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Cord Blood Platelet Aggregation; Quality Control by a Two-sample Technique

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ABSTRACT

Two blood samples were taken from the cords at 17 normal deliveries 2-4 min and 5-8 min after birth, respectively. The difference in platelet count between early and late samples in platelet-rich plasma was <5% in nine cords (Group A), and greater in eight cords (Group B). Platelet aggregation studies on the early and the late blood samples showed consistent results within each cord in Group A but not in Group B. The correlations between the responses were high for Group A. The aggregation responses were also slightly but significantly higher in the late samples in this group ($p<0.01$ in Group A; n.s. in Group B). The differences between responses in early and late blood samples could not be explained by acid-base dissimilarities. The variability in cord blood platelet aggregation results can be greatly reduced by platelet counting in PRP of two independent blood samples, accepting only samles with concordant platelet counts (<5% difference).

INTRODUCTION

Platelet aggregation of cord blood is one of the tools used for investigation of haemostasis in the newborn. Decreased aggregability of platelets in response to ADP, collagen and especially epinephrine compared with that in adults has been reported in normal cord blood (1,6,10). Differences in the conditions of the mothers, and in the pregnancies, the type of obstetric analgesia and the drug intake influence the results profoundly (1,3,4,9,11). Technical differences, such as the sodium citrate concentration in relation to the haematocrit, may play a role (12).

Discrepancies between results in different studies may be due to partial activation and/or aggregation of platelets during the course of delivery or during the process of platelet preparation. Activation in the vessel or during blood collection are expected to reduce the platelet count in whole blood. Platelet activation during the preparation procedure, e.g. during centrifugation will only be detected if the platelets are counted in

the final platelet rich plasma (PRP). As the normal variation in the number of platelets in PRP varies within wide ranges, partial activation and/or aggregation resulting in a decreased platelet count will not be disclosed in a single specimen, but will probably result in a difference in platelet count between two specimens from the same cord. The aim of this study was to examine the relation between accordance in platelet count and accordance in aggregation responses in two independent blood samples from the same cord in a well-defined group of normal mothers and their healthy infants.

SUBJECTS AND METHODS

For inclusion in the study, the mothers had to be healthy non-smokers with normal pregnancies ending in a normal vaginal delivery at gestational weeks 38-41. Further criteria were that only N_2O-O_2 and/or pudendal nerve block were given as obstetric analgesia, and that no drugs had been taken during the last month prior to delivery. The mothers were especially questioned concerning intake of non-steroid anti-inflammatory drugs. Seventeen infants with Apgar scores ≥ 8 at 1 and 5 min and a birth weight appropriate for gestational age were accepted. Without disturbing the normal delivery, two independent blood samples were drawn from the clamped cord by puncturing the umbilical vein 2-4 and 5-8 min after delivery, the second sample closer to the placenta. With a 16G siliconized cannula, 9 ml of blood were aspirated into a syringe containing 1 ml of sodium citrate (3.2%). The sample was accepted only if it was obtained easily within 20 sec. After blood sampling, all handling of the two samples from the same cord was simultaneous. The platelet-rich plasma (PRP) was prepared by centrifugation for 15 min at 140G at room temperature. The platelet count of the PRP was determined on a Thrombocounter C (Coulter Electronics, Hialeah, Florida, USA). A platelet suspension of 300 x 10^9/l was obtained by diluting the PRP with platelet-poor plasma (PPP), which was yielded after spinning down the rest of the sample for 10 min at 2750G.

Platelet aggregation was performed in a Payton dual-channel aggregometer at 37°C, with magnetic stirring at 900 rpm within 30-90 min post partum. After a stable base-line had been established for half a minute, aggregation was induced in 0.25 ml of the platelet suspension by ADP (Sigma, Stockholm, Sweden) 1.1 or 3.3 µmol/l, collagen (Hormon-Chemie, Munich, GDR) 1.0 or 5.5 mg/l, or epinephrine (ACO, Stockholm, Sweden) 8.3 µg/l -final concentration in the cuvette. Light-transmission changes (ΔOD) were recorded and expressed in per cent of the PRP-PPP light-transmission difference.

Maximal aggregation, and for collagen also the duration of the lag phase (sec), were measured at all recordings. Qualitatively the recordings were analyzed according to type of curve, i.e. reversible or irreversible aggregation. As the amounts of platelet suspension obtained after preparation were too small in some cases, all stimuli could not be tested in all cords. All aggregations performed in the 17 cords are included in the following description of the results. Means and standard deviations are given. Significance analysis

was performed by paired t-tests.

RESULTS

PLATELET COUNT

The platelet count in PRP varied widely from one cord to another (365-1140 x 10^9/l), but there was no systematic difference between the early and the late samples (Table 1). The variation in the difference in platelet count between the early and the late sample, expressed as per cent of the count in the early sample, was also considerable (-25.5 - +21.8 %). In nine of the 17 cords this difference was less than 5% (Table 1). In the following presentation we have divided the 17 cords arbitrarily into two groups - Group A, with a difference in platelet count between the early and the late cord blood sample of <5%, and Group B, with a difference of >5%.

Table 1. Platelet count in PRP of early and late cord blood samples. (Mean ±SD)

Cord No.	Early samples	Late samples	Difference/ early sample %	Group
1	890	885	- 0.6	A
2	440	380	- 13.6	B
3	850	840	- 1.2	A
4	1140	960	- 15.8	B
5	740	740	± 0	A
6	625	660	+ 5.6	B
7	390	475	+ 21.8	B
8	700	750	+ 7.1	B
9	652	659	+ 1.1	A
10	700	670	- 4.3	A
11	700	680	- 2.9	A
12	777	771	- 0.8	A
13	533	479	- 10.1	B
14	520	525	+ 1.0	A
15	660	740	+ 12.1	B
16	490	365	- 25.5	B
17	1035	995	- 3.9	A
Total	697 ± 201	681 ± 187		
Group A (n=9)	763 ± 149	752 ± 140		
Group B (n=8)	622 ± 235	601 ± 210		

PLATELET AGGREGATION

Qualitative description

Epinehrine (8.3 µg/l) produced no visible reactions (n = 8).

Collagen 1.0 mg/l (n = 16) produced no response in 11 cords and irreversible aggregation in one. In the remaining four cords reversible aggregation was obtained.

Collagen 5.5 mg/l (n = 12) caused reversible aggregation in one case and irreversible

aggregation in 11 cases.

ADP 1.1 µmol/l (n = 15) produced reversible aggregation in 12 cases. In one cord no reaction was seen. In the remaining two cords, both in Group B, reversible aggregation was noted in the early sample, while in the late sample no significant aggregation was seen.

ADP 3.3 µmol/l (n = 17) produced reversible aggregation in 13 cords and irreversible aggregation in four.

There was no difference between the early and the late sample with respect to qualitative responses to any of the stimuli tested in Group A, while in Group B there was slight inconsistency for ADP 1.1 µmol/l.

Quantitative results

The number of samples studied was sufficient for statistical evaluation in the case of ADP 1.1 and 3.3 µmol/l and of collagen 5.5 mg/l. In Group A the early and the late samples from the same cord showed very similar peak aggregation responses to these stimuli (Fig.1), the k values (slopes) for the regression lines quite close to 1 (0.81-1.01) and correlation coefficients relatively high (r = 0.85-0.95). There was also a strong correlation between the early and late samples with respect to the lag phases in response to collagen 5.5 mg/l (r = 0.99).

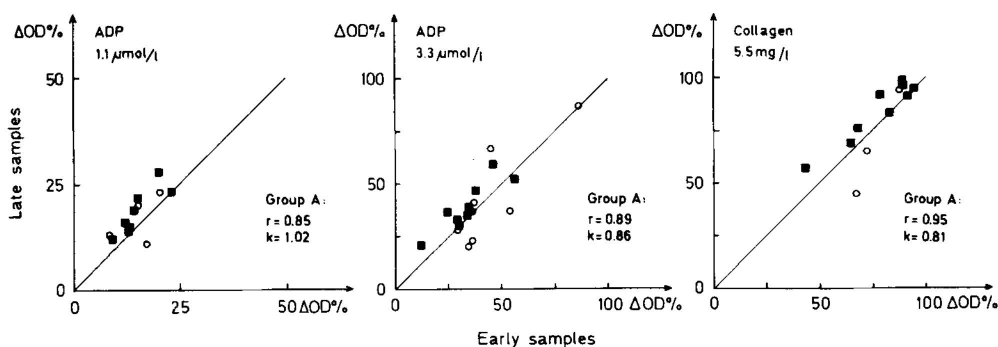

Fig.1 Relationship between maximal aggregation (ΔOD %) in early and late blood samples in response to ADP 1.1 µmol/l, ADP 3.3 µmol/l and collagen 5.5 mg/l. Group A (■) and Group B (o). The line represents the equation x = y.

As also seen in Fig.1, the relations between the early and the late samples were less consistent in Group B with k values varying from 0.59-2.25 and r = 0.53-0.98.

There was a small but systematic difference in the maximal ΔOD between the early and late samples in Group A, with a significantly lower peak aggregation in the early sample for all three tested stimuli (Table 2, Fig.1). A similar significant difference, with a shorter lag phase for the late sample, was also found in response to collagen 5.5 mg/l in Group A ($p<0.01$) but not in the total group. No significant difference between the early and the late samples was found in Group B ($p>0.1$ for all stimuli) (Table 2).

Table 2. Aggregation responses (ΔOD) to ADP and collagen (Mean ±SD)(n.s. = non significant).

Stimuli		Maximal aggregation (ΔOD)		
		Total	Group A	Group B
		(n=12)	(n=8)	(n=4)
ADP 1.1 μmol/l	early sample	14.9 ±4.5	14.9±4.5	15.0±5.1
		$p<0.02$	$p<0.01$	n.s.
	late sample	18.0±5.3	18.6±5.4	16.8±5.7
		(n=17)	(n=9)	(n=8)
ADP 3.3 μmol/l	early sample	38.9±16.0	33.9±12.5	44.6±18.4
		n.s.	$p<0.01$	n.s.
	late sample	40.9±17.6	39.4±12.0	42.5±23.1
		(n=12)	(n=9)	
Collagen 5.5 mg/l	early sample	77.3±15.0	77.8±16.7	
		n.s.	$p<0.01$	
	late sample	80.3±17.8	84.3±14.3	

Acid base data are given in Table 3. pCO_2 was significantly lower in the late samples.

Table 3. Acid-base data for all samples and for groups A and B. (Mean ±SD)(n.s. = non-significant).

pH		Before aggregation	After aggregation
Total (n=15)	early sample	7.43±0.06	7.58±0.09
		n.s.	n.s.
	late sample	7.43±0.05	7.59±0.10
Group A (n=8)	early sample	7.43±0.05	7.59±0.07
		n.s.	n.s.
	late sample	7.43±0.05	7.59±0.07
Group B (n=7)	early sample	7.43±0.07	7.57±0.11
		n.s.	n.s.
	late sample	7.43±0.06	7.58±0.13
pCO_2			
Total (n=15)	early sample	3.70±0.39	2.72±0.47
		$p<0.001$	$p<0.02$
	late sample	3.59±0.43	2.59±0.48
Group A (n=8)	early sample	3.76±0.34	2.69±0.37
		$p<0.05$	n.s.
	late sample	3.67±0.33	2.58±0.33
Group B (n=7)	early sample	3.63±0.45	2.75±0.60
		$p<0.01$	$p<0.01$
	late sample	3.50±0.53	2.60±0.64

DISCUSSION

During the first minutes after birth tremendous biological changes occur in the child and the placenta, many of which may be expected to influence the very complex factors underlying platelet aggregaoility. If platelet activation occurs during delivery, the number of circulating platelets might be decreased. Platelet activation can also take place during blood sampling and preparation of platelet suspensions (8). This is evident from our findings of differences in platelet counts. In two independent blood samples taken at an interval of 3-5 min there was no systematic difference between the early and the late samples but a randomly distributed difference between -26 and +22 %. The cut-off point was set at 5% for two reasons:

1. The distribution of the differences was completely symmetrical but still significantly different from a normal distribution. Nine cases were clustered around a mean of -1% (range -5 to +3%). The remaining eight cases were evenly distributed in the ranges +5 to 30% (four cases) and -5 to -30% (four cases). This fits with the hypothesis that those clustered around the mean represent differences between two biologically identical specimens (Group A), while the others represent differences between one specimen with a reduced number of platelets and another with the original platelet count.

2. The cut-off limits had to exceed the empirical counting error with the counter used in the hospital (1-3%).

The very high correlations between the aggregation responses in the early and the late samples in Group A, obtained at quantitative analysis, are in agreement with the conclusion that no significant change in platelet aggregability had taken place. The more inconsistent results in Group B suggest, on the contrary, that changes in platelet aggregability had occurred in one or both samples. This functional alteration might be due to a release of small amounts of ADP from activated platelets, resulting in a temporary unresponsiveness of some of the platelets to aggregating agents (7).

The interesting finding of a small but systematic difference, with higher platelet aggregability in the late sample, was only made in Group A (<5% difference in platelet count). This indicates that these subtle quantitative changes in platelet aggregability are only detectable in samples not exposed to activation. Platelet counting in PRP from two independent blood samples thus seems to effectively disclose platelet activation and is recommended for quality control.

The reason for the more pronounced aggregability in the late samples cannot be explained by these data. Acid-base changes could not answer for the difference, as the only significant discrepancy observed in pCO_2 between early and late samples would be expected to influence platelet aggregability in the opposite direction (2). Different physiological processes involved in the normal delivery, expulsion of the placenta and constriction of the umbilical vessels might result in the release of substances influencing platelet function, e.g. catecholamines (5), 5-hydroxytryptamine (5-HT) (14) and prostaglandins (13).

This study, in contrast to previous investigations in this field, combines extremely strict criteria for inclusion in the material with a two-sample technique for exclusion of activated samples. Using this technique cord blood aggregation studies in newborn infants can be possible to perform for scientific purposes.

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