Correspondence

Received: January 24, 1983

Accepted: April 29, 1983

Acta haemat. 1983;70:278-279

Developmental Changes in HbA₂ and HbF on Neocytes and Gerocytes in Normal Infants during the First Year of Life

A.	Achille	Iolascon
L.	Luciano	Pinto
B.	Bruno	Nobili
S.	Stefano	Cutillo

Napoli

Achille Iolascon, Luciano Pinto, Bruno Nobili, Stefano Cutillo, 1° Clinica Pediatrica, 1° Facoltà Medica Napoli, Via S. Andrea delle Dame 4, Napoli (Italy)

To the Editors,

We have read with interest the article by Graue et al. [1] concerning the separation and characterization of red blood cells from newborns and infants during the first trimester of life using dextran density gradient: mean cellular volume (MCV) and mean cellular haemoglobin concentration (MCHC).

We studied haemoglobin pattern modifications on neocytes and gerocytes during the 1st year of life to better define the developmental changes in HbF and HbA₂ levels during the first months of life.

We have examined 30 newborns from mothers with normal haematological parameters and haemoglobin pattern; 64 infants aged between 1 and 12 months and 20 control subjects. The screened children had no thalassaemic parents.

Blood samples, obtained during routine analysis, are collected on K₂EDTA, and processed daily. Mixture gradient and red cells separation procedures were according to Vettore et al. [2], with the following modifications: Percoll (Pharmacia, Uppsala) 35%, Meglamine diatrizoate (Selectographyn, 70%; Schering Berlin/Bergkamen) 18% and water 47% of final mixture.

The determinations were performed on neocytes (top layer) and gerocytes (bottom layer). The validity of this method of red cell separation has been assessed by means of the assay of different and known parameters, such as MCV, G-6-PD activity and erythrocyte creatine, that is in accord with the data reported in the literature [2–4].

HbA₂ was measured by microchromatography [5], and HbF by alkali denaturation [6]. Our data are summarised in table I. HbF levels decrease during the 1st year of life, reaching approximately 10% at 3–4 months on neocytes, and at 4–5 months on whole blood; at 1 year of age the HbF level is still higher than in adults.

The comparison of HbA₂ values of neocytes and

Table I. HbA₂ and HbF values (± DS) of red cells before (GR) and after density gradient separation of neocytes (N) and gerocytes (G) during the 1st year of life

Age
Number
of
subjects
HbA₂

HbF

N
G
GR
N
G
GR
At birth
30
0.64 ± 0.15
0.42 ± 0.07
0.49 ± 0.12
54.0 ± 8.8
72.0 ± 9.2
66.0 ± 7.6
1 month
10
0.91 ± 0.25
0.40 ± 0.12
0.72 ± 0.29
44.0 ± 5.7
68.0 ± 6.2
52.0 ± 5.5
2 months
6
2.07 ± 0.29
0.96 ± 0.15
1.14 ± 0.32
24.3 ± 6.8
37.5 ± 6.2
33.0 ± 6.5
3 months
8
2.13 ± 0.32

1.35 $\pm$ 0.19
1.55 $\pm$ 0.31
18.5 $\pm$ 5.5
25.6 $\pm$ 4.8
21.0 $\pm$ 6.1
4 months
8
2.27 $\pm$ 0.26
1.72 $\pm$ 0.25
1.93 $\pm$ 0.28
8.0 $\pm$ 3.3
13.6 $\pm$ 3.6
10.5 $\pm$ 3.5
5 months
6
2.39 $\pm$ 0.26
1.86 $\pm$ 0.30
2.18 $\pm$ 0.21
3.2 $\pm$ 1.1
5.6 $\pm$ 0.9
4.6 $\pm$ 0.9
6 months
6
2.50 $\pm$ 0.22
2.10 $\pm$ 0.32
2.25 $\pm$ 0.30
2.6 $\pm$ 0.4
3.9 $\pm$ 1.1
3.3 $\pm$ 0.9
7 months
5
2.50 $\pm$ 0.28
2.19 $\pm$ 0.31
2.28 $\pm$ 0.27
1.7 $\pm$ 0.5
3.3 $\pm$ 1.2
2.8 $\pm$ 1.1
8 months
5
2.48 $\pm$ 0.32
2.25 $\pm$ 0.29
2.34 $\pm$ 0.36
1.2 $\pm$ 0.4
2.3 $\pm$ 0.5
1.9 $\pm$ 0.7
9–10 months

5
 2.53 ± 0.26
 2.30 ± 0.28
 2.42 ± 0.34
 1.1 ± 0.6
 2.2 ± 0.6
 1.7 ± 0.4
 11–12 months
 5
 2.61 ± 0.24
 2.50 ± 0.28
 2.53 ± 0.29
 1.0 ± 0.3
 2.0 ± 0.5
 1.4 ± 0.4
 2–12 years
 20
 2.64 ± 0.28
 2.56 ± 0.24
 2.60 ± 0.30
 0.4 ± 0.2
 0.7 ± 0.3
 0.6 ± 0.2
 Correspondence
 279

gerocytes during the 1st year of life showed that HbA₂ reaches from the 2nd month a value equivalent to 80% of HbA₂ in the neocytes of adults; on overall red cell population a similar value is reached at 4–5 months, and in gerocytes at 6 months. Our data suggest the possibility of using this method of red blood cell separation for an early recognition of beta-thalassaemia traits.

Furthermore, Schmidt et al. [7], who studied the density distribution of RBC in infants, found a remarkable increase in reticulocytes of the overall population, especially in the top fractions on the 56th day; in this period we found a substantial increase of HbA₂ values on neocytes.

References

- Grauel, E.L.; Muller, S.; Gross, J.; Syllm-Rapa-port, I.: Separation and characterization of red blood cells from newborns and infants during the first trimenon of life using a dextran density gradient: mean cellular volume and mean cellular haemoglobin concentration. *Acta haemat.* 67: 102–108(1982).
- Vettore, L.; De Matteis, M.C.; Zampini, P.: A new density gradient system for separation of human red blood cells. *Am. J. Hematol.* 8: 291–297 (1980).
- Seaman, C; Wyss, S.; Piomelli, S.: The decline in energetic metabolism with aging of erythrocyte and its relationship to cell death. *Am. J. Hematol.* 8: 31–42(1980).
- Piomelli, S.; Lurinsky, G.; Wasserman, L.R.: The mechanism of red cell aging. Relationship between cell age and specific gravity evaluated by

ultracentrifugation in a discontinuous density gradient. *J. Lab. clin. Med.* 69: 661-674 (1967).

Huisman, T.H.J.; Schroeder, W.A.; Brodie, A.N.; Mayson, S.M.; Jackson, J.: Microchromatography of hemoglobins. III. A simplified procedure for determination of hemoglobin A₂. *J. Lab. clin. Med.* 86: 700-705(1975).

Singer, K.; Chernoff, A.I.; Singer, L.: Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. *Blood* 6: 413-418(1951).

Schmidt, G.; Gross, J.; Grauel, E.L.; Ihle, W.; Syllm-Rapaport, I.: Separation and characterization of red blood cells from newborn and infants during the first trimenon of life by means of a dextran density gradient, density distribution curves of erythrocytes, and reticulocytes. *Biol. Neonate* 37: 42-51(1977).

Metaxotou-Mavromati, A.D.; Antonoupolou, H.K.; Laskari, S.S.; Tsiarta, H.K.; Ladis, V.A.; Kattamis, C.A.: Developmental changes in HbF levels during the first two years of life in normal and heterozygous beta-thalassaemia infants. *Pediatrics, Springfield* 69: 734-738 (1982).

Acta haemat. 70: 279-280(1983)

Diagnosis of Metastatic Cancer from the Iliac Crest Biopsy