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THE IMPORTANCE OF HEMATOCRIT IN THE INTERPRETATION OF COAGULATION TESTS IN THE FULL-TERM NEWBORN INFANT

Federico Cerneca, Umberto de Vonderweid*, Roberto Simeone, Vincenzo Forleo*

*Servizio Analisi Chimico-Cliniche, Laboratorio di Patologia dell'Emostasi e *Centro di Neonatologia, Istituto per l'Infanzia, Trieste, Italy*

ABSTRACT

Background. **Hematocrit (HCT) is significantly higher in newborns than in adults, but this fact is not usually considered when performing coagulation tests in newborns. We studied 71 healthy full-term newborns and compared them to 100 healthy adults to test the hypothesis that correcting the anticoagulant-to-blood ratio for neonatal HCT would reduce the differences among the two populations.**

Methods. **PT, PTT, fibrinogen, platelets and factors II, VII, IX, X, V, VIII were measured in 71 healthy full-term newborns and 100 healthy adults. An anticoagulant-blood ratio corrected for HCT was used. In 16 newborns, a non corrected value was also used and results were compared with the corrected ratio.**

Results. **A significant difference was observed between newborns and adults in all tests with the exceptions of fibrinogen and factor V. In the 16 newborns from whom blood was collected without correcting in the anticoagulant, a significant difference was also found in all parameters but fibrinogen. A weak correlation linked the different variables.**

Conclusions. **After correction for HCT, neonatal PT and factors V, VII, VIII and IX were much closer to adult values; neonatal PTT and factors II and X were still definitely lower.**

Key words: hematocrit, coagulation tests, newborns

Normal values for neonatal coagulation tests reported in the literature are different from adult standards, but they also vary considerably among studies.[1-6] Such discrepancies may be ascribed, at least in part, to differences in the neonatal populations studied, i.e. gestational age distribution,[7] use of cord vs baby's blood,[1-5] age at sampling, vitamin K prophylaxis, type of feeding.[8,9] Another source of bias is the difference in hematocrit (HCT) values between adults and newborns, and even among newborns. The amount of anticoagulant commonly used (a ratio of 1:10 of blood) is based on a theoretical HCT of 40-45%. When the actual HCT is different, a correction should be made in order to maintain the plasma-to-anticoagulant ratio constant.

In our study, we tested the hypothesis that correcting for high neonatal HCT could reduce the differences between neonatal and adult coagulation parameters.

Materials and methods

Seventy-one healthy full-term, appropriate-for-age newborns (41 males, 30 females) were studied. Apgar scores at birth were normal: all were breast fed exclusively and had received 1 mg of vitamin K1 within 1 hour of birth. One hundred healthy adults (46 males, 54 females; age 18-45 years), receiving no medication, served as controls.

Neonatal blood samples (1 mL) were collected within 12 to 24 hours of birth from a dorsal

Correspondence: Dott. Federico Cerneca, Servizio Analisi Chimico-Cliniche, Laboratorio di Patologia dell'Emostasi, Istituto per l'Infanzia, via dell'Istria 65/1, Trieste, Italy.

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Table 1. Reference values for coagulation tests in the 71 healthy full-term newborns compared to the normal adult control values (values are expressed as means, standard deviations and standard errors of the mean).

	newborns (n = 71)			*adults (n = 100)*			
	mean	SD	SEM	mean	SD	SEM	p
Hct (L/L)	59.0	3.0	0.36	44.0	2.5	0.25	< 0.0001
PT (sec)	13.1	0.9	0.11	11.9	0.6	0.06	< 0.0001
PTT (sec)	35.0	4.5	0.53	28.8	2.7	0.27	< 0.0001
Plt (x10^9/L)	214	55	6.53	258	66	6.60	< 0.0001
Fbg (mg%)	251	51	6.05	262	44	4.40	0.134
FII (%)	73	7	0.83	100	15	1.50	< 0.0001
FV (%)	93	13	1.54	98	19	1.90	0.056
FVII (%)	88	12	1.42	95	18	1.80	0.005
FVIII (%)	113	38	4.51	92	21	2.10	< 0.0001
FIX (%)	86	18	2.14	94	16	1.60	0.003
FX (%)	72	10	1.19	97	15	1.50	< 0.0001

hand vein by means of a 1.5-in. 21 gauge straight needle. The blood flowed directly into a plastic tube containing 3.2% buffered sodium citrate. The anticoagulant-to-blood ratio was calculated each time on the basis of HCT values determined immediately before. HCT values were measured by a Coulter JT2 (Coulter Electronics, Milano, Italy) on a venous blood sample. Blood was immediately centrifuged (3000 g for 20 min at 4°C) and tested after platelet poor plasma had been removed. Coagulation screening tests for prothrombin time (PT), expressed in seconds (Thromborel S, Behring), and activated partial thromboplastin time (PTT), expressed in seconds (Dade FS), each required 50 uL of plasma. Fibrinogen (FBG) was measured as a thrombin clottable protein using 10 ul of plasma. The following coagulation factor assays were performed with human deficient plasma and 30 to 100 uL of each infant's plasma.

Factor II-, VII-, IX-, X-, V- and VIII-deficient plasma was obtained from Dade, and the results were expressed as a percentage of pooled plasma from 30 normal individuals (13 males, 17 females; age 15-40). Platelet (PLT) counts were evaluated using a Coulter JT2. Sixteen of the 71 newborns (10 M, 6 F) were also investigated as previously mentioned. In these cases, blood was collected at a fixed 1:10 v/v ration of anticoagulant/blood.

Adult plasma was tested with the same

Table 2. Comparison of coagulation tests in 16 healthy full-term infants with and without correction of the anticoagulant-to-blood ratio for Hct.

	newborns (n = 16) with correction			*newborns (n = 16) without correction*			
	mean	SD	SEM	mean	SD	SEM	p
PT (sec)	13.0	0.7	0.17	15.7	0.8	0.20	< 0.0001
PTT (sec)	35.0	4.0	1.00	44.0	3.1	0.78	< 0.0001
Fbg (mg%)	250	31	7.75	242	32	8.00	0.4782
FII (%)	71	5	1.25	55	7	1.75	< 0.0001
FV (%)	93	10	2.50	70	9	2.25	< 0.0001
FVII (%)	88	10	2.50	64	9	2.25	< 0.0001
FVIII (%)	106	26	6.50	87	23	5.75	0.0365
FIX (%)	86	11	2.75	60	11	2.75	< 0.0001
FX (%)	70	9	2.25	54	8	2.00	< 0.0001

Table 3. Correlation coefficient r and p values between FII and FX and between these and the other clotting factors in healthy full-term newborns.

	r	p
FII-FV	+0.5686	< 0.0001
FII-FVII	+0.3219	0.0062
FII-FVIII	+0.1708	0.1638
FII-FIX	+0.1500	0.2293
FII-FX	+0.4039	0.0005
FX-FV	+0.3552	0.0024
FX-FVII	+0.3474	0.0030
FX-FVIII	+0.2632	0.0301
FX-FIX	+0.1978	0.1115

method as that employed for neonatal samples. A two-ways Student's t-test was utilized to compare neonatal values with those found in the adults.

Results

Table 1 reports the mean ± 1 SD, the number of samples tested and the p-value. As expected, neonatal and adult HCT values were significantly different ($p < 0.0001$). The distribution of PT and PTT values in neonatal and adult specimens was also significantly different ($p < 0.0001$), but newborn PT was always comparable to adult values and newborn PTT was very close to the upper limit of the adult range.

Platelet count was also different ($p < 0.0001$), although the values observed in newborns still fell within the normal adult range. Fibrinogen did not differ in the two groups ($p = 0.134$). As occurred with for PT and PTT, the level of each clotting factor was significantly different in the two populations ($p < 0.01$); only FV did not differ significantly ($p = 0.056$), but the mean newborn values for the majority of the clotting factors fell within the normal adult range. The amount of factor VIII is quite variable among newborns: it is extremely high in some, lower in others. Factors II and X are the only items whose activity is definitely lower in newborns than in adults.

When blood samples collected with a corrected anticoagulant ratio were compared with those collected without an anticoagulant ratio corrected for HCT (see methods), significant differences were observed for PT, PTT, FII, FVII, FIX, FX, FV ($p < 0.0001$), and FVIII ($p = 0.0365$). No difference was found for fibrinogen ($p = 0.4782$) (Table 2).

As reported in Table 3, a significant correlation was also found among the various factors: the lower the values for FII and FX, the lower the value of other factors.

Table 4. Reference values for coagulation tests in healthy full-term infants according to Andrew et al, 1987.

	newborns			*adults*		
	mean	SD	n	mean	SD	n
PT (sec)	13.00	1.43	61*	12.4	0.78	29
aPTT (sec)	42.90	5.80	61	33.5	3.44	29
Fbg (g/L)	2.83	0.58	61*	2.78	0.61	29
FII (U/mL)	0.48	0.11	61	1.08	0.19	29
FV (U/mL)	0.72	0.18	61	1.06	0.22	29
FVII (U/mL)	0.66	0.19	60	1.05	0.19	29
FVIII (U/mL)	1.00	0.39	60*	0.99	0.25	29
FIX (U/mL)	0.53	0.19	59	1.09	0.27	29
FX (U/mL)	0.40	0.14	60	1.06	0.23	29

Note. All factors except fibrinogen are expressed as units per milliliter.
**do not differ statistically from adult values.*

Discussion

Since bleeding problems are relatively frequent during the neonatal period, it is very important to compare individual patient coagulation tests with appropriate reference values. Most data in the literature report markedly reduced coagulation factor activity in the first few days of life with respect to adult standards. This is especially true for vitamin K-dependent factors, whose levels are described as being as low as 30-50% of adult values, even following vitamin K prophylaxis.[10-14] In our opinion no convincing biological explanation has been offered for this phenomenon, which might be at least partially related to technical problems like HCT and blood collection quality.

Newborn and adult specimens should not be compared for coagulation performance without correcting for HCT, which is significantly higher in the former group. As shown in Table 2, adjusting this ratio brings the values observed in newborns near to those found in adults.

Our experience suggests that, after this correction is made, neonatal coagulation tests and individual clotting factor levels are much closer to adult standards than has been previously

reported in the literature. The value distribution in the two populations is usually different, but for PT and factors V, VII, VIII and IX the mean newborn values are usually within the normal adult reference range. When fibrinogen and platelet values are also considered, the overall difference is of modest clinical significance.

Conflicting results have been reported;[6] Andrew et al. (Table 4) observed clotting factors levels that differ from ours in spite of a very similar study population (use of baby's blood, age at sampling, vitamin K prophylaxis, type of feeding). If their data are compared with those we obtained without correcting for the HCT value, the difference is much smaller, indicating the importance of adjusting the anticoagulant/blood ratio for HCT.

There is another reason why correcting for HCT is important during the neonatal period. After the first few days of life, and especially in sick newborns, HCT tends to drop quite rapidly. If this correction is not routinely performed, it is possible to overlook a true reduction in clotting activity which may be clinically relevant.

In conclusion, our data suggest that HCT represents a bias when performing coagulation tests in newborns, and this technical problem may at least partially explain the differences between newborn and adult coagulation tests reported by a number of authors.

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