Platelets and platelet-derived growth factor in closure of the ductus arteriosus

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The crucial role of platelets in the permanent closure of the ductus arteriosus has recently been elucidated in an animal model; however, clinical studies investigating the impact of platelet count on ductal patency have conflicting results. Our aim is to compare platelet count, indices and serum platelet-derived growth factor levels in preterms with and without ductal patency. Preterms with a gestational age of 27-30 weeks followed up during a twelve-month period in the neonatal intensive care unit of Adnan Menderes University Hospital were enrolled. Infants underwent echocardiographic evaluation starting on the second day and subsequently assessed every other day until ductal closure was achieved, or upon clinical signs of reopening. Platelet-derived growth factor was measured on the second and fifth days of life. Eleven very low birth weight infants who subsequently required medical treatment for patent ductus arteriosus were compared with twenty-three infants with closed ductus. Although platelet count and indices were similar, median serum platelet-derived growth factor levels on day 5 were significantly lower among babies who subsequently required medical treatment for ductal patency (874.6 vs 1099.6 pg/ml). The current study points out a possible association between serum platelet-derived growth factor levels and ductal closure. Our results suggest that platelet-derived growth factor may play a role in ductal closure independent from platelet count and might be used as an adjunct surrogate for prediction of future need for treatment for hemodynamically significant patent ductus arteriosus in preterm infants.

Key words: echocardiography, newborn, patent ductus arteriosus, platelet-derived growth factor, platelet count.

In healthy term infants, closure of the ductus arteriosus occurs via two consecutive mechanisms. Initial functional closure, mediated mainly by prostaglandin E2 withdrawal and oxygen exposure, is followed by subsequent vascular remodeling that achieves anatomical closure[1]. Permanent closure requires the activation of a ubiquitous cell signaling cascade, including intramural hypoxia, formation of intimal cushions, migration of smooth muscle cells and platelet-vessel wall interactions[1].

The crucial role of platelets in the permanent closure of the ductus arteriosus has been demonstrated successfully by Echtler et al[2]. in an animal model. However, clinical studies investigating the impact of platelet count on ductal patency have reported conflicting results.[3-8] It is speculated that platelet dysfunction, rather than low platelet count, is the main contributory factor in the failure of the ductus to close in preterm infants[4-7]. To clarify the role of platelet dysfunction in ductal patency, we used the platelet-derived growth factor molecule as a surrogate. As one of the agents stored in the alpha granules of platelets that is released upon activation[9-14], platelet-derived growth factor is proposed as the key element affecting the process of ductal closure at the cellular level, for several reasons.

Firstly, anatomical closure involves migration

of vascular smooth muscle cells into the subendothelium, as well as increased deposition of collagen[15-17]. Migration is dependent on increased fibronectin levels and further facilitated by particular chemotactic agents[15-17]. Platelet-derived growth factor is one of the chemoattractants that readily promotes migration of vascular smooth muscle cells through the extracellular matrix, thus making a significant contribution to anatomical closure[12,15-18]. Another recent study disclosed possible interactions between platelet-derived growth factor and fibronectin molecules[19].

Definitive anatomical closure requires highly specialized interplay of vascular smooth muscle cells, endothelial cells and fibroblasts[20]. The responses of fibroblasts to platelet-derived growth factor, documented by Seppä et al.[21], provide a second line of support for our hypothesis. The group successfully showed that the release of platelet-derived growth factor from platelets results in recruitment of fibroblasts to that area, with subsequent mitogenic activity facilitating rapid proliferation.

Platelet-derived growth factor additionally promotes stimulation of hyaluronan synthetase in mesothelial cells[22]. In preterm infants, induction of this enzyme within the ductal wall triggers synthesis of hyaluronan, which not only contributes to cellular migration of smooth muscle, but also makes up the intimal mounts that occlude the lumen[15-17].

Finally, in addition to its role in anatomical closure, platelet-derived growth factor may play a part in functional closure, since it is involved in the regulation of vascular tone in adults[13].

Material and Methods

This prospective study was conducted in the neonatal intensive care unit of Adnan Menderes University Hospital. The trial was approved by the local ethics committee, and infants were enrolled after written parental consent was obtained. Preterm infants born at 27-30 weeks gestational age and followed up during the period January 2012-January 2013 were eligible for inclusion in the study.

In all cases, parenteral nutrition was initiated on the first day at 100 ml/kg/day and increased, up to 160-180 ml/kg/day, in accordance with urinary output and density. Along with parenteral nutrition, enteral feeding was started on day 1 of life and gradually increased. Dopamine was used for hypotensive infants in whom fluid treatment failed. The empirical antibiotic choice for preterm infants in our unit was ampicillin and gentamicin.

Nasal continuous positive airway pressure or mechanical ventilation was initiated, depending on the severity of respiratory distress. Surfactant (Survanta®, Abbot) was administered to babies with respiratory distress syndrome within the first hour of life at a dose of 4 ml/kg, and a repeat dose administered at the sixth hour in case of need.

Sample Collection

A complete blood count and serum C-reactive protein (CRP) measurement at 6 h of life was performed as part of routine premature care in our unit. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes and subsequently analyzed using a Siemens Advia 2120-i hematology analyzer device (Siemens Healthcare Diagnostics, GmbH, Marburg, Germany). Serum CRP levels were measured with a commercially available kit using the turbidimetric method (Tokyo Boeiki, Prestige 24i, Tokyo, Japan).

To determine serum platelet-derived growth factor concentrations, 0.5 ml blood samples were withdrawn on days 2 and 5. Samples were centrifuged and serum was carefully collected with plastic transfer pipettes, aliquoted into polystyrene tubes and stored at -80°C. Collected samples were subjected to enzyme-linked immunosorbent assay (ELISA) for human platelet-derived growth factor-BB using a commercial diagnostic system (eBioscience, Bender MedSystems GmbH, Vienna, Austria). The calculated overall intra- and interassay coefficients of variation were 4.0% and 8.4%, respectively.

Echocardiographic evaluation

Echocardiographic evaluation of the infants was performed by a pediatric cardiologist, starting on day 2 of life; they were subsequently followed up every other day until ductal closure was achieved, or upon clinical signs of reopening. Echocardiographic examinations were conducted with the commercially available Philips HD11 XE ultrasound system (Philips Medical Systems, Eindhoven, the Netherlands),

using an 8-13 MHz transducer. Measurements were performed by the same researcher. Averages of three to five consecutive readings for vessel diameter and flow velocity integrals were determined. M-mode measurements of the left atrium-to-aortic root ratio and two-dimensional measurements of ductal diameters were obtained from the parasternal view. Ductal diameters were measured at the pulmonary end, median part and aortic end of the ductus using color flow. Doppler studies included aortic and left pulmonary artery blood flow, transmitral inflow and ductal shunt.

All echocardiographic data were digitally recorded and later reviewed by the same researcher who performed all measurements and calculations. Intraobserver variability was assessed in a randomly selected subset of ten patients by repeating the measurements on two occasions. Variability was calculated as mean percentage error, derived as the difference between the two sets of measurements divided by the overall mean of the observed values with both measurements and found to be 2.9 ±1.6%.

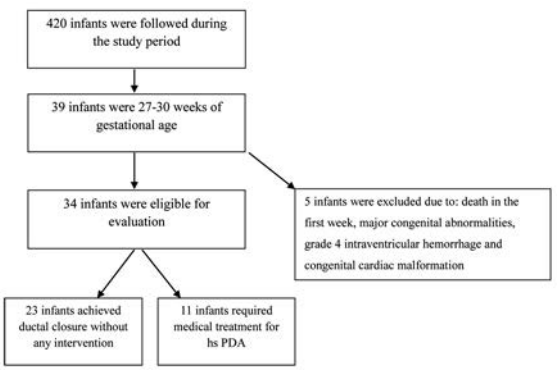

Fig. 1. Algorithm for identification of the study groups.

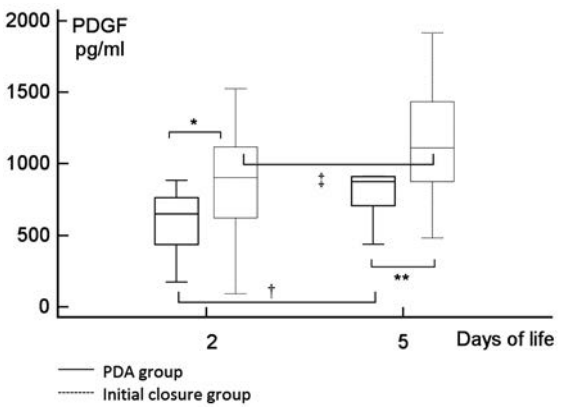

Fig. 2. Serum platelet-derived growth factor (PDGF) levels (pg/ml) of the groups on days 2 and 5.
(*p=0.11, **p =0.036, †p =0.0002, ‡p =0.0002)
p-values are calculated by the Mann Whitney-U independent samples test (*,**) and the Wilcoxon test for paired samples (†,‡).

Diagnosis of "hemodynamically significant patent ductus arteriosus"

Infants with ductal diameters >1.5 mm and left atrium-to-aortic root ratios >1.5 with retrograde diastolic flow in the aorta and diastolic flow in the pulmonary artery >0.20 m/sec on day 5 were classified as "hemodynamically significant patent ductus arteriosus"[23]. In the event hemodynamically significant patent ductus arteriosus was detected, treatment decisions were implemented according to the infant's clinical status. Increase in respiratory distress, need for increase in mechanical ventilation parameters, apnea, resting tachycardia, metabolic acidosis, hyperdynamic precordium, bounding pulse, systolic or continuous murmur at the upper left sternal border, hypotension, signs of pulmonary edema on chest X-ray or pink secretions from the endotracheal tube were the clinical signs taken into account for treatment decisions. Oral ibuprofen (Pedifen®, Atafarm, Istanbul, Turkey) was given for medical treatment (initial dose of 10 mg/kg, followed by 5 mg/kg at 24 and 48 hours).

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software (MedCalc Software, Belgium). Comparison of variables was performed with Fisher's exact test and the Mann Whitney-U independent samples test. The Wilcoxon test for paired samples was used to assess the differences between platelet-derived growth factor levels on days 2 and 5. Data were considered statistically significant at $p<0.05$.

Results

Thirty-nine infants were eligible for inclusion during the study period. Among these, five were excluded due to death in the first week, major congenital abnormalities, grade 4 intraventricular hemorrhage or congenital cardiac malformation (Fig. 1). Twenty-three cases achieved ductal closure without medical intervention (initial closure group). Eleven infants required medical treatment for hemodynamically significant

Table I. Demographic Data of the Study Groups

	Initial closure group (n=23)	PDA group (n=11)	*p*
Birth weight (g)	1298 (1110-1495)	1150 (1010-1480)	*
Gestational age (weeks)	30 (29.7-30)	29.5 (28-30)	*
Male	13 (59)	7 (63)	*
Vaginal delivery	6 (26)	2 (18)	*
APGAR, 5-minute	8 (7-9)	7 (7-9)	*
Surfactant need	23 (100)	11 (100)	*
Premature rupture of membranes >24 h	2 (9)	0	*
Antenatal steroids†	9 (39)	4 (36)	*
Chorioamnionitis	3 (13)	1 (9)	*

Data are represented as median (IQR) and n (%); *p*-values are calculated by the Mann Whitney-U and Fisher exact tests. *$p>0.05$, †Complete course

patent ductus arteriosus in the subsequent days during follow-up in the neonatal intensive care unit (PDA group). Four cases required a second course of ibuprofen; none of the infants required surgical intervention.

Demographic data of the groups are illustrated in Table I. Platelet count, mean platelet volume, platelet mass and platelet distribution width values were similar between the groups (Table II). Platelet-derived growth factor levels increased significantly from day 2 to day 5 in both groups. Additionally, the median platelet-derived growth factor level in the initial closure group was significantly higher than that in subjects requiring treatment for ductal patency on day 5 of life (1099.6 vs. 874.6 pg/ml, p=0.036) (Fig. 2). The median platelet-derived growth factor level on day 2 of life was also higher in the initial closure group (899.5 vs 651.6 pg/ml), but this difference was not statistically significant.

When a receiver operating characteristic curve analysis was performed for platelet-derived growth factor level on day 5, the best cutoff value to predict need for treatment for ductal patency was found to be 909 pg/ml (area under curve: 0.773, sensitivity: 91.7%, specificity: 71.1%, 95 % CI: 0.63-0.87, +LR: 3.17, -LR: 0.12, p=0.0003)

Discussion

In the current study, a gradual increase in serum platelet-derived growth factor levels in very low birth weight infants was demonstrated from day 2 to day 5 of life. However, PDGF levels on day 5 were higher in the initial closure group than in subjects subsequently requiring medical intervention for ductal patency. This difference is of particular interest, since platelet counts and indices were similar between the groups.

Recently, Echtler et al.[2] showed clear histological evidence of platelet accumulation within the lumen of the ductus arteriosus in newborn mice via intravital microscopy, and higher incidence of persistent patency of the ductus arteriosus in congenitally thrombocytopenic mice. The investigators additionally evaluated premature infants in their clinic retrospectively, and identified thrombocytopenia as a major risk factor for ductal patency. In contrast to these findings, the closure rate of the ductus arteriosus was similar in thrombocytopenic (n=19) and non-thrombocytopenic (n=99) infants in a study from Japan.[6] A large-scale study of 1,350 infants with birth weights <1500 g by Sallmon et al.[5] did not reveal a link between platelet count on day 1 after birth and patent ductus arteriosus. In light of the histologic findings of Echtler et al[2]., Sallmon et al.[6] concluded that impaired platelet function due to immaturity and critical illness, rather than low platelet numbers, contributes to ductal patency.

To our knowledge, the current study on very low birth weight infants is the first report highlighting a possible association between serum platelet-derived growth factor levels and ductal patency, and calls for further

Table II. Platelet Counts and Indices of the Groups

	Initial closure group	PDA group	*P*
Platelets, X 10^{12}/L	220 (183.5-273.2)	208.5 (181.5-235.5)	*
MPV, fl	9 (8.1-9.8)	9.1 (8.6-10)	*
Platelet mass	1936 (1608.1-2485.8)	1998.7 (1709.1-2277.3)	*
PDW (%)	54 (51.1-58.1)	56.4 (53.6-60.8)	*
PDGF day 2 (pg/ml)	899.5 (493.99-1072.84)	651.69 (438.71-760.9)	*
PDGF day 5 (pg/ml)	1099.6 (754-1273.99)	874.69 (704.50-909.38)	0.036
CRP, mg/dl	0.17 (0.3-6)	0.82 (0.2-36)	*

MPV: mean platelet volume, *Platelet mass*: MPV x platelet count, *PDW*: platelet distribution width, *PDGF*: platelet-derived growth factor. Data are represented as median (IQR); *p*-values are calculated by the Mann Whitney-U test. *$p>0.05$

clinical investigation in a larger patient cohort. Platelet-derived growth factor is stored in the alpha granules of platelets, and extrusion of the granule content occurs upon activation. Increase in platelet-derived growth factor within the ductal lumen is a critical process that contributes to functional and anatomical closure via several mechanisms. It is possible that significant enhancement of the local platelet-derived growth factor to a certain threshold provides a proper microenvironment for appropriate progression of cellular events, facilitating successful closure. Moreover, the serum platelet-derived growth factor level on day 5 of life may be a helpful adjunct for predicting whether potential treatments would be effective in hemodynamically significant patent ductus arteriosus cases.

Limitations and Future Directions

The current study has several limitations. First, the gestational age of our cases was in the range of 27 to 30 weeks. However, the behavioral pattern of the ductus may vary in infants at different maturation stages. Infants who are 27-30 weeks old usually need less inotropic and pulmonary support than do ≤26-week-old babies; they merely face significant metabolic and biochemical derangements that yield clearly different consequences, interfering with ongoing ductal closure at the cellular level. Platelet-derived growth factor levels in immature infants should be further investigated by future studies.

Secondly, in our study, intervention was indicated only in case of an apparent clinical sign of hemodynamically significant ductus arteriosus with a dynamic follow-up trend of sonographic data, rather than one static snapshot. This alternative allowed our group the chance of spontaneous closure instead of the implementation of medical treatment as soon as hemodynamically significant ductus arteriosus was diagnosed. Almost all infants requiring treatment were classified as grade 2 or 3 according to the McNamara staging system[23]. Comparison of grade 4 cases with infants who successfully achieved closure soon after birth in a larger study group may reveal a more striking difference.

Finally, all infants who required treatment responded to ibuprofen, and closure of the ductus was achieved in the first few weeks of life in these cases. Platelet-derived growth factor levels in non-responders require further investigation.

Conclusions

An increase from day 2 to day 5 in serum platelet-derived growth factor levels was observed in very low birth weight infants. In addition, platelet-derived growth factor levels on day 5 were higher in the initial closure group than in subjects subsequently requiring medical intervention for ductal patency. Further clinical investigation in a large cohort should improve our understanding regarding this association.

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