CLINICAL STUDY

Quantitative ultrasound for the assessment of osteopenia in preterm infants

Alessandro Rubinacci, Guido E Moro¹, Günther Boehm¹, Francesca de Terlizzi², Gian Luigi Moro and Ruggero Cadossi²

Bone Metabolic Unit, Scientific Institute, San Raffaele Hospital, Via Olgettina 60, Milan 20132, Italy, ¹Department of Perinatal Pathology, Macedonio Melloni Maternity Hospital, 20129 Milan, Italy and ²Laboratory of Clinical Biophysics, Igea, Carpi, 41012, Italy

(Correspondence should be addressed to A Rubinacci; Email: a.rubinacci@hsr.it)

Abstract

Objective: To evaluate the potential role of quantitative ultrasound (QUS) investigation in assessing the osteopenia of prematurity.

Design: QUS parameters measured at the time of discharge were related to the anthropometric characteristics and age (postnatal and gestational) of 51 (34 female and 17 male) preterm infants fed fortified human milk.

Methods: QUS evaluation was performed at the humerus (h) by measuring two parameters: ultrasound velocity (hSOS, in m/s) and bone transmission time (hBTT, in μ s). A group of 43 term infants (29 female and 14 male) was also evaluated.

Results: In preterm infants, significant correlations were found for hSOS and hBTT vs gestational age ($r = 0.504$, 0.477 , $P < 0.0001$), length ($r = 0.641$, 0.594 , $P < 0.0001$) and weight ($r = 0.580$, 0.562 , $P < 0.0001$) at birth, and length ($r = 0.341$, 0.332 , $P < 0.05$) and weight ($r = 0.331$, $r = 0.362$, $P < 0.05$) at QUS measurement. In preterm infants, both QUS parameters were negatively correlated with age ($r = -0.536$, $P < 0.0001$, $r = -0.443$, $P < 0.001$) and were significantly lower than in the term infants (hSOS: 1664 ± 42 m/s vs 1734 ± 28 m/s, $P < 0.0001$; hBTT: 0.58 ± 0.24 μ s vs 1.06 ± 0.15 μ s, $P < 0.0001$) even when adjusted for body length ($P < 0.05$). In preterm infants, hSOS was also negatively correlated with postconceptional age ($r = -0.322$, $P < 0.05$).

Conclusions: This study suggests that bone mineral accrual is mainly determined by the development *in utero*, and that prematurity induces a halt in the bone development process in the early postnatal period. QUS parameters are correlated with the severity of prematurity and might therefore have clinical applications when bone maturation in early life needs to be determined.

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Introduction

Postnatal increase in the bone mineral mass of preterm babies is less than that expected *in utero* (1). Major efforts are therefore devoted to the re-establishment of the normal bone development process to guarantee the accrual of the genetically determined peak bone mass that represents an effective achievement for the prevention of osteoporosis in adulthood. Unfortunately, bone mineralization is one of the major challenges of neonatal management in low (LBW) and very low birth weight (VLBW) infants, and osteopenia is recognized with increasing frequency (2–4). Despite the likelihood of a spontaneous resolution of the osteopenia at the appendicular (5, 6) as well as at the axial skeleton (7, 8), several studies in former preterm infants have demonstrated persisting lower bone mineral content (BMC) (9, 10), particularly at the hip (11).

Single-photon (SPA) in the past (12–14) and dual-energy X-ray absorptiometry (DXA) today (6, 7,

15–17) have been applied to assess mineral accretion in LBW and VLBW infants. Despite the great advantage over SPA in terms of accuracy and reproducibility and the low radiation exposure (18), DXA does not overcome the typical limits of absorptiometry in longitudinal studies in preterm infants because of the cumulative radiation dose and the stress of transport to and restraint in the unit. DXA is even limited by the fact that mineral accretion and bone growth are not parallel (2, 19, 20). This is particularly evident in preterm infants because the mineralized to total bone volume ratio increases continuously during the third trimester of gestation leading to a progressive change in volumetric bone mineral density (BMD) (2). Thus, the ratio of BMC to area (i.e. BMD, in g/cm^2) is not appropriate in the growing preterm infant where both BMC and bone area are increasing at different rates (2, 19–21). The same holds true for term infants whose bone mineral mass increases considerably during the first months of life despite the precipitous

drop in the total BMD (for review see 21). Any normalization attempt to create a volumetric density to remove the potential influence of bone size on BMD has failed, at least in childhood, because of low precision and lack of diagnostic sensitivity (22). In addition, the conversion equation to derive total calcium content from BMC based upon the perfect agreement between BMC and ash weight (23) must be confirmed after the neonatal period in view of the major changes in bone growth and mineral deposition that occur after birth (2).

Conversely, quantitative ultrasound (QUS) measurement of bone quality could fit the requirements for a screening tool for the detection of osteopenia in prematurity and for the assessment of bone development (24) because (a) the infant does not require immobilization or restraint as QUS is very fast and the ultrasound probe is not disturbed by the infant's movements but moves accordingly, and (b) bone edge detection, a consistent handicap when bones with a low degree of mineralization are measured by X-ray absorptiometry methods, does not appear to be a problem for QUS: the device displays the ultrasound waveform and automatically detects the fastest waves that have been transmitted through the bones.

The present cross-sectional study was therefore designed to provide the anthropometric correlates of QUS evaluation in view of its potential application in assessing the osteopenia of prematurity. Thus, single-point QUS parameters, gestational age, postconceptional age and the anthropometric characteristics of the preterm infants were assessed. This study was aimed at further evaluating the feasibility of QUS evaluation in prematurity, by providing a measurement approach that might implement the information provided in preterm infants by tibial speed of sound (SOS), recently investigated (24). Single-point QUS (DBM Sonic 1200; Igea, Carpi, Italy), originally developed for the evaluation of adult phalanges (25), provides not only SOS but also the bone transmission time (BTT, in μ s) that is not affected by the thickness of the soft tissue (25). Moreover, a detailed evaluation of the ultrasound waves transmitted by this device has been described (26) and validated *in vitro* (27) as well as in the growing skeleton: single-point QUS assesses bone status at birth (25) and detects bone growth, pubertal bone development and gender differences in the bone development process between 3 and 21 years of age (28, 29).

Materials and methods

Subjects

Fifty-one preterm infants (34 females and 17 males) and 43 term infants (29 females and 14 males) appropriate for gestational age and admitted to the Department of Perinatal Pathology, Macedonio Melloni

Maternity Hospital of Milan in the period from March 1999 to July 2001 were enrolled in the study after oral informed consent was given by the parents. The study was carried out according to the ethical principles stated in the declaration of Helsinki.

For preterm infants, information was available on age (range 0–19 weeks of life), length at birth (range 31–46 cm), weight at birth (range 590–2950 g), gestational age (range 24–36 weeks), and length and weight at QUS measurement. For term infants, information was available on age (range 0–1 weeks), length at birth (range 46–54 cm) and weight at birth (range 2790–4900 g). The weight of the infants was determined to the nearest 10 g. Length was determined by two measurements using a measuring board with fixed head-board and movable foot-board. Ultrasound dating scans were available for most of the infants, but not all. Since no major discrepancies were found between ultrasound and clinical dating data according to the method of Dubowitz *et al.* (30), infants were referred on the latter. Infants were considered appropriate for gestational age if birth weight and length at birth were >10th but <90th centile of the data of Battaglia & Lubchenco (31). All infants were without malformations, signs of infection or other major clinical problems and were discharged from the hospital in good clinical conditions. None of the preterm infants required mechanical ventilation: only a few of them developed mild respiratory distress and were supported by continuous positive airway pressure in the first days of life. Enteral feeding was started within the first 3 days of life with fresh maternal milk or with donor milk from the hospital human milk bank. Supplemental parenteral nutrition was necessary at least up to the end of the first week of life. When the infants were able to tolerate a milk volume intake of 100 ml/kg per day, human milk was fortified with 3 g bovine milk protein fortifier (Eoprotin; Milupa GmbH, Friedrichsdorf, Germany) until the time of discharge from the hospital.

QUS measurement

To avoid discomfort or stress in the early period of life due to excessive handling, it was decided to postpone QUS evaluation to the time of the final evaluation of the clinical outcomes of prematurity. Therefore, preterm infants underwent QUS at discharge from the hospital when they reached a postconceptual age of at least 34 weeks. The term infants were evaluated at birth (within the first week).

The device employed (DBM Sonic 1200) consists of a central unit and a caliper. Two ultrasound coaxial probes are placed in the arms of the caliper and applied to the bone segment to be analyzed: one probe produces the ultrasound pulse (emitter) while the other receives it after transmission through the humerus. The hardware of the central unit analyzes the wave form of the received ultrasonic signal for a quantitative evaluation

of the characteristics of the wave form related to the transmission through the site of measurement. As the expected transmission speed of the ultrasound in the soft tissue is 1570 m/s, that part of the signal crossing the body segment with a higher velocity is defined as 'fast wave' and related to the transmission only through the bone tissue (Fig. 1); the slowest part is related to transmission through both soft tissue and bone tissue (reflections at the interfaces). Output analysis focused on the fast wave characteristics.

The device was adapted to measure the SOS at the humerus (hSOS) on the basis of the following formula:

$$v = \frac{d}{t_1 - t_0}$$

where d is the distance between the probes, t_0 is the instant at which the ultrasound signal is emitted and t_1 is the instant at which the highest amplitude point of the first peak of the signal is received after transmission (Fig. 1).

The device also measures the BTT at the humerus (hBTT) which represents the time-interval between the instant at which the fast wave of the signal is received after transmission (t_1) and the instant at which the part of the signal that transmits with a velocity of 1570 m/s is received (t_2) (see Fig. 1). It has been demonstrated by mathematical calculations that this parameter, also defined as the 'time frame', is independent of the amount of soft tissue around the investigated bone and is affected by its mineralization and architecture (25).

The humerus was chosen for the ultrasonic evaluation in infants: (a) to avoid the risk of scanning skeletal sites that had not yet undergone endochondral ossification; (b) to allow comparison with early literature on the osteopenia of the preterm infant that was mostly

obtained by SPA at the humeral diaphysis; (c) because the humerus of infants has a cross-sectional size comparable with the adult phalanx (the site of measurement used in adults); and (d) because at the time of measurement (gestational age ranging from 24 to 36 weeks and postconceptual age ranging from 34 to 43 weeks), it is conceivable that the ultrasounds have been transmitted through the ossified shaft. In fact, by the seventh week the primary ossification centre is seen histologically and a bony collar appears in the mid-shaft. At first, periosteal bone occupies more of the length of the diaphysis but, by the beginning of the fifth month, periosteal and endochondral bone formation are co-extensive. By the sixth month, growth of the bone takes place and ossification extends so that 79% of the length of the bone is occupied, at term, by ossified shaft and 21% by the cartilaginous extremities (32, 33).

The ultrasonic measurement involved an anteroposterior scan of the humerus in an orthogonal direction with respect to the axis of the humeral diaphysis, by gently placing the caliper on the arm of the preterm infant and searching for the highest hSOS that was recorded. Four scans were carried out for each infant: two on the left humerus and two on the right.

Statistical analysis

The precision of the magnitudes measured was determined by calculating the standardized coefficient of variation (sCV%): the CV% for a series of dual measurements with repositioning was normalized on the dynamic range calculated on the population of this study. The dynamic range was defined as the difference between the minimum value and the maximum value measured on the whole population. The sCV% was thus calculated by the formula:

$$\text{sCV}\% = \frac{\text{standard deviation} \times 100}{\text{dynamic range}}$$

Since duplicate measurements were obtained for all the subjects, the final value of sCV% was calculated as a root mean square (rms) sCV% accordingly to the following formula:

$$\text{sCV} = \sqrt{\sum_{j=1}^m \frac{\text{sCV}_j^2}{m}}$$

where m is the total number of subjects, $j = 1$ to m , and sCV_j is the sCV of the j subject.

In comparing the results of the ultrasound measurement on the left and right humerus of the patients, paired two-tailed Student's t -test were performed. Mean values and standard deviations were calculated for all the clinical and instrumental parameters and for each group. For comparisons between groups, the

Figure 1 Schematic representation of the graphic trace. hSOS: the arrow indicates the time value utilized to calculate ultrasound velocity; CUT OFF, the arrow indicates the time corresponding to the 1570 m/s ultrasound velocity; hBTT represents the time-interval between the previous two values.

two-tailed heteroscedastic Student's *t*-test was used with $P = 0.05$ as the minimum level of significance. The thresholds of $P = 0.01$, 0.005 and 0.0001 were used to differentiate the levels of significance.

The associations between the variables measured were performed by analysis of linear regression and the correlation coefficients were reported in terms of values of *r*. For each analysis of regression the level of significance (*P*) was also reported. The same thresholds of significance as given above were adopted in this case.

QUS parameters in term and preterm subjects adjusted for length at birth and length at measurement were calculated on the basis of the linear fitted equation for hSOS and hBTT vs length at birth and at measurement in preterm infants. Corrected values of QUS parameters were then compared by Student's *t*-test.

Results

QUS precision

The precision of the QUS measurement was tested on a group of six preterm infants with duplicate measurements: rms (sCV%) = 1.76 for hSOS and rms (sCV%) = 1.99 for hBTT. In the preterm subjects, the mean values of hSOS recorded on the left and right humerus were 1664 ± 42 m/s and 1663 ± 40 m/s respectively; the mean values of hBTT on the left and right humerus were 0.58 ± 0.24 μ s and 0.54 ± 0.23 μ s respectively. Since none of the side-related differences reached the significance level, it was decided to include in the subsequent analyses only the measurements performed on the left side for all the subjects for the sake of homogeneity.

Characteristics of the study group

Table 1 reports the mean $\pm$ S.D. values for the parameters of demographic, auxological and QUS characteristics of both the preterm and term subjects. For preterm infants, auxological data are reported both at birth and at the time of QUS measurement (at discharge). Three infants were severely premature with extremely low birth weight and gestational age. Nevertheless, they were included in the analysis because of the aims of the study, i.e. assessing QUS correlates with degree of the prematurity. The growth retardation of these infants was obviously related to the severity of their clinical condition.

The data were pooled irrespective of gender because no significant gender-related differences were observed in either term or preterm infants for all variables.

QUS in the preterm infants

The morphological analysis of the fast wave of the ultrasonic signal revealed that the wave form recorded in the preterm subjects differed from that recorded in the

Table 1 Demographic, auxological and QUS characteristics of the study population. Values are means $\pm$ S.D.

	Term infants	Preterm infants
Number	43	51
Gestational age (weeks)	40.0 ± 0.2	$32.1 \pm 2.9^*$
Age postpartum (weeks)	0.6 ± 0.5	$5.2 \pm 4.0^*$
Length at birth (cm)	49.7 ± 1.9	$40.2 \pm 3.9^*$
Weight at birth (g)	3466 ± 463	$1586 \pm 480^*$
hSOS (m/s)	1734 ± 28	$1664 \pm 42^*$
hBTT (μ s)	1.06 ± 0.15	$0.58 \pm 0.24^*$
Length at QUS (cm)	—	42.2 ± 3.8
Weight at QUS (g)	—	1978 ± 430

* $P < 0.0001$ compared with term controls.

infants born at term, where only one or two peaks appeared, instead of the expected three or more (Fig. 2).

Among preterm infants the ultrasonic parameters, i.e. hSOS and hBTT, were positively correlated with the auxological measurements. The tightness of the association slightly improved when length and weight at birth (Fig. 3) were included in the analysis instead of those taken at QUS measurement. In fact, the correlation coefficients for hSOS vs length at birth and at QUS measurement were $r = 0.641$ ($P < 0.0001$) and $r = 0.341$ ($P < 0.05$) respectively, and for hBTT vs length at birth and at QUS measurement they were $r = 0.594$ ($P < 0.0001$) and $r = 0.332$ ($P < 0.05$).

Figure 2 Ultrasound signal of (A) a preterm and (B) a term subject. The circles show the number of peaks in the hBTT interval.

Furthermore, the correlation coefficients for hSOS vs weight at birth and at QUS measurement were respectively $r = 0.580$, $P < 0.0001$ and $r = 0.331$, $P < 0.05$, and for hBTT vs weight at birth and at QUS measurement they were $r = 0.562$ ($P < 0.0001$) and $r = 0.362$ ($P < 0.05$). Significant positive correlations were even found for both hSOS and hBTT vs gestational age (Fig. 4A and B). However, the correlation for both hSOS and hBTT became negative when registered age was considered whereas only the former was retained when postconceptual age was considered (Fig. 4C and D). The results did not change when the three subjects who were extremely premature were removed from the analysis. All statistical significances of the associations were retained.

Comparison between QUS in preterm and term infants

For term infants, the QUS measurement was done almost at birth (within a week), and the demographic (age postpartum) and auxological data (length and weight) at birth and at QUS measurement were therefore corresponding. Both ultrasonic parameters were significantly ($P < 0.0001$) lower in preterm infants than in the term counterpart (Table 1). Comparison between preterm and term infants adjusted for length at birth and/or length at measurement showed that preterm infants had significantly lower values of hSOS and hBTT ($P < 0.05$ for both) with respect to term infants. When the three subjects who were

severely premature were removed from the analysis hBTT remained significantly lower ($P < 0.05$) in preterm than in term subjects. Both QUS parameters were significantly lower in preterm infants than in post-conceptual age term-matched controls (hSOS 1649 ± 54 m/s in preterm ($n = 7$) vs 1734 ± 28 m/s in term infants ($n = 43$) and hBTT 0.49 ± 0.31 μ s in preterm vs 1.06 ± 0.15 in term infants; $P < 0.01$ for hSOS and $P < 0.005$ for hBTT). However, the differences in the anthropometric characteristics (weight 2226 ± 969 g in preterm vs 3466 ± 463 in term infants; length 43.1 ± 6.7 cm in preterm vs 49.7 ± 1.9 in term infants; $P < 0.0001$ for weight and $P < 0.05$ for length) still remained significant in this subgroup.

By combining in a single plot the relationships between length at the time of QUS measurement and both ultrasonic parameters in preterm and term infants, it appeared that both ultrasonic parameters (Fig. 5) followed a similar linear trend with the length, even if the data of term infants was distributed higher compared with preterm infants.

Discussion

This study has demonstrated that both ultrasonic parameters, hSOS and hBTT, are correlated with the auxological characteristics of the preterm infants at birth and at the time of measurement, and are tightly associated with the severity of the prematurity.

Figure 3 (A) and (C) hSOS and (B) and (D) hBTT vs auxological parameters at birth for the preterm infants.

Figure 4 (A) and (B) hSOS and hBTT vs gestational age and (C) and (D) postconceptional age in preterm infants. n.s., not significant.

In considering that QUS is not only associated with BMC but that it implies different bone structural properties, these results should be evaluated in an extended frame that considers the developmental process of the selected skeletal site and the impact of birth before

term. The measurements were performed at the mid portion of the humerus where the ossification process is well documented (32, 33), and they were confirmed by the radiological images of the humerus that have been obtained in preterm babies of a similar state of development (34). Accordingly, the characteristics of the ultrasound signal in preterm babies showed a trace suggestive of having been transmitted through a mineralized bone as the signal is transmitted with a velocity higher than 1570 m/s. The reduced number of peaks in the fast part of the signal in the preterm compared with the term infants suggests a reduced transmission of the ultrasound waves due to a halt in the development of the structural and material properties of preterm infant bone.

Since there are no reference data in preterm infants at birth at different gestational ages and since the anthropometric characteristics of the two groups of infants were significantly different, this study did not provide a direct demonstration that QUS could detect disturbance in the process of bone development. However, the following observations suggest that this view is conceivable: (a) QUS parameters were significantly lower in the infants born preterm than at term even when adjusted for anthropometric parameters, and in preterm infants than in postconceptual age term-matched controls; (b) gestational age and auxological characteristics of the preterm infants were correlated with the QUS parameters in as much as the heavier and longer infants had faster hSOS and hBTT; and (c) age and QUS parameters were negatively correlated, thus indicating that the longer the time between birth before term and discharge (postnatal age) the

Figure 5 (A) hSOS and (B) hBTT vs length at QUS measurement in term (○) and preterm (●) infants.

earlier was the halt to bone development *in utero*. Since very low birth weight, short length and shortened gestation reflect extreme prematurity, it follows that QUS evaluation is able to proportionally detect the impact of preterm birth upon bone development, thus confirming the original observation of Nemet *et al.* (24) that even tibial SOS is negatively correlated to postnatal age. The fact that QUS evaluation of the humerus failed to confirm the sex-related difference in BMC revealed by SPA evaluation of the radius (14) might be due to the fact that these differences are site specific. It was, in fact, observed that the differences in bone density due to sex were significant only for the radius and femur and not for the humerus (34). Finally, there is some concern about the comparison between preterm and term infants because of the lack of homogeneity and of the limits of any correction factor. However, it might help in the clinical setting where there is the need to have some standard to refer to as an indication of normal bone development at term *in utero*. To this purpose, QUS parameters in term infants at birth are, at this state of knowledge, an acceptable reference with all the limits outlined.

Since the mechanism of the osteopenia of prematurity is still a matter of debate, as well as the underlying histologic defect, it is hard to say which material or structural characteristics are responsible for the different ultrasound transmission through the bone as a function of the presence and the degree of prematurity. Conceivably, the lower bone acquisition rate of the former reflects a bone modelling/remodelling imbalance between bone resorption and formation (35–37) that could determine bone mass rather than a bone mineralization defect, with subsequent reduction of the ultrasound transmission, at least in those not affected by serious illness or rickets, the incidence of which has decreased with improvements in care and nutrition (38). With both SPA and DXA it was impossible to disentangle the two separate processes that might be occurring in the preterm skeleton: reduced mineral density per unit of volume of bone tissue (mineralization defect) or reduced accrual of bone mass (osteopenia). However, radiologic (39, 40), histomorphometric (41), radiomorphometric (35), biochemical (35–37) and kinetic (42) evaluations have suggested that the latter condition, i.e. osteopenia, is the most likely feature of the metabolic bone disease of prematurity at the actual nutritional regimens. It might therefore account for the differences in ultrasound transmission whereas a mineralization defect or differences in bone size could be excluded. This hypothesis is sustained by the fact that birth before term determines a halt in the bone mass accrual, at least till the catch-up period takes place (4–6), rather than in the mineral accrual per unit of volume of bone tissue. In fact, (a) the increase in bone volume fraction (density) is the determinant feature of growing bone (43), and (b) the weights of the bones increase faster with age

during fetal development than do the volumes while percentage ash weights of the fetal skeletons show only a slight but significant increase with age (38). Finally, the hypothesis that QUS simply detects bone size differences can be rejected by considering that (a) body weight increases relatively faster than bone cross-sectional area (44), (b) studies in bones from pigs show no dependency of SOS from the thickness of cortical or trabecular bone slices (45), and (c) ultrasound velocity is largely dependent upon bone density rather than bone width (46, 47). Finally, microarchitectural (structural property) deterioration cannot be taken into account to explain the reduced ultrasonic transmission, as suggested by some (48) and questioned by others (49), because architectural adaptation in growing bone follows the increase in bone volume fraction (43) and is not activated at this early state of development.

Among the ultrasonic parameters, it appears that hBTT has a higher power of discrimination than hSOS when applied to the comparisons between preterm and term infants. The *t* value at Student's *t*-test analysis, indicating the amplitude of the overlap between the S.D. of the two populations, was in fact higher for hBTT ($t = 11.03$) than for hSOS ($t = 9.28$). The major hBTT specificity was likely due to the fact that this parameter is independent of the amount of soft tissue, thus solely representing the contribution of bone tissue to the ultrasound wave transmission. On the contrary, hSOS is affected by the amount of the soft tissue between the probes and it would remain less specific than hBTT in assessing the degree of osteopenia, unless a normalization method for soft tissue thickness (arm width) could be developed. By subtracting soft tissue inference in the assessment of hSOS, strong correlations between hSOS and cortical area have, in fact, been consistently demonstrated *in vivo* and in phantom simulations (26, 27, 46, 47).

In conclusion, the correspondence between the QUS outcomes and current knowledge should be recognized. Once ultrasound transmission pathways through a growing bone have been better clarified and reference values obtained, QUS might provide an efficient tool when bone maturation in early life needs to be determined.

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