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# Elevated platelet counts in children with osteogenesis imperfecta suggest that inflammation is present

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Abstract

Background

Elevated platelet counts are observed in cancer, auto-immunity and inflammation with concurrent illness. Pro-inflammatory cytokines are elevated in murine osteogenesis imperfecta (OI) models. We hypothesised that platelet counts might be elevated in children with moderate-severe OI.

Methods

We reviewed the hospital records of 71 children with moderate-severe OI, treated in the Sheffield Highly Specialised Severe, Complex and Atypical OI Service. Data relating platelet count (below/above average, above upper limit) to prior and concurrent events were summarised as event proportions per child. Additionally, we created platelet standard deviation (SD) scores to assess the whole group in relation to age-related trends.

Results

1206 platelet counts were recorded. Platelet SD scores were right-shifted by 0.89 SD overall. 49 of 71 (69%) patients had at least one platelet count above the normal range and 246 (20.4%) of all counts were above the upper limit of normal. Of these, 101 (41%) were high despite no confounding factors being present to explain them. For the 47 children with data at age less than 2 years, 89 (30.0%) platelet counts were above the upper limit of normal and 39 (44%) had no associated confounding factor. Elevated platelet counts were recorded most often for children with new or existing vertebral fractures.

Conclusions

Raised platelet counts were observed in association with new and healing vertebral fractures, but also (41%-44%) in the absence of identified pro-inflammatory factors or events. We speculate that these findings are evidence for a pro-inflammatory component to OI that could be a target for therapeutic intervention.

**Introduction**

Osteogenesis imperfecta (OI) is a heritable group of disorders occurring in 1/15-20,000 births [1]. It is characterised by altered bone mass, architecture and material properties with resultant increased fracture rate [2]. OI has a wide spectrum of clinical severity ranging from apparently normal, through significant bony abnormalities with multiple fractures, to perinatal mortality [3]. Patients affected by OI may also exhibit a range of other abnormalities including joint laxity, abnormal dental development (dentinogenesis imperfecta), aortic root dilatation and blue sclerae [4]. Infants with severe OI are occasionally observed to have recurrent unexplained low grade fevers (own unpublished observations).

Mutations in type I collagen genes are the commonest cause of OI and result in synthesis of abnormal pro alpha chains, part of the collagen fibre [5]. These chains are structurally unable to fold correctly into normal collagen fibrils. The fibrils are in turn unable to pack together and orientate themselves adequately leading to a less stable structure [6]. Bone mineralisation occurs based on this abnormal extracellular matrix template [7]. Bone turnover is increased. [8]

Platelet counts have been reported to be elevated in a variety of settings where inflammation is present [9], including cancer [10], infection [11] and inflammatory bowel disease [12]. In this study, we looked at platelet counts as a potential marker of inflammation in children with severe OI. We hypothesised that platelets would be high in children with OI and that raised platelets would be more common in infants under age 2 years where additional forms of input such as surgical stabilization of the limbs are not possible.

**Methods**

This was a retrospective cohort analysis of children who attended the nationally-commissioned Sheffield Children's Hospital's Severe, Complex and Atypical Osteogenesis Imperfecta Highly Specialised Service (SCA OI HSS) spanning the period 2001-2017. Ethical permission for the study was not sought, as per UK Health Research Authority guidance, because all the data were already collected and were anonymised prior to use. The service provides multidisciplinary team management of children with OI from England and Ireland who meet the following eligibility criteria:

- multiple early life fractures

- 6 or more vertebral crush fractures

- multiple rodding or Ilizarov surgery of limbs

- intractable bone pain

- cranio-cervical, skull base or spine deformity requiring surgery

- unusual (non-collagen gene) forms of OI.

The children's hospital records, pathology and digital radiology records were accessed. Of the 123 children enrolled into the SCA OI HSS since its inception in 2011, 71 were identified as appropriate

for the project and 52 excluded as either they had never had any blood tests at Sheffield Children's Hospitals or only had blood tests associated with surgery. The 71 children identified are/were under regular review at Sheffield. For those starting bisphosphonates during infancy, pamidronate was given seven times in their first year of life, extending to 3 monthly after age 1 year; they had blood tests at every cycle. Those taking zoledronic acid (none aged less than 2 years) had 3-6 monthly cycles and blood tests. Children taking risedronate were seen in outpatient clinic every 6 months and had blood tests every 12 months. All children had 6-monthly dual energy x-ray absorptiometry (DXA) scans and spinal radiographs 12-monthly to monitor bone mass and vertebral fractures. Some of the children had shared care plans in place where they received some of their treatment at their local hospital but still underwent regular review at Sheffield.

Platelets were coded into 3 "bands" as either above the upper limit of normal, or above average but below the upper limit of normal, or below average for age and sex. An age and sex-related z-score was also calculated for each platelet count, based on age and sex compared to our laboratory reference range with the assumption that the upper and lower limits of the range represent the 2.5$^{th}$ and 97.5$^{th}$ centiles, i.e. -2 and +2 standard deviations respectively, with the standard deviation being one quarter of the range and the average value being the midpoint.

In addition, for each platelet count in each child, the following was recorded:

- Age of the child at the time of the blood test
- Time since the child had started bisphosphonate treatment
- Number of vertebral fractures present on radiographs at that time
- Number of new vertebral fractures (since last lateral spine radiograph or DXA)
- Number of long bone fractures since last platelet count
- Length of time since latest long bone fracture
- Whether the child had a concurrent illness at the time of the platelet count (defined as either a vomiting illness, pyrexia or other trigger in their early warning score* or a child deemed too unwell to receive their bisphosphonate treatment)
- When the child's latest surgery was and the length of time since that surgery
- Creatinine as a marker for renal injury
- White cell counts and neutrophils as markers of infection
- Bone specific alkaline phosphatase and general alkaline phosphatase
- Urine N-terminal cross-linked telopeptide of type I collagen (NTx)
- Type and dose of bisphosphonate treatment

OI type, date of birth, type and dose of bisphosphonate treatment and sex were recorded for each participant. If a fracture date was not documented, it was estimated as the first of the month prior to the latest platelet count. *In utero* fractures were recorded as having occurred on the child's date of birth.

For each child we recorded the unchanging demographic data as well as summary variables for the proportion of platelets across the record that fell into each platelet count group, and the proportion of platelet counts for each child that was associated with a "confounding factor" within each "band".

Confounding factors were defined as any of the following:

- New or existing vertebral fracture on latest scan
- New non-vertebral fracture since last platelet count
- Surgery since last platelet count
- Concurrent illness at time of platelet count

We undertook a subgroup analysis of those who started treatment before age 2 years to address our hypothesis that elevated platelets would be more evident at a younger age.

All data were entered and held in Excel; the collated summary data field was exported and analysed using standard methods – F-test of multiple means, Student's t-test and ANOVA for multivariate analysis of categorical and continuous variables - in DataDesk 7.0.2.

**Results**

71 children with OI met the eligibility criteria for this study. All children were less than 16 years old at the time of data collection. All had received bisphosphonates. Demographic data are shown in Table 1. Platelet z score for the entire set of measurements was plotted against age (figure 1a); the histogram of the total set of platelet values was right-shifted by 0.89 standard deviations (figure 1b). The kurtosis for the OI platelet distribution is 1.35 suggesting a general lack of outliers, and skewness is 0.76 suggesting a slight rightward skew, as seen in Figure 1b. For all children, 246 (19%) of 1206 platelet counts were above the upper limit of normal (as opposed to the expected percentage of 2.5% for a normal distribution), and 592 (49.1%) were between average and the upper limit of normal. In children under age 2 years, 89 (30%) of 297 platelet counts were above the upper limit of normal, and 156 (52.5%) were between average and the upper limit of normal.

The "by-individual" proportion of platelets (mean ±SD) within the 3 "bands" - platelets below average; above average and within the normal range; or above the normal range (ULN) and their relationship with the main confounding factors in turn are shown in Table 2a (whole group) and Table 2b (children aged less than two years).

Amongst the whole group, there were significant differences in the proportions of platelet counts within each band for children with non-vertebral fractures, existing vertebral fractures and the presence of one or more "confounders". The pattern of associations with fracture suggested that higher platelet counts – above average, or above the upper limit of normal – were associated with prior fracture. The mean proportion of platelet counts with an associated confounder was significantly lower within the "Above ULN" band than in the "Below average" band ($p=0.0245$).

Amongst the children aged up to 2 years, there were significant differences in the proportions of platelet counts within each band for children with prior non-vertebral fractures, and with any confounder. In general, in keeping with the higher percentage of platelet counts within the "above ULN" band below age two years, the proportion of platelet counts within the "above ULN" band was higher for each confounder in those aged up to two years than for the entire group.

The proportion of platelet counts in each band associated with each of the fracture-related factors is shown in table 3. The distribution within the platelet bands was similar across the groups.

There was an inverse relationship between time since last fracture and platelet count, with the highest platelet z-scores recorded within 250 days of fracture. This is shown in Figure 2, which also shows that the overall shift in platelet counts persists well beyond one year after fracture. Platelet count varied by OI type as shown in Figure 3. ANOVA by OI type with platelet count as the outcome, and including time since last fracture, confirmed that platelets were increased but less so in types I, IV and V than in types III and VI.

**Discussion**

In this study, 69% of platelet counts were either above the upper limit of normal or above average for the whole cohort; in the children aged under two years (all of whom received pamidronate), this rose to 84%. This was reflected in a right-shift of platelet z-scores by nearly 1 standard deviation, with a rightward skew. Platelets thus appear to be significantly higher in patients with OI than the normal population. This was the case even when no confounding factors were present to explain this shift. This increase in platelet count has not previously been reported in children with OI. There were significant differences between the OI types, with types III and VI showing generally higher values than types I, IV and V. Type V OI results from a mutation in the promoter region of IFITM5 and is associated with hypertrophic callus formation around fractures. Type VI OI is caused by mutations in the SERPINF1 gene, and is characterised by florid osteomalacia and altered bone matrix.

The factors more strongly associated with a higher mean proportion of elevated platelet counts were fractures, both vertebral and non-vertebral, rather than concurrent illness or surgery. This applied to both the entire group and to those aged below two years. Whilst there were no clear differences to suggest that new as opposed to healing fractures were more strongly associated with elevated platelet counts, time from fracture did impact on platelet count, suggesting that the healing process has some influence.

Vertebral radiographs or DEXA scans were not available for every platelet count. Not all vertebrae were visible clearly enough to determine fracture status on each image as ascertained from the radiology reports and review of DXA images. Since not all platelet counts had an associated DXA or spine radiograph, and the timing of fractures in relation to platelet counts was variable, the combined number of “informative” platelet counts that were associated with a specific prior event type (e.g. existing vertebral fracture) could be less than the total number of counts for that individual. This means that the recording of “platelets with (any associated) confounder” may appear discrepant with the values recorded within individual category recordings.

The values for mean proportions associated with each confounder reflected the overall right shift of platelet values. Of note, however, the mean proportions within each band for the “confounder present” group did not summate to 1.0, suggesting that much of the variance in platelet count is not accounted for by the identified factors. This in turn suggests that there are other causes for an “inflammatory” response, which may contribute to the clinical phenotype in these severely affected patients. This potential inflammation in OI appears to be apparent throughout childhood, although more marked at an earlier age.

Raised platelets have been reported in other genetically-determined conditions affecting bone. In a case of a young boy with Camurati-Engelmann disease, platelets of 658 (150-400 reference range) were reported [13]. In Camurati-Engelmann disease, excessive release of TGFβ from bone is associated with hyperostosis, bone pain and increased bone turnover. TGFβ is intimately involved in bone remodelling [14]. It is produced in its inactive form by osteoblasts [15], incorporated into the bone matrix [16] and released by osteoclasts during bone resorption [17]. Increased TGFβ signalling has been observed in three different OI mouse models (crtp-/-; G610C; and lethal brtl), but not in one (non-lethal brtl). Anti-TGFβ antibody treatment reduced bone turnover and improved bone mass, architecture and biomechanics in two models [14].

Splenomegaly and increased myeloid lineage expansion, along with elevation of IL-1α and TNFα suggesting a chronic inflammatory stimulus were recently observed in another OI mouse model, the *oim* mouse. Targeted anti-TNFα therapy did not, however, alter bone resorption or improve fracture rate [18]..

TGFβ's influence is not limited to bone. Of particular interest, lung defects, including alveolar size, improved with TGFβ neutralising antibody treatment in the mouse models of OI. Severely affected infants with OI have both small chests and a persistent oxygen requirement. This may simply reflect ribs that are often initially gracile and sometimes fractured. The finding of raised platelets, particularly early in life, allows us to postulate that some of the observed lung disease may be due to inflammatory processes.

Therapeutic interventions for OI are determined primarily by severity of disease. Many children with OI have similar activity levels to their peers and may only require fracture management and physiotherapy [19]. They will also be screened for vertebral fractures, which may prompt a change of management and the introduction of bisphosphonate treatment. Other more severely affected children will require consistent orthopaedic and rehabilitation support as well as bisphosphonate treatment from an early age [20]. Bisphosphonates have been used as treatment for OI for decades, although treatment protocols vary between centres [20]. Bisphosphonates have shown a significant increase in bone mass density in the spine and elsewhere [21, 22] due to their anti-osteoclastic effects. However, studies have struggled to determine any true benefit in terms of fracture rate, quality of life or mobility in patients with OI on bisphosphonate treatment [21, 22]. Concerns have also been raised about the long term use of bisphosphonates, particularly with regards to the risk of atypical femur fracture in OI [23]. This study's finding of one or more potential inflammatory pathways active in OI could help in the development of more targeted and effective treatments for OI.

Our study has significant limitations; the data is based on a retrospective survey, albeit of a cohort of significant size. We have chosen specific potential confounders, based on the clinical course of these children; we may have chosen wrongly. In studies of older individuals without OI, there is no increase in the number of platelets following a fracture. Our recording of the potential confounders is incomplete in that the number of occasions when platelets were measured significantly exceeds the number of times when scans and radiographs were performed. Fracture ascertainment may be incomplete; we did not subject children to repeated skeletal surveys. Non-vertebral radiographs were taken only when a patient presented with a clinically apparent fracture; some older patients will have chosen to self-immobilise and not attend. The recording of the timing may not have been

accurate, but we applied the same "rules" regarding timing in relation to subsequent platelet counts for all subjects. The vertebral fracture data is only as accurate as the scan quality and time between each scan allows. Some subtle vertebral fractures may have been missed and not all vertebrae were clearly visible on each scan or radiograph. There may be other inflammatory confounding factors not identified here. Finally, we have no other confirmatory measures suggesting that the raised platelets are an inflammatory response.

Despite these limitations, the striking right-shift in the distribution of the platelet counts is suggestive of a previously unidentified process contributing to the clinical phenotype of these children. We are not able to explain much more than 50% of the variance in this increase. This increase may be caused by excessive remodelling of abnormal bone matrix leading to pro-inflammatory cytokine release. Further studies are required to define the extent and origin of this process and the affected pathways. There may be a therapeutic opportunity here to target what we believe to be an inflammatory process and improve outcome for these children, once that process is more clearly defined.

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**What is known about this topic**

Osteogenesis imperfecta is the commonest cause of inherited bone fragility, affecting 1 in 15-16,000 live births

Severely affected children have multiple fractures, progressive bony deformity, restricted mobility and chronic pain.

Treatment with bisphosphonates, regarded as standard care, has been reported to increase bone mass, restore vertebral architecture and, in some studies, reduce fracture frequency.

**What this study adds**

This study has identified a right shift in the distribution of platelets, more marked in the younger children

This right shift is not accounted for by prior or new fractures, or other concurrent illness

The results suggest that inflammation-related pathways could be targets for treatment in children with the more severe form of osteogenesis imperfecta

Figure legends

Figure 1a. Platelet count Z-scores plotted against age in years; the shaded area represents the normal range

Figure 1b. Histogram of platelet z score frequencies; normal distribution shown as red dashed line

Figure 2. Platelet count z-score plotted against time since fracture, with line of best fit

Figure 3. Box plot of platelet z-score by OI type, showing 95% CI around the mean (shaded area)

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