A inhibits formation of the apatite precursor mineral basic calcium phosphate, but does not dissolve basic calcium phosphate or hydroxyapatite when it is already formed [24, 25].

Our group has recently reported age-related serum fetuin-A levels in healthy children and we have also recently confirmed the kinetics of the protein as a negative acute-phase reactant in children ([19] and Shroff et al., in press). The present data, however, demonstrate that fetuin-A levels in younger children are even lower than those reported in adult patients with renal failure as an independent risk factor of adverse outcome [17]. In that study, cardiovascular mortality was significantly raised in patients with fetuin-A levels of 0.20–0.54 g/l [17] suggesting that fetuin-A may influence vascular calcification in adults [26].

We have also recently demonstrated that low fetuin-A levels are associated with increased vascular stiffness and calcification in children with renal failure [19]. Moreover, in that study, there was an inverse relationship between fetuin-A levels and high sensitivity CRP (hs-CRP) [19] confirming fetuin-A as a negative acute-phase reactant. We suggest that this area is worthy of further study in JDM, since it could influence long-term cardiovascular morbidity and/or mortality.

It is clear that calcification in childhood is an essential component of skeletal growth and maturation, and correspondingly levels of fetuin-A may necessarily need to be low in the young to contribute to the pro-calcific environment required for normal skeletal growth. In healthy children, this would clearly be advantageous and could represent an important evolutionary metabolic adaptation integral to growth. Indeed, the observed difference in osteopontin in children vs adults in the present study may reflect this higher bone-mass turnover in the growing skeleton, although this remains speculative. In chronic inflammatory disease in children, this finely controlled balance may become upset because fetuin-A has been reported to be a negative acute-phase protein [17–19]. Thus, a fall in serum fetuin-A in JDM patients could be one factor that may tilt the Ca$^2+$ X P balance in damaged inflamed tissue towards ectopic calcification [27, 28].

In the present study, changes in fetuin-A due to chronic inflammation could not be demonstrated, probably because most patients in all disease groups (JDM and JIA) were well controlled and had low acute-phase responses (Table 1), although one caveat is that we did not measure hs-CRP. We recently have demonstrated that there is a tight inverse relationship between fetuin-A and hs-CRP in healthy children and children with renal failure [19],

![Fig. 3. Plasma osteopontin levels (nanograms per litre) in JDM patients and controls. Kruskall–Wallis test revealed significantly lower plasma osteopontin levels in healthy adults compared with any of the other groups: JDM patients with calcinosis (JDM ca+); or without calcinosis (JDM ca–); or healthy adolescents (Age 8.3 yrs (5.3–17.4 yrs); Age 9.4 yrs (4.4–13.2 yrs); Age 14.5 yrs (14.0–17.0 yrs)); or healthy adolescents (Age 14.5 yrs (14.0–17.0 yrs)); or healthy adolescents (Age 14.5 yrs (14.0–17.0 yrs)). There was a modest but highly significant positive correlation ($R = 0.39$, $P < 0.0082)$ between serum fetuin-A and plasma osteopontin irrespective of clinical diagnosis.](image-url)
emphasizing that ongoing sub-clinical inflammation could be directly linked to the development of calcinosis in JDM by lowering fetuin-A. In that context, another limitation of our study, which could account for the lack of apparent effect of chronic inflammation, was that fetuin-A and/or osteopontin levels could have been altered by therapy, because most patients (13/15 in the calcinosis group and 13/15 in the non-calcinosis group) were treated with immunosuppressive therapy at the time of blood sampling. We were unable to overcome this limitation since only limited samples from untreated children were available to us from the JDM National Registry and Repository for this study. However, although this impacted to some extent on the powering of our study, we did not detect any evidence of selection bias relating to those patients not included that would have influenced these results.

Low fetuin-A and a corresponding low osteopontin could explain why younger children with JDM are at greater risk of calcinosis, and speculatively may provide an explanation for why this is rarely seen in adults with dermatomyositis. It also would provide a logical scientific rationale for recommending aggressive anti-inflammatory treatment to treat and prevent calcinosis in JDM, not only to control local inflammation but also to avoid an additional negative effect of systemic inflammation on a low fetuin-A level in young patients.

In addition to being significantly younger at disease onset based on the data from the whole data set of 212 patients, the overall impression was that the 15 patients with calcinosis in this study had had a more severe disease course, inferred from the more aggressive use of cyclophosphamide and infliximab in that group (Table 1). Moreover, in previous reports, patients with calcinosis had significantly higher Child Health Assessment Questionnaire (CHAQ) scores than patients without calcinosis, indicating that this complication is associated with a more unfavourable functional outcome [6]. In the present study, this difference in treatment between the two JDM groups could be a confounding variable and may have affected fetuin-A levels causing a rise in this protein in the calcinosis group.

Our data suggest that fetuin-A levels alone do not determine whether a child with JDM will develop clinically overt calcinosis since we did not observe any statistically significant difference in plasma fetuin-A levels in those with calcinosis compared with those without, although we did observe a lower median fetuin-A level in those with calcinosis than those without (0.24 g/l vs 0.30 g/l; Table 2). This latter observation may be explained by the younger age of the calcinosis patients, given the significant correlation between age and serum fetuin-A levels that we observed. It is also remarkable that two of the JDM patients with calcinosis had lower fetuin-A levels than ever reported before. It is likely, however, that relatively small patient numbers resulted in inadequate power to detect such a small difference in fetuin-A between calcinosis and non-calcinosis groups. Indeed, power calculations predicted that differences in fetuin-A of 0.2 g/l would have been detected with 15 patients in each study limb, a difference greater than that observed in our study. Another limitation is that those children classified as not having calcinosis may have had sub-clinical calcification that was not detected. To address this important point and to allow quantification of calcinosis we are currently designing a radiological calcification mapping protocol, a tool that we hope to apply to future studies of this nature to allow correlation with serological parameters.

The molecular basis of pathological ectopic calcification remains unclear. This may occur due to changes in the aforementioned Ca × P product; by up-regulation of osteochondrogenic markers on cells not normally expressing those signals; and/or by down-regulation of inhibitory signals such as fetuin-A, osteopontin (OPN) or matrix GLA protein [14]. For example, in vitro treatment of vascular smooth muscle cells with elevated phosphate results in loss of their smooth muscle cell lineage markers with simultaneous gain of osteochondrogenic markers such as alkaline phosphatase, osteocalcin and osteopontin [27, 28]. Whether or not products of chronic inflammation could exert a similar effect on vascular smooth muscle, skeletal muscle cells or skin is not yet known. We speculate that negative acute-phase responses causing lowering of calcification inhibitors such as fetuin-A and/or OPN, combined with pathological expression of pro-calcific cell surface signals on skeletal muscle and/or subcutaneous cells could provide a plausible molecular model to explain the extent and duration of calcinosis in JDM. In support of this hypothesis, our data suggest that the pattern and distribution of inflammation is likely to influence ectopic calcification, since the JIA patient group did not have clinically overt calcinosis despite having comparable levels of fetuin-A to the JDM calcinosis patients. Future studies of these putative mechanisms are thus warranted in JDM.

In conclusion, we have demonstrated age-dependent changes in serum fetuin-A, a major inhibitor of ectopic calcification, which could be a contributing factor in the aetiology of ectopic calcification in JDM. Future work will examine the relationship of this novel observation to other parameters, which may predict future development of calcinosis and cardiovascular morbidity.

### Rheumatology key messages

- Risk of calcinosis is higher in younger children with JDM.
- This risk could be explained by age-dependent differences in fetuin-A, a major calcification inhibitory protein.

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### Disclosure statement

The authors have declared no conflicts of interest.

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