

Serum immunoglobulin G subclasses and serum immunoglobulin A in acute bronchiolitis in infants

Carlsen KH, Mellbye OJ, Fuglerud P, Johansen B, Solheim AB, Belsnes D, Danielsen A, Henrichson L. Serum immunoglobulin G subclasses and serum immunoglobulin A in acute bronchiolitis in infants.

Serum IgG subclasses and Serum IgA were studied in 43 infants with acute bronchiolitis and 20 healthy infants. IgG subclasses were determined by a capture ELISA and IgA was quantified by turbidimetry. IgG1 concentrations were significantly lower in infants with bronchiolitis than in normal infants. The other IgG subclasses and IgA did not differ between the groups. The subgroups of infants with bronchiolitis who had previously suffered from otitis media or bronchitis, had significantly lower IgG2 than the other infants with bronchiolitis. The same was found for infants with bronchiolitis who had suffered from three or more lower respiratory tract infections. In infants who had suffered from upper or lower respiratory infections before the acute bronchiolitis, IgA was significantly higher than in infants without previous respiratory infections. Ten infants with bronchiolitis (23%) had IgG1 deficiency, that is values below the lower reference limit calculated in a population of healthy Norwegian infants. No healthy infants had any IgG1 deficiency. No infant with bronchiolitis had IgG2 or IgG3 deficiency. The low IgG1 values found in infants with acute bronchiolitis, may be one cause for infants to be more susceptible to RS virus infections.

**K.-H. Carlsen¹, O. J. Mellbye²,
P. Fuglerud⁴, B. Johansen³,
A. B. Solheim⁵, D. Belsnes⁵,
A. Danielsen⁵, L. Henrichson⁵**

¹Voksntoppen Children's Institute for Asthma and Allergy, ²Institute of Immunology and Rheumatology, ³Department of Chest Medicine, Rikshospitalet, ⁴Nycomed A/S, Statistical R&D, ⁵Nycomed Pharma, Oslo, Norway

Kai-Håkon Carlsen, Voksntoppen Children's institute for Asthma and Allergy, Uliveien 14, N-0394 Oslo, Norway

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Acute bronchiolitis is a common severe, acute respiratory infection in infancy, frequently requiring admission to hospital, and is most often caused by respiratory syncytial (RS) virus (1). The predominant symptoms are those of bronchopulmonary obstruction (BPO), common to both acute bronchiolitis and bronchial asthma. We have previously reported abnormalities in total immunoglobulin levels in infants with acute bronchiolitis, and especially in infants with bronchiolitis later developing bronchial asthma (2,3). Low levels of serum immunoglobulin (Ig) G subclasses have been reported in several different clinical conditions, mostly among children, including recurrent respiratory infections, bronchial asthma and congenital cardiac disease (4–7). Recently, we reported reference values of IgG subclasses and IgA in Norwegian children from infancy to adolescence (8), measuring IgG subclass levels by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies to subclass-specific antigenic determi-

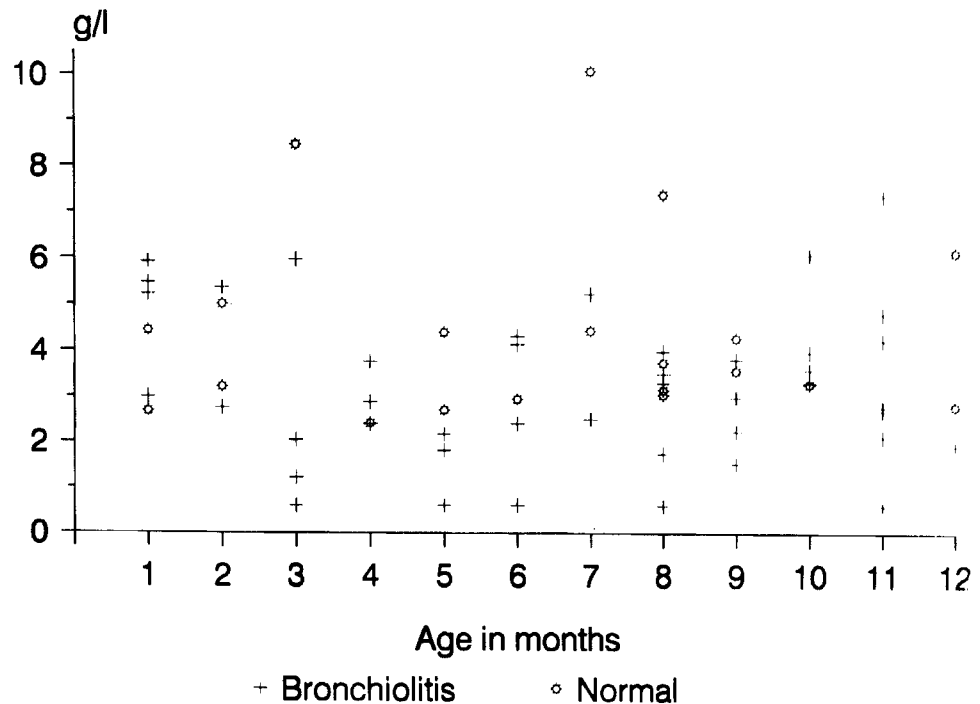
nants on the IgG molecule. IgA was quantified by a turbidimetric method. In the present study we report the levels of IgG subclasses and IgA in infants suffering from acute bronchiolitis, using the same assays for analysing the IgG subclasses and IgA.

Material and methods

Study population

Infants with acute bronchiolitis – Venous blood samples, drawn from 43 infants, suffering from acute bronchiolitis, were tested for serum IgG subclass concentrations and total IgA levels. The infants suffering from acute bronchiolitis were admitted to the Paediatric Department of Ullevål Hospital, Oslo, or seen by two paediatric pulmonologists in Oslo. The diagnosis of acute bronchiolitis was made using the criteria of Court (9): "... illness mainly affecting infants. Rapid respiration, dyspnea, wheezing, chest recessions, cough,

Fig. 1. IgG1 subclass concentrations in 43 infants with bronchiolitis and in 20 healthy infants according to age in months.



rhonchi and rales are very frequent. Fever is very frequent, but high fever uncommon."

The blood samples for determination of IgG subclasses and IgA were drawn from the infants with acute bronchiolitis during the acute phase of the illness. Median time from admission to hospital to sampling was one day (range 0–9 days, 95% CI 1–2 days) for 41 infants admitted to hospital. For two infants with bronchiolitis seen by two paediatric pulmonologists, sampling was done on the day of consultation with acute symptoms.

Four of the infants with bronchiolitis were prematurely born, two at 27 weeks', one at 31 weeks' and one at 36 weeks' gestation. The other infants with bronchiolitis as well as the control infants were born at term. The infants with acute bronchiolitis included in the study were allowed to have had no more than one episode of acute bronchopulmonary obstruction prior to the acute bronchiolitis. Ten infants had one episode, whereas the remaining infants with acute bronchiolitis had no previous episodes of bronchopulmonary obstruction.

Nasopharyngeal secretions were examined by immunofluorescence for respiratory virus antigens in the same manner as in previous studies (2, 3) in 37 infants. Respiratory syncytial (RS) virus infection was diagnosed in 21 infants (56.8%), and parainfluenza virus infection was found in three patients (8.1%). No virus antigens were detected in 13 patients (35.1%).

The infants admitted to hospital with acute bronchiolitis had a median body temperature at

admission of 38.3°C (range 36.8–40.5°C, 95% CI 37.8–38.9°C), and a median respiratory rate of 60 per min (range 40–90 per min; 95% CI 60–70 per min). Their obstructive score was 4 (70%) – 5 (30%), according to the Aas score, with a minimum score of 0; maximum score of 6 (10). Respiratory chest recessions were noted in all but one infant at the time of admission.

Blood gas analyses at the time of admission (27 infants) showed a median pH of 7.36 (range 7.25–7.43), a median Base Excess of –1 mmol/l (range –6.1 mmol/l – +6.8 mmol/l), a median pCO₂ of 5.7 kPa (range 4 kPa–9.5 kPa), and a median O₂-saturation (SaO₂) of 83.5% (range 72% – 96%). Of the 43 infants with bronchiolitis, 34 required supplementary oxygen on admission to hospital, and seven infants received intravenous fluids.

Control group – A group consisting of 20 healthy infants, recruited from two health care centers in Oslo served as control group. The control children were not allowed to have suffered from any lower respiratory tract infections or any episodes of bronchopulmonary obstruction. They did not have a history of recurrent upper respiratory tract infections or atopy. They had no signs of respiratory illness at the time of drawing blood samples.

Quantification of IgG subclasses – IgG subclasses were determined by a capture antibody ELISA using the method described by Bird and coworkers (11), and with modifications as described previ-

Table 1. IgG subclass and IgA concentrations (g/l) in infants with acute bronchiolitis (n = 43) and in normal infants (n = 20). Values are given as medians with ranges in parentheses and 95% confidence intervals on the lower line. For statistical calculation, concentrations below the limits of detection were assigned the same rank. The IgG1 concentrations in the infants with bronchiolitis are significantly lower than for the healthy infants ($p = 0.016$).

	Acute bronchiolitis (n = 43)	Normal infants (n = 20)	p-values
IgG1 (g/l)	2.9 (<1.20–7.4)	3.7 (2.4–10.1)	0.016
95% CI	2.3–3.8	3.1–4.4	
IgG2 (g/l)	0.50 (0.31–2.1)	0.65 (0.26–1.8)	n.s.
95% CI	0.42–0.61	0.50–0.83	
IgG3 (g/l)	0.25 (0.10–1.4)	0.31 (0.09–0.67)	n.s.
95% CI	0.20–0.34	0.24–0.49	
IgA (g/l)	0.27 (<0.1–1.3)	0.19 (<0.1–0.66)	n.s.
95% CI	0.17–0.42	0.12–0.41	

ously (8). Briefly, microtitre plates (Nunc, Roskilde, Denmark) were coated with 100 µl/well of the following murine monoclonal antibodies: anti-IgG1 (clone 2B6 = HP6069, O. Mäkelä, Helsinki, Finland, and Zymed Laboratories Inc., San Francisco, USA); anti-IgG2 (clone AC3-AA11 = HP6014, Oxoid, Basingstoke, UK); anti-IgG3 (clone CC4-DC10 = HP6050, Oxoid, Basingstoke, UK); and anti-IgG4 (clone CB2CC5 = HP6025, ICN, High Wycombe, UK). After incubation with test and standard samples, the wells were incubated with a peroxidase-conjugated rabbit antibody specific for human IgG (Nycomed, Oslo, Norway). Enzyme activity was detected with 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) (Boehringer, Mannheim, Germany) as substrate. Dilutions of Nycomed's reference serum which had been calibrated against the WHO reference serum 67/97, were used as standards. The lower limit of reading was 1.2 g/l for IgG1, 0.25 g/l for IgG2, 0.04 g/l for IgG3 and 0.04 g/l for IgG4.

Quantification of total IgA – IgA was quantified by turbidimetry, using a Behring Turbitimer turbidimeter, and Behring Turbiquant anti-IgA antiserum and control serum (Behringwerke AG, Marburg, Germany). The lower reading limit for IgA was 0.1 g/l.

Statistical methods – The results are given as medians, ranges and non-parametric 95% confidence intervals (95% CI) based upon medians, calculated by the Bernoulli-Wilcoxon method (12). Differences between two groups were tested by the two-sample Wilcoxon midrank sum test, and when number of groups compared exceeded two, the Kruskal-Wallis test was employed (12). Differences in proportions between groups were tested

by Fisher's exact test. P-values below 5% were considered to be statistically significant (twosided tests). IgG subclass and IgA values below the lower reference limit (8) were considered as IgG subclass or IgA deficiency. Values below the lower reading limits of the analysis were given the same rank when tested.

Ethical considerations – The study was approved by the regional medical ethics committee.

Results

IgG subclass concentrations and IgA concentrations in infants with acute bronchiolitis compared to normal infants

The 43 infants with acute bronchiolitis and the 20 healthy infants did not differ significantly in age distribution according to month. Fig. 1 shows the single values for IgG1 in the infants with acute bronchiolitis and the healthy infants according to age in months. As seen from Table 1, IgG1 concentrations were significantly lower in infants with acute bronchiolitis than in normal infants ($p = 0.016$). The range of IgG1 values in infants with acute bronchiolitis varied from <1.2 to 7.4 g/l, and in healthy infants from 2.4 to 10.1 g/l (Table 1, Fig. 1). The other subclasses did not differ significantly between the infants with acute bronchiolitis and the healthy infants.

Of the 43 infants with acute bronchiolitis, 39 infants had IgG4 levels below the lower reading limit, whereas 14 of the 20 healthy infants had IgG4 levels below the lower reading limit of 0.04 g/l (Fisher's exact test: n.s.). IgG4 levels are therefore not included, and IgG4 levels below 0.04 g/l do not allow any conclusion as to IgG4 deficiency in this age group.

No significant differences were found in IgG subclass levels or in IgA levels related to the detection of RS virus or parainfluenza virus infection.

IgG subclasses and IgA in infants with bronchiolitis relationship to sex and previous respiratory infections

No significant differences in IgG subclasses and IgA values were found between boys and girls with bronchiolitis.

Significantly lower IgG2 values were found among the infants with bronchiolitis who had suffered from previous otitis media ($n = 8$, median = 0.38 g/l; 95% CI: 0.36–0.53 g/l) than among the infants with bronchiolitis without previous otitis media ($n = 35$, median = 0.55 g/l, 95% CI: 0.44–0.85) ($p = 0.03$). The same was found among those who had suffered from bronchitis ($n = 21$, median = 0.44 g/l, 95% CI: 0.38–0.51) compared to those without ($n = 22$, median = 0.75 g/l, 95% CI: 0.43–

1.00) ($p = 0.01$). This was not found for IgG1, IgG3 or IgA. Infants with bronchiolitis without previous upper respiratory tract infections at all had lower IgA ($n = 16$, median = 0.14 g/l, 95% CI: 0.05–0.19) than those with one to three upper respiratory tract infections ($n = 18$, median = 0.31 g/l, 95% CI: 0.20–0.46), and those with four or more upper respiratory tract infections ($n = 9$, median = 0.53 g/l, 95% CI: 0.39–0.89) ($p \leq 0.01$). No significant difference was found for IgG subclasses in this respect.

IgG subclass and IgA deficiency

IgG subclass deficiency was found more often in infants with acute bronchiolitis than in healthy infants; that is IgG subclass levels below the lower reference levels as calculated in a population of healthy Norwegian infants (8). Ten infants (23%) with bronchiolitis had IgG1 concentrations below the lower reference limit, in contrast to none of the healthy infants ($p = 0.015$, Fisher's exact test). One healthy infant had IgG3 level below the lower reference limit, but this was not found in any of the infants with bronchiolitis. No infants with or without bronchiolitis had IgG2 levels below the lower reference limit.

IgA concentrations did not differ significantly between the infants with acute bronchiolitis and the normal infants (Table 1). The number of infants with IgA deficiency was not calculated as the calculated lower reference limit was below the reading limit of the IgA analysis for this age group (8). Nine infants with bronchiolitis had IgA levels below the reading limit whereas four of the normal infants had IgA levels below the reading limit of 0.1 g/l (ns).

Discussion

Several authors have reported the occurrence of low levels of IgG subclasses in lower respiratory tract disease, such as bronchial asthma (4, 13, 14) and recurrent sinopulmonary infections (15). To our knowledge, however, nobody has reported such findings in infants with acute bronchiolitis.

In the present study the infants with acute bronchiolitis were tested during the acute phase of their illness, with blood samples drawn the first day after admission. A respiratory virus infection was diagnosed in 65% of the infants with bronchiolitis, but at this early stage of the illness, the virus infection most probably had not affected their immunoglobulin levels. As seen from their SaO₂, their obstructive score and body temperature, they were acutely ill. The infants with acute bronchiolitis were matched with a control group of healthy in-

fants. It would have been preferable to match each control subject to each patient with respect to age and sex. However, this was not possible to accomplish due to limited access to blood samples from healthy infants. The age distribution did not differ between the index subjects and the control subjects. This is most important, as immunoglobulin levels change according to age during the first year of life. Therefore, we find it appropriate to compare IgG subclasses and IgA between the patient and the control group in the present study.

Acute bronchiolitis occurs most frequently at six months of age (2), at the time when the protection by maternal antibodies is very low. The low levels of IgG1 found in the present study in infants with bronchiolitis, may possibly render the infants more susceptible to the RS virus infection (16, 17), which was diagnosed in more than half of the index subjects of the present study. As the samples were taken during the early phase of the acute bronchiolitis, it seems improbable that the low levels of IgG1 are due to the virus infection at this early phase. Since the main part of the IgG consists of the IgG1 subclass, low levels of this subclass may render the infants more prone to respiratory infections. This is also indicated by previous studies (2) which demonstrated that infants contracting acute bronchiolitis had more middle and lower respiratory tract infections than control infants, and that they contracted their first respiratory tract infection at an earlier age.

Antibodies against viruses are usually of the IgG1 and IgG3 subclass isotypes (18). IgG1 is the predominant isotype produced following RS virus infections (16, 17). The low levels of IgG1 subclass during the acute phase of bronchiolitis may indicate that these infants had low levels of RS virus-specific IgG1 subclass antibodies at the time they contracted acute bronchiolitis. Antibodies of the IgG3 subclass isotype are reported to protect against other types of virus infections (hepatitis B and herpes viruses), but not against RS virus infections (18).

Although the IgG4 subclass only constitutes two to six percent of the total IgG protein, specific IgG4 subclass antibodies have also been described to increase after certain virus infections (18). However, as many infants with acute bronchiolitis and normal infants in the present study had IgG4 concentrations below the lower reading limit, no conclusions can be drawn as regards levels of total IgG4 protein due to limitations of the assay sensitivity. Lee et al. demonstrated that IgG4 subclass synthesis in infants and small children increases more slowly towards adult levels during childhood than the other IgG subclasses. This explains the difficulties in confirming low levels of IgG4 in in-

fancy in the present study as well as in other studies (18,19).

IgG2 subclass antibodies are thought to protect against microbial agents with polysaccharide antigens, including *Streptococcus group A*, *Streptococcus pneumoniae* and *Haemophilus influenzae type b* antigens (18,20). Since these microbial agents are not associated with bronchiolitis, as expected, no significant differences in IgG2 subclass levels between infants with acute bronchiolitis and normal infants were found.

However, when looking into differences within the group of infants with acute bronchiolitis, we found that infants who had suffered from otitis media or bronchitis prior to the acute bronchiolitis had lower IgG2 concentrations than those without. The same was found for those with one to three or more previous upper respiratory tract infections compared with those without any upper respiratory tract infections. This indicates that low levels of the IgG2 subclass isotype render infants and children more susceptible to bacterial infections. This is in agreement with Smith et al. (13,14) who described low concentrations of IgG2 in children with recurrent chest symptoms such as bronchitis and recurrent infections, and Umetsu et al. (15) who found low IgG2 concentrations in 15 children with recurrent sinopulmonary infections. Our findings of lower IgG2 antibodies in infants with bronchiolitis and previous otitis media or bronchitis are in accordance with the results of de Baets et al., who recently described lower levels of specific IgG1 and IgG2 antibodies against pneumococcal type 3 polysaccharide antigens in children with recurrent bronchitis compared to healthy children (21). In normal children the levels of specific IgG2 antibodies were higher than the specific IgG1 antibodies, whereas children with recurrent bronchitis had higher levels of specific IgG1 antibodies to pneumococcal type 3 polysaccharide antigens than specific IgG2 antibodies. De Baets et al. interpreted this as signs of a defective humoral immune response against polysaccharide antigens, being an important cause of recurrent bronchitis in childhood.

IgA concentrations did not differ between the infants with acute bronchiolitis and the control infants, but among the infants with acute bronchiolitis, IgA levels were higher in infants with previous respiratory infections, probably as a result of immune stimulation. This is the opposite of what was found for IgG2 subclass concentrations.

In conclusion, the concentration of IgG1 subclass antibodies is diminished in infants contracting acute bronchiolitis. This is probably associated with a lower immune defence against the RS virus infection. IgG2 concentrations are lower in infants

with bronchiolitis who had otitis media or bronchitis prior to the bronchiolitis, possibly connected with a lower specific humoral immune response to bacterial polysaccharide antigens. The low levels of IgG subclasses may be due to a diminished response to the antigens in question (lack of recognition?) or on the other hand to a reduced ability to produce specific IgG subclass protein. As bronchiolitis often is followed by recurrent bronchopulmonary obstructive episodes and asthma, further studies are required to determine whether low IgG1 subclass levels may give rise to recurrent obstructive episodes after the acute bronchiolitis, or whether IgG subclass deficiencies may occur due to the effect of different lymphokines.

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