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Concentrations of main serum opsonins in early infancy

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Abstract

The evolution of the main serum opsonins in neonates and infants of varying gestational age was investigated to provide reference values for these opsonins in early infancy. Serum concentrations of immunoglobulins, IgG subclasses, C3, C4 and fibronectin were serially measured from birth until the age of 6 months in term and preterm infants. Measurements were performed by rate nephelometry. Five hundred and sixty six neonates (gestational age 26–41 weeks) were examined at birth, 233 at 1 month, 218 at 3 months, and 147 at 6 months, respectively. The same measurements were performed in 54 pairs of neonatal/maternal samples and in 230 apparently healthy adults. Gestational age had a significant impact on serum IgG, IgG subclasses, C3 and C4 up till the third month, and on fibronectin until the first month. No such impact was observed for IgA and IgM. Sixteen per cent of the neonates had IgM concentrations higher than 0·2 g/l at birth, suggesting that the critical concentration of serum IgM at birth for suspected intrauterine infection should be reconsidered. Concentrations of all opsonins at birth were significantly lower than adult reference values. They only approached or even reached adult values by the third or the sixth month.

Data from analysis of the neonatal and the corresponding maternal sera indicate that there is a preferential active transplacental transport of IgG subclasses in the order of IgG1, IgG3, IgG2 and IgG4. These results show that concentrations of immunoglobulins, C3, C4 and fibronectin undergo changes during the first months of life, depending not only on the infants' postnatal age but also on gestational age.
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The ability of serum to opsonise invading bacteria is an essential defensive mechanism in infants. The integrity of this function depends mainly on the serum concentrations of certain proteins, collectively known as opsonins. These comprise: immunoglobulins; the C3, C4 complement components; and fibronectin. Serum opsonic activity in neonates, especially those born prematurely, may be low compared with adult values, because of low titres of maternally derived IgG and IgG subclass specific antibodies,[1–6] or because of insufficient production of C3, C4[7–8] and fibronectin.[9–11] Gestational age reference values are therefore needed for the clinical interpretation of opsonin measurements in early infancy. Published values have not included all opsonins and refer to relatively small series of infants. This is probably because there are certain limitations in the determination of serum proteins in early infancy, mainly arising from the amount of blood required. However, the recent development of rapid and accurate methods requiring minimal quantities of blood, such as nephelometry, the wide application of these measurements is now practicable.

Methods

Five hundred and sixty six neonates (315 males and 251 females), appropriate for gestational age, were studied. Gestational age was determined by prenatal ultrasonography, when available, or by accurate maternal dates, and confirmed by neonatal examination (Ballard score). The infants were divided into three groups on the basis of gestational age:

Group I consisted of 99 preterm infants ranging from 26 to 30 weeks (mean gestational age 28·37 (1·4) weeks and birthweight of 1125 (264) g.

Group II comprised 207 infants ranging from 31 to 37 weeks (mean 33·4 (1·9) weeks and birthweight of 2011 (497) g.

Group III comprised 260 term infants (mean gestational age 39·5 (1) week and birthweight of 3440 (417) g.

All neonates with evidence of intrauterine or perinatal infection, intrauterine growth retardation, major congenital abnormalities and uncertain gestational age were excluded from the study. Informed consent was obtained from all parents.

Blood samples were collected through an indwelling umbilical arterial catheter or by venepuncture during routine procedures within the first 24 hours of birth. Blood samples were also collected from 54 mothers 12 hours after delivery and their corresponding neonates of 37–42 weeks' gestation.

Of the 566 infants enrolled in the study, 233, 218, and 147 were followed up to the end of the first, third, and sixth month, respectively. Infants who had either received intravenous immunoglobulin or double volume exchange transfusion, or who developed infections during the study period were excluded from the follow up. Follow up included clinical examination, collection of data about infections, and measurement of serum opsonin concentrations.

Table 1 Percentiles of serum concentrations of immunoglobulins in three groups of infants from birth until 6 months of age

Postnatal age	Group	n=	IgG percentiles					IgM percentiles					IgA percentiles				
			5	25	50	75	95	5	25	50	75	95	5	25	50	75	95
Birth	I	99	2·80	3·88	4·96	5·93	7·53	0·0	0·06	0·08	0·16	0·36					
	II	207	5·08	6·64	8·02	9·87	12·19	0·0	0·04	0·09	0·15	0·31					
	III	260	6·90	9·65	11·40	12·85	15·32	0·07	0·09	0·13	0·17	0·28					
	p*				<0·001					<0·001							
1 month	I	54	2·25	3·21	3·75	4·22	5·60	0·25	0·41	0·48	0·68	0·89	0·0	0·08	0·12	0·17	0·26
	II	104	2·84	3·71	4·50	5·36	7·56	0·22	0·39	0·47	0·65	0·88	0·0	0·0	0·09	0·13	0·29
	III	75	4·16	5·35	6·23	7·44	10·17	0·21	0·39	0·59	0·79	1·05	0·0	0·10	0·12	0·21	0·32
	p*				<0·001				NS						<0·05		
3 months	I	54	1·07	1·94	2·50	3·27	4·48	0·28	0·45	0·58	0·77	1·30	0·0	0·13	0·19	0·23	0·35
	II	104	1·20	2·11	3·09	3·83	5·80	0·35	0·52	0·69	0·97	1·41	0·01	0·09	0·19	0·24	0·41
	III	60	2·75	3·76	4·06	4·82	7·06	0·43	0·68	0·86	1·09	1·46	0·11	0·18	0·24	0·32	0·39
	p*				<0·001					<0·01					NS		
6 months	I	35	2·03	3·40	3·97	4·65	6·71	0·57	0·79	0·96	1·26	1·77	0·06	0·17	0·27	0·35	0·44
	II	79	2·05	2·97	4·17	5·08	7·51	0·42	0·67	0·99	1·30	1·72	0·01	0·15	0·22	0·39	0·62
	III	33	3·47	4·30	4·91	6·09	7·30	0·60	0·80	0·95	1·15	1·62	0·16	0·22	0·26	0·35	0·43
	p*				NS					NS					NS		
Adults		230	8·74	10·80	12·30	14·20	16·90	0·64	1·03	1·40	1·86	2·99	1·03	1·60	2·05	2·54	3·47

*Significance of difference among the three groups using the Kruskal-Wallis one way analysis of variance; NS not significant.

Serum and plasma samples were stored at −70°C until analysed. All parameters were measured in serum except for fironectin which was measured in plasma. All measurements were performed by rate nephelometry – fixed time nephelometry – using the Behring Nephelometer Analyser (BNA). Data obtained from 230 healthy blood donors were used as adult reference values.

STATISTICAL ANALYSIS

Percentiles (5th, 25th, 50th, 75th, and 95th) of the measured variables for each gestational age group at birth and at each postnatal age were calculated. The Kruskal-Wallis one way analysis of variance was used to evaluate the difference of values among various gestational age groups. The Wilcoxon rank sum test for paired samples was used to compare the respective concentrations in neonatal and maternal sera.[12] Data were analysed using the SPSS for MS Windows (version 5.0.1, International Use, SPSS Company) software package.

Results

Total IgG subclass values at birth differed significantly among the different groups (tables 1 and 2). A wide range of individual values of IgG2 and IgG4 was observed. The relative concentrations of IgG subclasses were similar in all gestational age groups (70%, 20%, 7% and 3% for IgG1, IgG2, IgG3 and IgG4, respectively). Serial IgG measurements showed that concentrations decreased progressively until the third month in all gestational age groups, and then increased again until the sixth month (table 1). The pattern of IgG1 was similar to that of IgG, while IgG3 reached the lowest concentrations at the end of the first month and increased thereafter. IgG2 and IgG4 concentrations declined during the first three months and remained low until the sixth month (table 2).

IgA was undetectable by rate nephelometry (<0·07 g/l) in 99% of the infants at birth, but at six months of age it could be measured in all infants.

IgM concentrations at birth showed a wide range of individual values. In 90 of the 566 (16%) neonates, values were higher than 0·2 g/l. Concentrations increased rapidly, reaching adult reference values by six months (table 1).

At birth, mean concentrations of C3 and C4 corresponded to 0·60–0·79 and 0·41–0·59 of the respective adult reference values, depending on gestational age. During the following months, concentrations of these complement

Table 2 Percentiles of serum concentrations of IgG subclasses in three groups of infants from birth until 6 months of age

Postnatal age	Group	n=	IgG1 percentiles					IgG2 percentiles					IgG3 percentiles					IgG4 percentiles				
			5	25	50	75	95	5	25	50	75	95	5	25	50	75	95	5	25	50	75	95
Birth	I	99	1·48	2·08	3·11	3·99	6·16	0·0	0·60	0·85	1·16	1·60	0·13	0·19	0·26	0·41	0·55	0·0	0·0	0·11	0·19	0·35
	II	207	0·76	3·88	5·13	6·59	8·59	0·40	0·86	1·32	1·80	3·01	0·16	0·37	0·49	0·66	1·01	0·0	0·08	0·18	0·34	0·67
	III	260	3·36	5·81	7·41	8·93	12·0	0·67	1·36	2·14	3·02	5·22	0·26	0·48	0·70	0·93	1·48	0·0	0·09	0·23	0·44	0·97
	p*				<0·001					<0·001					<0·001					<0·001		
1 month	I	54	1·17	1·85	2·20	2·86	4·54	0·34	0·67	0·86	1·07	1·64	0·04	0·16	0·24	0·30	0·42	0·0	0·0	0·11	0·16	0·26
	II	104	1·20	2·20	2·92	3·79	5·08	0·10	0·96	1·16	0·63	1·69	0·16	0·22	0·28	0·39	0·62	0·0	0·0	0·08	0·17	0·31
	III	75	2·76	3·36	3·90	4·89	6·36	0·43	0·76	1·11	1·47	2·54	0·20	0·26	0·32	0·46	0·71	0·0	0·0	0·09	0·20	0·34
	p*				<0·001					<0·001					<0·01					<0·05		
3 months	I	54	0·60	1·02	1·43	1·89	3·95	0·0	0·0	0·45	0·75	1·04	0·0	0·11	0·18	0·34	0·57	0·0	0·0	0·0	0·0	0·09
	II	104	0·60	1·35	1·89	2·76	3·82	0·0	0·0	0·55	0·76	1·17	0·11	0·16	0·27	0·46	0·82	0·0	0·0	0·0	0·0	0·04
	III	60	1·51	2·02	2·57	3·36	5·13	0·0	0·48	0·67	0·79	1·36	0·13	0·26	0·32	0·46	0·92	0·0	0·0	0·0	0·0	0·10
	p*				<0·001					<0·001					<0·001					NS		
6 months	I	35	0·78	2·02	2·57	3·24	4·94	0·0	0·0	0·45	0·75	1·09	0·01	0·22	0·33	0·49	0·78	0·0	0·0	0·0	0·0	0·07
	II	79	1·17	1·81	2·86	3·79	5·86	0·0	0·0	0·40	0·76	0·95	0·04	0·26	0·39	0·54	0·86	0·0	0·0	0·0	0·0	0·10
	III	33	2·10	2·43	3·68	4·38	5·84	0·0	0·0	0·40	0·67	1·62	0·18	0·32	0·45	0·75	1·00	0·0	0·0	0·0	0·0	0·12
	p*				NS					NS					NS					NS		
Adults		50	2·98	4·60	5·63	7·49	9·86	1·32	2·20	2·73	3·81	5·61	0·31	0·54	0·68	0·83	1·25	0·0	0·25	0·40	0·75	1·02

*Significance of difference among the three groups using the Kruskal-Wallis one way analysis of variance; NS not significant.

Table 3 Percentiles of serum concentrations of C3, C4, and fibronectin in three groups of infants from birth until 6 months of age

Postnatal age	Group	n=	C3 percentiles					C4 percentiles					Fibronectin percentiles				
			5	25	50	75	95	5	25	50	75	95	5	25	50	75	95
Birth	I	68	0·13	0·26	0·37	0·64	0·77	0·04	0·09	0·11	0·15	0·24	0·08	0·09	0·11	0·14	0·19
	II	131	0·28	0·37	0·47	0·58	0·81	0·08	0·12	0·13	0·17	0·24	0·08	0·10	0·13	0·17	0·21
	III	260	0·35	0·45	0·53	0·62	0·74	0·10	0·13	0·15	0·18	0·24	0·09	0·12	0·15	0·18	0·23
	p*				<0·001					<0·001					<0·05		
1 month	I	37	0·30	0·37	0·53	0·69	0·85	0·10	0·12	0·17	0·23	0·32	0·11	0·16	0·21	0·24	0·34
	II	68	0·32	0·44	0·50	0·62	0·81	0·11	0·13	0·16	0·22	0·34	0·11	0·18	0·23	0·28	0·37
	III	76	0·41	0·54	0·64	0·72	0·90	0·12	0·19	0·22	0·27	0·36	0·13	0·18	0·22	0·29	0·41
	p*				<0·001					<0·001					NS		
3 months	I	35	0·35	0·49	0·64	0·73	0·95	0·11	0·13	0·16	0·25	0·31	0·14	0·22	0·25	0·33	0·36
	II	61	0·48	0·59	0·70	0·77	0·92	0·11	0·16	0·22	0·28	0·37	0·17	0·24	0·28	0·33	0·39
	III	60	0·55	0·69	0·76	0·87	1·02	0·16	0·22	0·26	0·32	0·47	0·18	0·22	0·30	0·35	0·45
	p*				<0·001					<0·01					NS		
6 months	I	19	0·75	0·80	0·85	0·97	1·12	0·18	0·21	0·23	0·27	0·32	0·20	0·21	0·32	0·35	0·40
	II	38	0·62	0·71	0·82	0·94	1·05	0·16	0·23	0·31	0·39	0·47	0·24	0·29	0·31	0·35	0·40
	III	33	0·64	0·76	0·87	1·01	1·24	0·18	0·23	0·31	0·40	0·55	0·23	0·28	0·31	0·37	0·44
	p*				NS					NS					NS		
Adults		230	0·49	0·64	0·72	0·81	1·03	0·15	0·22	0·26	0·32	0·41	0·08	0·26	0·34	0·43	0·63

*Significance of difference among the three groups using the Kruskal-Wallis one way analysis of variance; NS not significant.

components increased, reaching adult values by the third or sixth month of age. A wide variation of values within each gestational age group was evident especially among neonates with a gestational age of less than 35 weeks (table 3).

Fibronectin concentrations were less than half of the adult values at birth and then showed a rapid increase, approaching adult reference values by the sixth month (table 3).

The significance of the difference in opsonin concentrations among the various gestational age groups is shown in tables 1 to 3.

Statistical analysis of the results obtained from 54 paired neonatal and maternal sera showed that IgG2 and IgG3 titres in neonates were similar to those of their mothers. Significant differences were observed for total IgG, IgG1, IgG4, IgM, C3, C4 and fibronectin ($p<0·01$). The neonatal: maternal concentration ratio for IgG, IgG1, IgG2, IgG3, IgG4, C3, C4 and fibronectin was 1·22, 1·41, 0·97, 1·08, 0·86, 0·59, 0·49, and 0·52, respectively.

Discussion

Gestational age is expected to influence mainly the serum concentrations of those opsonins which are actively transferred across the placenta – namely, IgG and IgG subclasses. The impact of gestational age on the other non specific opsonins, such as C3, C4, and fibronectin, has not been adequately investigated.[13–16] In our study serial measurements of these opsonins showed that gestational age had a significant impact on concentrations of IgG, IgG subclasses, C3 and C4 until the third month. This impact was not extended further than the first month on fibronectin. On the other hand, gestational age had no significant impact on IgA and IgM concentrations either at birth or subsequently.

Findings regarding the transplacental transfer of IgG subclasses are controversial.[2 3 5 17 18] Hay *et al*[18] reported an impairment in transplacental transfer of IgG2 while more recent studies generally indicate that all IgG subclasses are freely transferred through the placenta.[2 3 5 17] Our data indicate that there is a preferential active transplacental transport of IgG subclasses in the order of IgG1, IgG3, IgG2, and IgG4. Furthermore, results from serial measurements of IgG subclasses suggest that IgG3 is produced earlier than IgG2 and IgG4. This supports the findings of Morell *et al*[17] and Oxelious *et al*,[9] who studied healthy infants ranging in age from 10 days to 4 months.

It is worth noting that IgM concentrations at birth were higher than 0·2 g/l in 16% of our neonates, a level so far considered to be the normal upper limit of normal for this age.[20] These neonates were neither small for gestational age nor had any evidence of intrauterine infection. Similar results were obtained by Conway *et al*[21] in about 35% of the studied neonates. These findings suggest that the critical level of serum IgM at birth for suspected intrauterine infection should be reconsidered.

So far published data do not provide reference values for C3 and C4 in early infancy. We found that C3 and C4 concentrations at birth were around half to two thirds those of adult reference values, depending on gestational age. Values increased with postnatal age, reaching adult values by the third or sixth month and not by the second year or later in childhood, as has been reported before.[13 14 22] As in earlier relevant studies[13 15] we also observed a wide range of individual values even within each gestational age group.

Data concerning fibronectin in early infancy are also limited. Two earlier papers report that fibronectin concentrations were determined in only small groups of infants ranging from 1 to 8 months of age.[10 16] In our study fibronectin concentrations at birth were around half the adult values, but serial measurements showed a rapidly rising pattern.

In general, reported values of IgG, IgG subclasses, C3, C4 and fibronectin vary widely.[3 4 9 10 13 14 23] A major factor for this could be the different individual methods of measurement used. We used rate nephelometry, which is a reliable and accurate method and produces results within a few hours. Data on measurements of serum opsonins using this method are very limited.[10] Valetta *et al*,[10] who also used nephelometry to measure fibronectin

in the cord blood of 76 term and preterm neonates, reported values comparable with those of our study. From both studies it is evident that fibronectin values determined by nephelometry are higher than those found by other methods, such as electroimmunoassay[9 11] and enzyme immunoassay.[16] On the contrary, concentrations of C3 and C4 measured by nephelometry in our study were lower than those previously reported.[13 14] We believe that rate nephelometry is an appropriate method for assessing complement components because it is not affected by the presence of breakdown products.

In conclusion, our results show that concentrations of immunoglobulins, C3, C4 and fibronectin undergo significant changes during the first months of life, depending not only on the infants' postnatal age but also on their gestational age. Therefore, gestational age reference values are required for clinical interpretation of the values of these opsonins in early infancy. We believe that our results circumscribe the evolving pattern of the main serum opsonins, especially that of the IgG subclasses, C3, C4 and fibronectin.

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