Original article

Arch Argent Pediatr 2024;122(1):e202303001

Prevalence of osteopenia of prematurity before and after implementing an early strategy with the use of calcium and phosphate

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ABSTRACT

Introduction. With the use of aggressive parenteral nutrition in very low birth weight infants, alterations in calcium and phosphate metabolism were detected. In 2016, a prevention strategy was implemented through calcium phosphate monitoring and early supplementation. Our objective was to study whether this strategy reduces the prevalence of osteopenia and to identify associated risk factors.

Population and methods. Quasi-experiment comparing the prevalence of osteopenia between two groups: one after implementing the calcium phosphate monitoring and supplementation strategy (01/01/2017–12/31/2019) and another prior to such intervention (01/01/2013–12/31/2015).

Results. A total of 226 patients were included: 133 in the pre-intervention period and 93 in the post-intervention period. The overall prevalence of osteopenia was 26.1% (95% CI: 20.5–32.3) and it was reduced from 29.3% (95% CI: 21.7–37.8) in the pre-intervention period to 21.5% (95% CI: 13.6–31.2) in the post-intervention period, with no statistical significance (p = 0.19). In the multivariate analysis, the NEOCOSUR score for risk of death at birth, use of postnatal corticosteroids, and the intervention period were independently associated with osteopenia. Being born after the intervention reduced the probability of alkaline phosphatase > 500 IU/L by 71%, regardless of the other variables included in the model.

Conclusion. Calcium phosphate monitoring and early supplementation is a protective factor against the development of osteopenia in very low birth weight infants.

Keywords: *osteopenia; preterm newborn infant; calcium; phosphate; very low birth weight infant.*

doi: http://dx.doi.org/10.5546/aap.2023-03001.eng

To cite: Carrascal Gutiérrez MO, Janis MC, Brener Dik PH, Galletti MF, Mariani GL. Prevalence of osteopenia of prematurity before and after implementing an early strategy with the use of calcium and phosphate. *Arch Argent Pediatr* 2024;122(1):e202303001.

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Funding*: None.*

Conflict of interest*: None.*

Received*: 1-19-2023*
Accepted*: 5-9-2023*

INTRODUCTION

Advances in perinatal care have increased the survival of very low birth weight (VLBW) preterm infants, giving rise to the challenge of achieving a sequelae-free hospital discharge.[1,2] In preterm infants (PTIs), calcium (Ca) and phosphate (P) reserves are scarce; after birth, it is difficult to achieve an adequate intake due to several reasons: a delay in reaching full enteral feeding, insufficient mineral content in breast milk (BM), intestinal immaturity limiting absorption, and limited mineral intake in parenteral nutrition (PN).[3–7] These situations favor the development of osteopenia of prematurity (OP).[8,9] The prevalence of OP is 20% to 30% in VLBW infants and 50% to 60% in infants with a birth weight < 1000 grams.[5,10] Associated risk factors include low birth weight (LBW); prolonged administration of PN; administration of postnatal corticosteroids, xanthines, furosemide, and alterations in vitamin D metabolism.[10–12]

Postnatal growth restriction continues to be common despite recommendations combining "aggressive" PN (macronutrient intake since the first day of life) with early administration of BM.[13–17] When these recommendations were implemented, a high prevalence of hypophosphatemia, hypercalcemia, and hypokalemia was observed during the first week of life.[18,19] This condition was described as refeeding syndrome,[18–21] related to insufficient Ca and P intake, and it has been suggested that it would favor the progress towards osteopenia of prematurity.[22]

In 2016, at the neonatal intensive care unit (NICU) of Hospital Italiano de Buenos Aires, a modification was introduced in nutritional support protocols: Ca and P intake was started from birth, through standard PN, and serum values were monitored during the first 48 hours of life. By optimizing mineral intake, refeeding syndrome would be reduced.[5,23,24] The hypothesis of this study was that, by preventing calcium phosphate disorder in the first 2 weeks of life, the prevalence of OP would also decrease. The primary objective of the study was to compare the prevalence of OP between 2 groups of VLBW infants who received early PN, differentiated by early Ca and P monitoring and supplementation. The secondary objectives were to compare the prevalence of early hypophosphatemia/hypercalcemia and the requirement for intravenous (IV) correction of phosphate between both groups, and to identify OP-associated risk factors.

METHODS

Study type and design: before-and-after study comparing the prevalence of OP between 2 groups of VLBW infants who received early and "aggressive" PN, differentiated by early Ca and P monitoring and supplementation during their stay in the NICU. Infants born between 01/01/2013 and 12/31/2015 were included in the pre-intervention group and those born between 01/01/2017 and 12/31/2019, in the post-intervention group.

Study population: all VLBW infants were included. Patients referred from other health facilities, those with congenital malformations, those who died in the first 4 weeks of life, and those born in 2016 were excluded, as this was the time in which both strategies may have overlapped.

Intervention: all included patients started receiving PN since their admission to the NICU; enteral feeding with BM was indicated as soon as it was available. The hospital does not have a breast milk bank. In the pre-intervention period, initial PN was given without the addition of Ca and P, continuing with individualized PN. In the post-intervention period, the initial PN contained 48 mg/kg of Ca and 38 mg/kg of P (1:1 molar ratio). A permanent supply of PN bags was ensured. The change was communicated to the health care team in charge of these patients. Ca and P monitoring was standardized and the requirement for corrections was agreed upon. After a 1-year washout period, the new nutritional strategy was implemented as standard practice.

Primary outcome variable: OP was defined as an alkaline phosphatase (AP) level > 500 IU/L at any time prior to discharge (requested as per clinical criteria).

Study variables: time of first phosphatemia value, lowest phosphatemia value and highest calcemia value, requirement for phosphate supplementation and rapid corrections of phosphate, AP value.

Hypercalcemia was defined as an ionized calcium value > 1.35 mmol/L, while hypophosphatemia was defined as a value < 4 mg/dL, with values < 2 mg/dL considered severe hypophosphatemia.

Demographic variables and/or variables related to clinical course were collected, such as birth weight (BW); LBW; gestational age (GA); NEOCOSUR score (probability of neonatal death, score from 0 to 1: where 1 is the maximum probability);[25] sepsis;

necrotizing enterocolitis (NEC) (Bell staging 2/3); intraventricular hemorrhage (IVH); bronchopulmonary dysplasia (BPD) (oxygen requirement at 36 weeks); postnatal administration of corticosteroids, xanthines, and furosemide; and duration of PN.

Analysis of results

Measures of central tendency and dispersion were described for quantitative variables based on distribution. Results were reported for dichotomous variables with frequency measures. Continuous variables were compared using the t test or the Mann Whitney test based on their distribution, while categorical variables were compared using the χ^2 test or Fisher's exact test based on assumptions.

The null hypothesis of equality in the prevalence of OP between both groups was assessed. A univariate analysis was done to compare the remaining variables between both groups. To identify OP-associated risk factors, a bivariate analysis was performed to compare 2 groups according to whether or not they had presented the primary outcome. A multivariate logistic regression analysis was used to adjust for potential confounders. Variables arising from the univariate analysis ($p < 0.05$) and/or reported in the bibliography as associated with OP were included as adjustment variables. The model that best explained the results was selected on the basis of post-estimation tests: pseudo R2, goodness of fit, ROC curve for the model. The crude and adjusted odds ratio (OR) with their 95% confidence interval (CI) and *p* value were reported. Any *p* value < 0.05 was considered statistically significant. The STATA 13 software was used for analysis.

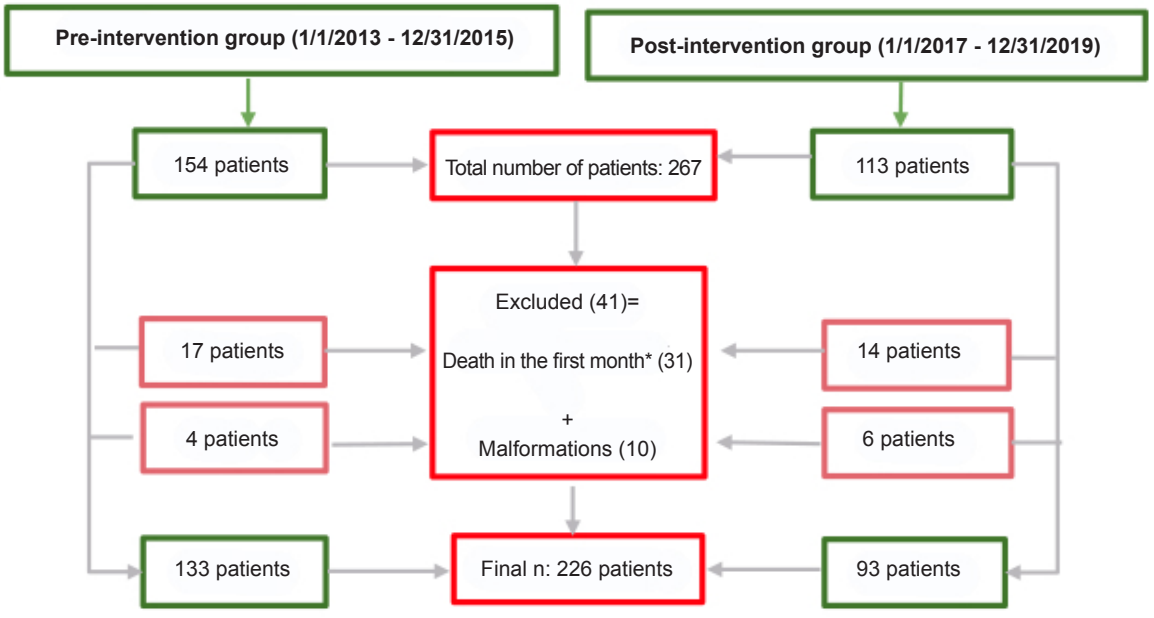
A consecutive non-probabilistic convenience sampling was performed, without estimating the sample size and analyzing 100% of VLBW infants that met the inclusion criteria, born in each period.

The study was approved by the Ethics Committee for Research Protocols.

RESULTS

A total of 267 VLBW infants were born during the study periods. A total of 226 patients were included (*Figure 1*), while 41 were excluded because they did not meet the inclusion criteria. Their mean GA was 29.6 ± 2.4 weeks and their median BW was 1138 grams (945–1330). *Table 1* shows the demographic and baseline characteristics; the only difference shown is the

Figure 1. Flow chart of patients



** Causes of mortality in order of frequency: sepsis, enterocolitis or intestinal perforation, pulmonary hemorrhage, pulmonary embolism, hydrothorax.*

administration of postnatal corticosteroids.

The variables related to calcium and phosphate metabolism and clinical course are shown in *Table 2*. The samples for AP measurement were collected at a median of 4 weeks with an interquartile range (IQR) of 3–6 (pre-intervention: median of 4 weeks and IQR of 3–6; post-intervention: median of 5 weeks and IQR of 3–7). The median AP level for the overall study population was 397 IU/L (306–517). The 75th percentile, which was estimated to assess the biological plausibility of the selected cut-off point, was 517 IU/L. The overall prevalence of OP was 26.1% (95% CI: 20.5–32.3) with 99% (n = 58) of AP samples > 500 IU/L collected after 3 weeks of life, while it was 45.2% (95% CI: 33.5–57.3) in the subgroup of infants with a birth weight < 1000 grams. In the pre-intervention

Table 1. Demographic and baseline variables associated with osteopenia by period

Variable	Pre-intervention period (n = 133)	Post-intervention period (n = 93)	*p* value
GA (weeks), mean (SD)	29.7 (2.3)	29.6 (2.3)	0.88*
BW (grams), median (IQR)	1170 (960–1345)	1095 (920–1310)	0.15**
Low birth weight, n (%)	46 (34.6)	31 (33.3)	0.84***
Male sex, n (%)	68 (51)	43 (46)	0.47***
NEOCOSUR score, median (IQR)	0.13 (0.05–0.29)	0.11 (0.04–0.35)	0.93**
Postnatal GCs, n (%)	17 (12.8)	23 (24.7)	**0.021*** **
Caffeine, n (%)	102 (76.7)	69 (74.2)	0.67***
Furosemide, n (%)	51 (38.3)	37 (39.8)	0.83***
Days of PN, median (IQR)	10 (8–14)	13 (8–19)	0.12**

GA: gestational age; BW: birth weight; GCs: glucocorticosteroids; PN: parenteral nutrition; SD: standard deviation; IQR: interquartile range; n: number.

** t test.*

*** Mann-Whitney test.*

**** χ^2 test.*

Table 2. Variables associated with calcium and phosphate metabolism and clinical course by period

Variable	Pre-intervention period n = 133	Post-intervention period n = 93	*p* value
Calcium and phosphate metabolism			
AP > 500 IU/L, n (%)	39 (29.3)	20 (21.5)	0.19***
AP in IU/L, median (IQR)	385 (303–529)	404 (314–483)	0.89**
First P measurement in days of life, median (IQR)	2 (1–4)	1 (0–1)	**< 0.001****
Lower P in mg/dL, median (IQR)	2.8 (2–3.6)	3.3 (2.9–3.6)	**< 0.001****
Higher iCa in mMol/mL, median (IQR)	1.43 (1.37–1.53)	1.47 (1.41–1.54)	0.051**
Hypercalcemia iCa > 1.35 mMol/mL, n (%)	109 (82)	90 (96.7)	**< 0.001**
Hypophosphatemia < 4 mg/dL, n (%)	107 (80.4)	83 (89.2)	0.08***
Severe hypophosphatemia < 2 mg/dL, n (%)	27 (20.3)	1 (1.08)	**< 0.001****
Rapid correction of P, n (%)	19 (14.3)	0 (0)	**< 0.001****
P supplementation, n (%)	92 (69)	67 (72)	0.64***
Morbidities associated with clinical course			
BPD, n (%)	30 (22.5)	29 (31.1)	0.14***
IVH, n (%)	21 (15.8)	18 (19.3)	0.48***
Sepsis, n (%)	23 (17.3)	23 (24.7)	0.17***
NEC, n (%)	5 (3.8)	12 (12.9)	**0.02**

******** *P: phosphate; iCa: ionized calcium; AP: alkaline phosphatase; BPD: bronchopulmonary dysplasia; IVH: intraventricular hemorrhage; NEC: necrotizing enterocolitis; IQR: interquartile range; n: number.*

** t test.*

*** Mann-Whitney test.*

**** χ^2 test.*

***** Fisher's test.*

period, it was 29.3% (95% CI: 21.7–37.8); and it reduced to 21.5% (95% CI: 13.6–31.2) in the post-intervention period. In the subgroup of infants with a birth weight < 1000 grams, it decreased from 52.6% (95% CI: 36.5–68.1) to 37.8% (95% CI: 23.5–54.7). These differences were not statistically significant for both strata. The minimum phosphatemia level, the prevalence of severe hypophosphatemia, and the requirement for rapid P corrections showed significant differences, just like the difference in days to the first phosphatemia measurement, favoring the post-intervention group. Regarding neonatal morbidities related to prematurity, there were no differences between groups in terms of BPD, IVH, and sepsis. A significant increase in NEC was observed in the post-intervention period.

Table 3 shows the results of the univariate analysis according to the presence or absence of OP. Patients with OP were smaller and more immature and had a higher baseline risk of death. The group of patients with OP received additional corticosteroids, diuretics, and PN for a longer period and had a higher incidence of late-onset sepsis and NEC. The values corresponding to calcemia, phosphatemia, and the requirement for rapid P corrections were statistically different.

Lastly, a multivariate logistic regression analysis was performed (*Table 4*). The baseline risk of death (NEOCOSUR score), receiving postnatal corticosteroids, and the period during which the birth occurred were independently associated with OP. Being born during the post-intervention period reduced the probability of AP > 500 IU/L by 71%, regardless of the other variables included in the model. *Figure 2* shows the corresponding ROC curve.

DISCUSSION

This study shows that, for VLBW infants receiving "aggressive" PN, the implementation of supplementation and close monitoring of Ca and P is a protective factor against the development of OP. This is a common complication in the studied population with variable prevalence according to their GA and BW, with those born before 28 weeks of GA and with a BW < 1000 grams being the most affected ones.[9,12,20] These results are consistent with those reported in the bibliography, both in the overall population and among PTIs with a birth weight < 1000 grams. Although such reduction was not statistically significant after the intervention, it is possible that the sample size was underpowered. In

Table 3. Univariate analysis by presence of primary result (alkaline phosphatase > 500 IU/L)

Variable/AP > 500	No (n = 167)	Yes (n = 59)	*p* value
GA weeks, median (IQR)	30 (29–32)	29 (27–30)	**< 0.001****
BW grams, median (IQR)	1205 (1005–1355)	940 (800–1125)	**< 0.001****
Low birth weight, n (%)	55 (33)	22 (37.3)	0.54***
Male sex, n (%)	78 (46.7)	33 (56)	0.22***
NEOCOSUR score, median (IQR)	0.08 (0.03–0.23)	0.36 (0.14–0.7)	**< 0.001****
Postnatal GCs, n (%)	17 (10.2)	23 (39)	**< 0.001*****
Caffeine, n (%)	121 (72.5)	50 (84.7)	0.06***
Furosemide, n (%)	50 (30)	38 (64.4)	**< 0.001*****
Days of PN, median (IQR)	10 (7–14)	16 (10–27)	**< 0.001****
Lower P in mg/dL, median (IQR)	3.2 (2.7–3.9)	2.7 (2–3.1)	**< 0.001****
Higher iCa in mMol/mL, median (IQR)	1.43 (1.38–1.53)	1.5 (1.41–1.57)	**0.008****
Severe hypophosphatemia < 2 mg/dL, n (%)	15 (9)	13 (22)	**0.009*****
Rapid correction of P, n (%)	9 (5.4)	10 (17)	**0.006*****
Late-onset sepsis, n (%)	23 (13.7)	23 (39)	**< 0.001*****
NEC, n (%)	8 (4.8)	9 (15.2)	**0.018******
Post-intervention period, n (%)	73 (43.7)	20 (33.9)	0.19***

A: gestational age; BW: birth weight; GCs: glucocorticosteroids; PN: parenteral nutrition; NEC: necrotizing enterocolitis; P: phosphate; iCa: ionized calcium; IQR: interquartile range; n: number.

** t test.*

*** Mann-Whitney test.*

**** χ^2 test.*

***** Fisher's test.*

Table 4. Univariate and multivariate models. Likelihood of alkaline phosphatase > 500 IU/L

Variable	Univariate OR (95% CI)	Univariate *p* value	Multivariate aOR (95% CI)	Multivariate *p* value
NEOCOSUR score	56.9 (15–213)	0.001	46.3 (9–221)	**0.001**
Severe hypophosphatemia (P < 2 mg/dL)	2.8 (1.2–6.4)	0.01	0.9 (0.28–2.85)	0.87
PN days	1.06 (1.02–1.08)	0.001	1.03 (0.99–1.05)	0.10
Postnatal GCs	5.6 (2.7–11.6)	0.001	3.8 (1.3–10.8)	**0.01**
NEC	3.6 (1.3–9.7)	0.013	1.22 (0.28–5.3)	0.78
Post-intervention period	0.66 (0.35–1.22)	0.19	0.29 (0.12–0.7)	**0.006**

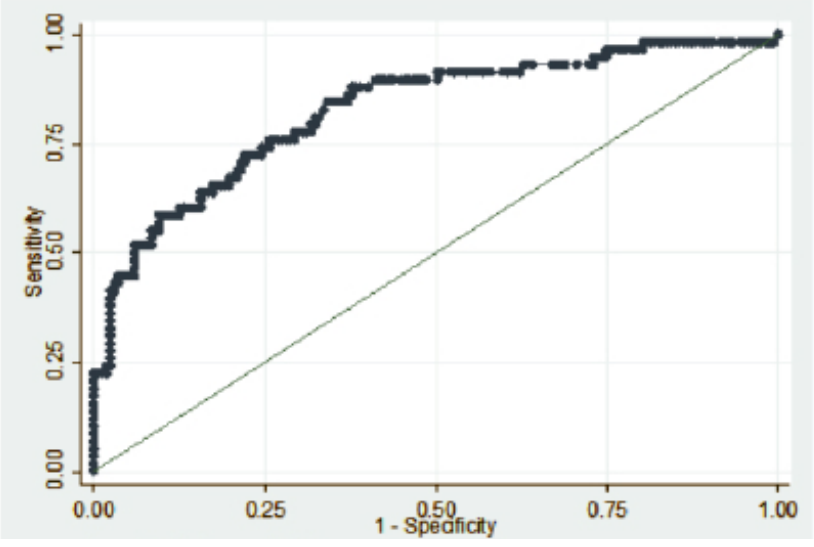
n = 225; pseudo R2 = 0.26; p = 0.001; goodness of fit p = 0.6 (Hosmer-Lemeshow test).
Area under the ROC curve of the model = 0.82.
OR: odds ratio; aOR: adjusted odds ratio; PN: parenteral nutrition; GCs: glucocorticosteroids; NEC: necrotizing enterocolitis.

addition, the multivariate model showed that being born in the post-intervention period was a protective factor against OP, regardless of the other variables included. These findings may have a highly relevant clinical impact.

One aspect that is worth noting is the value defined as diagnostic of OP. Ninety percent of blood AP is produced by bone. AP may reflect bone turnover and is used as a parameter of mineralization.[26,27] Although some authors used higher cut-off points (700–800 IU/L),[28] a recent study reported that a value of 500 IU/L has a 100% sensitivity and an 80% specificity.[5] The 75th percentile of AP was 517 IU/L, which gives biological plausibility to the selected cut-off point.

The association between calcium and phosphate intake and the development of OP is still a matter of debate. Some authors have reported the relationship between an insufficient intake of these micronutrients during the first 8 weeks of life and the development of OP among newborn infants with a GA < 30 weeks and extremely LBW.[1] The presence of hypophosphatemia is one of the earliest markers of altered calcium and phosphate metabolism that could be related to the development of OP and may occur from early postnatal life.[12] In this study, it was observed that an early mineral intake was associated with lower chances of hypophosphatemia with requirement of phosphate correction, which may have contributed to the reduction in OP.

Figure 2. ROC curve for the multivariate model



Area under the ROC curve = 0.82.

Calcemia values were higher in the post-intervention group. This may be due to the early administration of Ca in PN from the day of birth and to the fact that the doses administered may exceed the needs at this stage.

The baseline characteristics of both groups were similar, except for the use of postnatal corticosteroids, so they were comparable. The deleterious effect of corticosteroids on bone mineralization among PTIs is well known and an independent association with OP was established.[29–31] However, there was a trend toward a decrease in OP in the post-intervention group, which might have been more pronounced without the difference in the practice of postnatal corticosteroid administration. We did not observe significant differences in terms of morbidity associated with prematurity, except for a higher incidence of NEC in the post-intervention group. This complication prolongs the days of PN and delays the progression of enteral feeding, leading to a state of increased risk of OP. Even so, the post-intervention group showed a lower prevalence of OP, despite having a higher proportion of such risk factor. This supports the importance of early Ca and P supplementation as a preventive intervention for OP.

In the univariate analysis, patients with a diagnosis of OP were more immature and smaller, in addition to having a higher risk of death according to their NEOCOSUR score. They also showed a higher frequency of postnatal corticosteroid use, diuretic use, days of PN, late-onset sepsis, lower phosphatemia levels, and higher calcemia levels. All these factors have been associated with the development of OP.[10–12] In the multivariate analysis, it was observed that being born in the post-intervention group served as a protective factor against OP. On the contrary, postnatal corticosteroid use and baseline severity estimation were observed to be independent risk factors. These results are especially encouraging considering that a change in behavior, such as Ca and P supplementation and monitoring from the first hours of life, may have an impact on the clinical course and development of comorbidity in the long term.

This study has certain limitations. Certain practices and clinical course outcomes associated with the outcome measures could have been modified in the study period, such as the use of non-invasive ventilation, neurodevelopmental care, etc. These variables were not collected, so their impact cannot be analyzed. Another limitation may be the time at which the highest AP value was measured; some authors consider that its measurement in the third week of life is a predictor value; others consider that the best time would be between 4 and 8 weeks of life.[22,28] Due to the observational design of this study, it was not possible to establish a specific time for sample collection. Such variability may result in a collection bias, although 99% of samples in our population with AP > 500 IU/L were collected after the third week of life. Given the observational nature of this study, it is not possible to warrant the causality between both outcome measures. However, some of Hill's criteria for causation are met, making the observed association relevant.[32] Consistent with previous studies,[5,23] we observed temporality and biological plausibility in the different correlations in both periods and in a robust multivariate model according to the post-estimation tools used.

Based on these findings, it may be concluded that optimizing the use of calcium and phosphate in VLBW infants receiving PN is independently associated with a lower probability of OP. This practice may show benefits in their long-term clinical course. Nevertheless, prospective studies confirming these findings would be useful. ■

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