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ORIGINAL ARTICLE

Risk factors and biochemical markers in metabolic bone disease of premature newborns

Factores de riesgo y marcadores bioquímicos de la enfermedad metabólica ósea del recién nacido prematuro

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Abstract

Background: Metabolic bone disease (MBD) of prematurity is a complication of multifactorial aetiology, which has been increasing, due to progressive decrease in mortality of preterm newborns. The aim of the study was to analyze risk factors of severe MBD and its analytical markers. **Patients and Method:** Retrospective study involving preterm infants less than 32 weeks gestational age and/or weight less tan 1,500 g born between january 2012 and december 2014. Comparison was made according to the presence of severe MBD. **Results:** 139 patients were recruited. Mean value of 25(OH)D3 was 70.68 ± 25.20 nmol/L, being higher in patients born in spring-summer than in autumn-winter (80.94 ± 25.33 vs 61.13 ± 21.07; p = 0.000). Levels of 25(OH)D3 were similar in patients with severe MBD compared with the rest of patients (65.61 ± 26.49 vs 72.07 ± 24.89, P = 0.283). Higher levels of alkaline phosphatase (AP, IU/L) (1314.19 ± 506.67 vs 476.56 ± 188.85; p = 0.000) were found in these patients. Cutoff point of AP 796.5 IU/L (S 95.2%, specificity 92.4%) was calculated by ROC curve. The risk factors most associated to severe EMO were restricted fetal growth, birth weight, duration of ventilation therapy and parenteral nutrition. **Conclusions:** AP levels were the best marker of severe MBD development. EMO risk increases with the number of risk factors and lower levels of 25(OH) D3. Levels of 25(OH)D3 higher than 70nmol/L appear to protect from the development of severe MBD, even in patients with multiple risk factors.

Keywords:
Prematurity,
Metabolic bone disease,
alkaline phosphatase,
sensitivity,
specificity

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Introduction

Metabolic bone disease (MBD) is the new designation for osteopenia of prematurity, a complication in premature children, characterized by a reduction of osteoid tissue and bone mineral component (BMC) and by biochemical alterations of phospho-calcium metabolism. This condition may be favored by several nutritional and biomechanical factors, such as prolonged nutrient deficiency, immobilization and parenteral nutrition, as well as the intake of antagonistic medications with bone metabolism[1-3]. Although in the past the incidence of this pathology was similar or even greater than the current one, the progressive reduction of mortality in the last years and the growing clinical experience, allowed for a systematic search of osteopenia and MBD (affecting the premature ones) as a usual clinical practice in centers with experience in neonatology. All this is allowing the pathology to be more and more frequently diagnosed and to be treated early, even in mild forms that were previously not detected[4].

The period of greatest development of the skeleton is during fetal life, mainly at the end of the third trimester[5,6]. The newborn is prematurely deprived of these inputs necessary for proper mineralization. Also, chronic placental involvement alters the transport of phosphate, which could condition osteopenia in children with intrauterine growth restriction[7,8].

The current incidence of MBD is unknown. In recent studies, we could find this pathology in slightly more than half of the newborns (NB) with less than 28 weeks of gestational age (GA) or newborn's weight (NBW) less than 1000 grams, and 1 of every 4-5 children under 1500 grams[9,10]. It is closely associated with GA, NBW, and the type of feeding with delayed enteral inputs and with the severity of the total process. Thus, the risk of bone involvement is greater in premature infants under a serious clinical situation, taking into account that an extreme prematurity is linked with a very low weight[2,3].

The pathology usually starts its developing process from 4 weeks of age, with a wide clinical variability, from mild asymptomatic forms to classic rickets. If demineralization is severe, it could favor multiple fractures and alterations of phospho-calcium in the patient's metabolism[11].

The diagnosis is basically analytical, so it is necessary to make a number of determinations in preterm infants (PTNB) with suspected risk factors for the disease. Any parameter can be considered isolated as MBD marker, although the most commonly used is the serum alkaline phosphatase. The figures that determine the diagnosis are not clearly defined in the literature, but in some series it is accepted that above 500 IU/L as a high sensitivity and specificity, and in others, above 700 IU/L[1,12,13].

The diagnosis of MBD will be confirmed by a densitometry, however its availability is scarce in neonatal units. Despite the advances in the knowledge of this disease, the analytical cuttoffs markers which determine a high suspicion of MBD are not clear yet, and although the risk factors are well defined, it is not clear is there are other protective factors that may prevent the MBD development, despite it could be presented along with multiple risk factors[14-16].

Although MBD is a self-limiting disease over time, recovery could last up to 2 years[17]. Some studies give us evidence of postnatal growth delays at 8-12 years of age[17,18]. Therefore, if it prevention fails, the presence of fractures, dolichocephaly and delays in growth rate, as well as other long-term effects, such as osteopenia in adulthood, would be favored[16,19].

Hence, the present study is focused in analyzing the risk factors associated with the development of severe MBD and the analytical markers of the disease, aiming to find cutoffs in the different biochemical parameters that allow to identify the development of this bone disease.

Patients and Methods

A retrospective observational, descriptive and analytical study was performed, including all PTNB infants less than 1,500 g of NBW and/or less than 32 GAW (Gestational Age Weeks), who were born between January 2012 and December 2014 and who were admitted to the Neonatal Unit of Miguel Servet Children's Hospital, Zaragoza, Spain (center that registers 4000-4500 deliveries and 80-100 PTNB less than 1500 g or 32 GAW per year).

Patients who died or were transferred from this center before discharge and those who did not undergo an analytical extraction due to a serious clinical situation at that time or failure to apply the protocol, were excluded. The process of inclusion of patients is detailed in figure 1.

The diagnostic-therapeutic protocol of this center includes the performance of blood analysis with calcium, phosphorus, 25 (OH) vitamin D3 and alkaline phosphatase (AP) at 3-4 weeks of life, or earlier (if there is clinical suspicion of osteopenia or fractures), due to the presence of bone deformities, bone palpation pain, a chance finding on X-ray for another reason or analytical alterations, such as hypophosphatemia, hyperphosphaturia, hypocalcemia or hypercalciuria, detected in another control. The samples are processed immediately after their extraction in the biochemistry laboratory, of the hospital itself. Subsequently, serial

checks are performed every 2-3 weeks until hospital discharge. If the AP is greater than 800 IU/l, or if there are ionic alterations or images suggesting MBD or fractures in the X-rays, the paratohormone (PTH) is added to assess the stimulation of the bone remodeling. The wrist x-ray is performed with AP above 500 IU/l and on clinical suspicion of MBD or fractures cases, in order to assess if there are radiological signs of osteopenia (in our sample we defined patients with severe MBD due to the radiological presence of metaphyseal changes with rarefaction, fraying and cup formation of the growth plate or periosteal detachment of the shaft). Those with radiological signs of MBD or with elevated AP above 800 IU/L are treated with oral solutions of calcium carbonate (30-50 mg/kg/day) and phosphorus (starting at 10-20 mg/kg/day and increasing up to 100 mg/kg/day).

In our center, parenteral nutrition (PN) is started on the first day of life in all patients with less than 32 GAW and/or NBW less than 1500 g, with initial calcium inputs of 50-60 mg/kg and phosphorus of 40-50 mg/kg, adjusted according to analytical controls. In order to start with enteral nutrition (EN) as soon as possible, an attempt of feeding patients with breast milk or donated breast milk from a bank is made, and PN is suspended from 120 ml/kg EN.

From the ingestion of 100 ml/kg of milk, the fortification of the milk begins. The usual vitamin D intake for neonates is 400 IU per day. To obtain all the needing data, a review of the patients' medical records was carried out. Antenatal variables and the immediate neonatal period (single or multiple pregnancy, gender, birth reason, prenatal corticoid administration, gestational age, anthropometry at birth, Apgar test, resuscitation in the delivery room, neonatal evolution variables, respiratory and hemodynamic support and their duration, use of diuretics and other medications, time of onset of EN and PN withdrawal), analytical markers

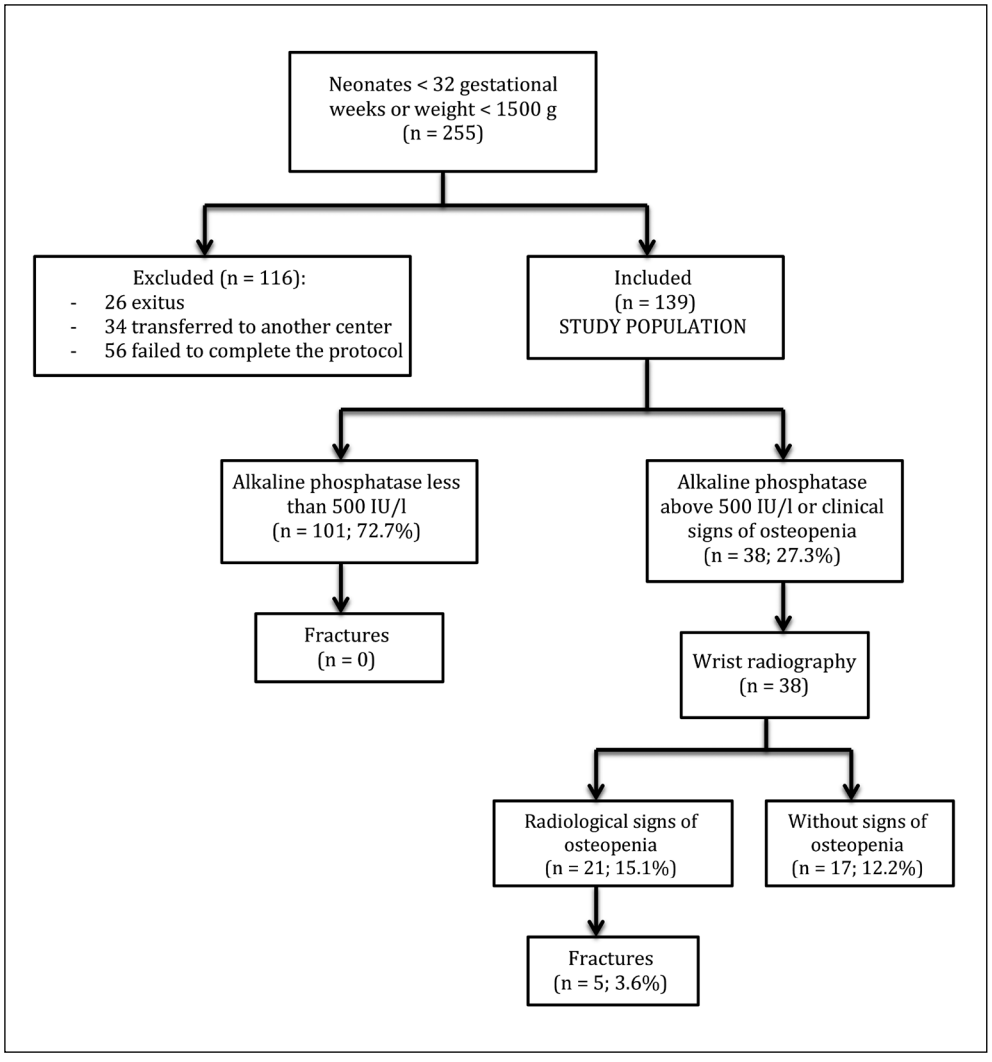

Figure 1. Flow diagram of patient inclusion.

of phospho-calcium metabolism (ions, 25 (OH) vitamin D, AP and PTH) and radiological presence of severe MBD or fractures.

Statistical analisys

All data were recorded and analyzed using the statistical package SPSS statistics 21.0.

An initial descriptive study was carried out to know frequencies, measures of central tendency and measures of dispersion.

The analytical study was performed by Kolmogorov-Smirnov and Saphiro-Wilk tests for normality analysis of the quantitative variables, and U Mann-Whitney, Kruskal-Wallis and Wilcoxon tests were used for the quantitative variables, because they were nonparametric samples, while the qualitative variables were compared by Chi-square test or Fisher's exact test.

A multivariate analysis of logistic regression was performed with significant variables in the univariate analysis.The study was approved by the Ethics Committee of Scientific Research of Aragon (CEICA).

Table 1. Demographic characteristics of the global sample

Characteristics	Muestra global
Male. n (%)	67 (48.2%)
Birth weigth in grams. median (range)	1250 (480-1495)
Gestational age weeks. median (range)	29.28 (24-36.86)
Newborns from multiple gestation. n (%)	49 (35.3%)
Neonates < 28 gestational weeks or weigth < 1000 grams. n (%)	74 (53.2%)
Intrauterine growth restriction. n (%)	30 (21.6%)
Neonates. n (%)	
Small for gestational age	15 (10.8%)
Appropiate for gestational age	122 (87.8%)
Large for gestational age	2 (1.4%)
Antenatal steroids ≥ 2 doses. n (%)	96 (69.1%)
Gestational maternal pathology. n (%)	66 (47.4%)
Preeclampsia or eclampsia	30 (21.6%)
Chorioamnionitis	22 (15.8%)
Thyroid pathology	8 (5.7%)
Pregestacional or gestacional diabetes	6 (4.3%)
25(OH)vitamine D3 in nmol/l. median (range)	69.21 (25.5-145)
Alkaline phosphatase in IU/l. median (range)	480 (189-2911)
Paratohormone in pg/ml. median (range)	80.05 (37.2-123.4)
Total days in Neonatal Intensive Care Unit. median (range)	38 (0-160)
Radiological signs of osteopenia. n (%)	21 (15.1%)
Fractures. n (%)	5 (3.6%)

Qualitative variables expressed in: n (percentage); Quantitative variables Expressed in: median (rank).

Results

A sample of 139 patients was obtained, whose inclusion process is shown in figure 1.

Table 1 describes the demographic data of the overall sample. A 15.1% of patients (n = 21) had radiological signs of osteopenia, of which 13 (61.9%) were male and 8 (38.1%) were female, with no statistical differences between genders. 5 neonates (3.6%), all of them male, and born with NBW less than 1000 g or before 28 GAW, presented one or more fractures during their stay in the Neonatal Unit, finding a statistical association between male gender and the development of fractures (9.2% vs 0%, p = 0.024).

The variables studied and their association with the development of osteopenia or fractures are shown in Table 2. Considering the set of variables associated with severe MBD, we found a statistical association between the sum of the number of related factors (RF) and the development of severe MBD (without severe MBD 4.71 ± 1.54 AP, severe MBD 6, 81 ± 1.63 RF, p = 0.000). However, we observed that, with an exception of one patient, the remaining infants who had 6 or more RF, but had 25 (OH) vitamin D3 values above 70 nmol/l, they did not develop severe MBD.

A multivariate logistic regression analysis was performed with the statistically significant variables in the univariate analysis, finding that the only factor that was independently associated with the development of severe BMD was intrauterine growth retardation (OR 9.65, 95% CI 3.48 -26.76, p <0.001), this fact totally detached with the rest of variables, including birth weight, mechanical ventilation duration (MV) or PN.

The mean value of 25 (OH) vitamin D3 in the global sample was 70.86 ± 25.20 nmol/L. Patients born during spring and summer (n = 71) had values significantly higher than those born in autumn-winter (n = 68) (80.94 ± 25.33 nmol/l vs 61.13 ± 21.07 nmol/l; P = 0.000). No differences were found in the PTH and AP values between the different stations.

The values of 25 (OH) vitamin D, AP and PTH as a function of the presence or absence of osteopenia or fractures are shown in Table 3. No alteration was found in the calcium values (normal values (NV) considered in this center: 8.5-10.5 mg/dL) and phosphorus (NV 4.5-7.5 mg/dL) in any patient during the time period studied.

The ROC (Receiver Operating Characteristic) curve calculated the optimal AP cutoff point for the diagnosis of MBD with radiological alteration, which in our sample was 796.5 IU/l, rating a sensitivity of 95.2% and a specificity of 92.4% (figure 2). It was not possible to estimate the diagnosis of fractures, due to the small number of patients in this group.

Table 2. Risk factors associated with the presence of osteopenia and fractures

	Osteopenia (n = 21)	No osteopenia (n = 118)	p	Fractures (n = 5)	No Fractures (n = 134)	p
Birth weigth in grams, median (range)	780 (480-1430)	1.275 (530-1495)	< 0.001	650 (550-820)	1.100 (480-1495)	0.001
Intrauterine growth restriction, n (%)	13 (61.9%)	17 (14.4%)	< 0.001	4 (80%)	26 (19.4%)	0.008
Antenatal steroids ≥ 2 doses, n (%)	20 (95.2%)	76 (66.1%)	0.007	5 (100%)	91 (69.5%)	0.321
Mechanic ventilation, n (%)	13 (61.9%)	54 (45.8%)	0.173	5 (100%)	62 (46.3%)	0.024
Total days of mechanic ventilation, median (range)	17 (1-92)	5 (0-67)	0.072	17 (1-92)	5.5 (0-67)	0.183
Total days of respiratory support*, median (range)	39 (1-160)	12.5 (0-120)	0.001	59 (50-160)	13 (0-120)	< 0.001
Total days of parenteral nutrition, median (range)	30 (11-122)	14.5 (2-89)	< 0.001	47 (32-122)	16.5 (2-89)	0.002
Time to complete enteral nutrition in days, median (range)	19 (2-106)	3 (1-79)	< 0.001	30 (19-106)	4 (1-79)	0.002
Use of diuretics, n (%)	10 (47.6%)	10 (8.5%)	< 0.001	4 (80%)	16 (11.9%)	0.001
Use of inotropes, n (%)	9 (42.9%)	20 (16.9%)	0.016	3 (60%)	26 (19.4%)	0.061

*Total days of respiratory suppont includes days of mechanic ventilation + days of non invasive ventilation (BiPAP or CPAP) + days of oxygenotherapy.

Table 3. Values of biochemical markers according to development of osteopenia or fractures

	Osteopenia (n = 21)	No osteopenia (n = 118)	p	Fractures (n = 5)	No fractures (n = 134)	p
25(OH) vitamin D3 in nmol/l, median (range)	61.8 (25.5-79.9)	72 (29.5-145)	0.283	47.5 (37.2-79.9)	71.7 (25.5-145)	0.034
Alkaline phosphatase in IU/l, median (range)	1300 (763-2911)	437 (189-1173)	< 0.001	1485 (1383-2315)	465.5 (189-2911)	< 0.001
Paratohormone in pg/ml, median (range)	113.7 (17.7-820)	64.5 (4.1-101.6) *n = 14	0.134	517.5 (176.5-820)	41.1 (4.1-115.2) *n = 30	0.001

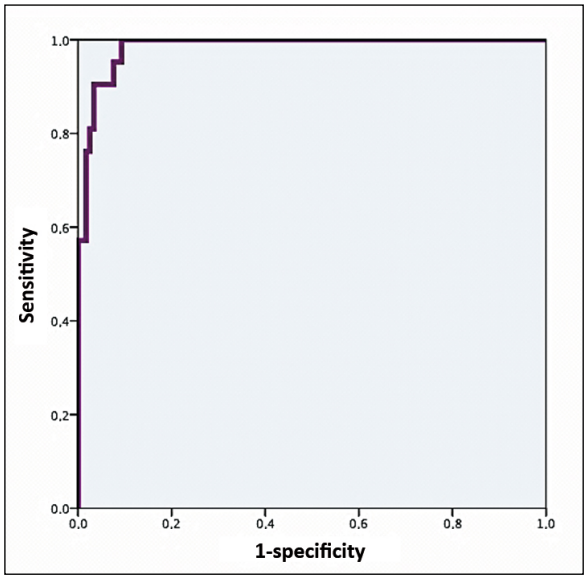

Figure 2. Receiver operator characteristic curve of alkaline phosphatase to predict osteopenia of prematurity.

Discussion

The growing advances in the intensive care of PTNB have led to a progressive decrease in mortality, which has a main consequence of causing more frequent morbidity of these patients, such as bronchopulmonary dysplasia, growth retardation, retinopathy, etc. One of these complications is osteopenia of prematurity[10,20,21].

We have found that prolonged parenteral nutrition, delayed oral feeding or the use of certain drugs as well, sum factors that predetermine prematurity itself, as well as low weight and/or intrauterine growth restriction, which ease the development of bone disease in these patients[6,22]. All these mentioned above actually is in accordance with other data published. In order to avoid this pathology, the main factor is prevention. It is highly important to prevent through early enteral feeding (or enteral nutrition EN), passive movilization through physical therapy

and a restrictive use of antagonistic medications with bone mineralization[3,9,16,22].

Regarding our patients, we have seen that those with a torpid neonatal evolution, are those with a more severe osteopenia, presenting an increased risk of developing fractures, which demonstrates the importance of avoiding these associations as far as possible, through non-respiratory management or through an invasive or early onset of trophic enteral nutrition. According to our results, we have observed that these patients could be considered 'safe' around their first month of life. Thus, patients who achieve exclusive enteral nutrition, along with parenteral nutrition withdrawal and respiratory symptoms support before 30 days of life, are clearly less likely to develop severe MBD.

The analytical figures from which there is osteopenia are not well defined at the moment in data already published, although the alkaline phosphatase is the most accepted biomarker for its diagnosis[1,13]. Along with this determination, we had not seen a relationship between the numbers of 25 (OH) vitamin D and PTH and the osteopenia development in our study, but we had seen it with those of alkaline phosphatase.

In addition, we have seen that in our patients a number of AP above 800 IU/L would be acceptable, being of an adequate clinical utility to identify newborns affected by severe MBD, presenting a good sensitivity and specificity. This is in contrast to other studies, in which it is considered as lower values around 500 IU/L[1,12,13,23].

In regard with our results, along with the fact that we do not find statistical association between osteopenia and vitamin D3 deficit, we consider that it is not a risk factor, but it could produce this pathology in an unfavorable context, espacially if it is associated with other factors, such as IUGR (intrauterine growth restriction), prolonged ventilation and immobilization, among others, which is supported by Robinson and Barrera, who already mentioned this in previous publications[7,8].

At birth, if patient is presented with vitamin D3 deficiency, we are facing 2 situations. On the one hand, it may happen that the infant does not present major problems and in this case the deficit is compensated by the early introduction of feeding with breast milk fortifiers. But, on the other hand, there is the opposite situation, in which the newborn has an important pathology. The expenditure is greater with a lower contribution, starting a series of calcium-saving mechanisms, among which the most important is the secondary hyperparathyroidism with calcium and renal phosphorus renal loss. If this situation is prolonged, bone disease is favored in prematurity[21,24]. Supporting this hypothesis, we have observed that at equal amounts of vitamin D, patients with multiple associated factors are those who develop the most severe disease.

In contrast, there are other types of patients that, despite accumulating numerous aggravating factors, have high vitamin D3 levels, which appears to be the hormone capable of blocking the release of PTH and subsequently a bone disease. This fact has already been described previously in several studies[2,6,25]. In line with these findings, the difference in vitamin D figures between spring-summer and fall-winter-born patients is striking. It should be verified that these results are maintained in other series and with larger sample sizes, which would imply that, if the birth occurs in the colder months of the year. It could be an additional factor to have a more severe form of MBD.

We also found lower values of 25 (OH) vitamin D in patients with fractures, but any statistical differences, probably due to the limited sample of children with fractures in our study.

In our sample we did not find patients with severe hypophosphoremias nor significant alterations in calcium despite radiological alterations, which was not expected. It could be due to the optimization of the exogenous contributions through the parenteral nutrition, that is carried out at an early stage during the last years, but it would be necessary to consider a more detailed study in the future regarding this topic.

The only variable that we found to be independently associated with the rest of the variables in the severe MBD development is IUGR, which confers a relative 10-fold higher risk of presenting severe MBD than preterm infants without IUGR. We would have to repeat this analysis with larger sample sizes in order to see more closely if these results are consistent. It is hypothesized that vitamin D3 deficiency conditions poor placenta implantation and that alteration in the trophoblast induces preeclampsia in mothers and IUGR[7,8,26]. Probably, the association between IUGR and maternal vitamin D3 deficiency leads to a decrease in intrauterine bone calcification, which, together with the postnatal complications that do not allow the compensation of this deficit by decreasing extra uterine contributions, makes these patients the most likely to have MBD[1,2,7,8,26].

All results obtained in this study allow us to confirm previous reports according to the risk factors that are most associated with the osteopenia of prematurity development. The main limitations that we found are the retrospective character, which makes some patients do not have all the variables under study, the small sample size especially in the subgroup of infants with fractures and the impossibility of performing densitometry to confirm the diagnostic suspicion by radiography.

It would be interesting to extend this research with a more complete prospective study in the future, in

which these findings could be related to values of bone densitometry during their admission and later during the follow-up in Neonatology.

Conclusions

Alkaline phosphatase levels are the ones that most reliably inform us about the metabolic bone state of the newborn, because we do not have densitometry, considered the gold standard test for BMC (bone mineral content) measurement.

The risk of bone metabolic disease increases to a greater number of risk factors and lower numbers of vitamin D3. Levels of 25 (OH) D3 above 70 nmol/l appear to protect against the development of this pathology, even in patients with multiple risk factors.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Financial Disclosure

The study has not received any funding for its implementation nor is it sponsored by any commercial enterprise.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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