Calculation of Lung Volume in Newborn Infants by Means of a Computer-assisted Nitrogen Washout Method

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ABSTRACT. A clinically adapted method for the calculation of the functional residual capacity in newborn infants has been developed. The method is based on a multiple breath nitrogen washout test, during which the ventilatory air flow and the nitrogen concentration signals are sampled by a minicomputer, which also performs the calculations. The ventilatory air flow is measured by a pneumotachometer connected to a face-out volume displacement body plethysmograph, and the nitrogen concentration by a nitrogen analyzer. The functional residual capacity volume is calculated from the sampled signals by adding the expired nitrogen volumes during each expiration, and finally dividing this sum by the initial alveolar nitrogen concentration. Before the calculations, the sampled signals are adjusted regarding nitrogen analyzer delay and plethysmograph characteristics. The method presented is designed to minimize the test equipment influence on the baby's respiration and also to inhibit the necessity of pneumotachometer compensations normally connected with washout methods. Furthermore, the calculated breath-by-breath values of end-expiratory nitrogen concentration, nitrogen volume, inspired and expired tidal volume, are stored on disk for further analysis and resimulation of the test. The method has been tested on a mechanical lung model and on washouts from healthy newborn infants. The model tests indicate that the accuracy and the reproducibility of the method are good, and the results from the infants are in good agreement with previously obtained results. (*Pediatr Res* 18:1160-1164 1984)

Abbreviations

FRC, functional residual capacity
TGV, thoracic gas volume

Following birth, the fetal lung fluid is gradually replaced by air, and an adequate lung function is soon established. Normally, this is an extremely efficient process. Disturbances in this process, e.g. the inability to develop an appropriate lung volume, lead to neonatal respiratory disease. The extent to which an adequate lung volume has been established can be determined by meas-

uring FRC. Despite its central importance, measurements of FRC are seldom used clinically in neonatal medicine, because of the lack of suitable methods.

Therefore, in order to facilitate such measurements, we have developed a clinically adapted computer-assisted plethysmographic nitrogen washout method for the calculation of FRC in newborn infants, which reduces the problems connected with ordinary washout methods (3, 8, 15). It also enables further analysis of the collected washout data, for instance nitrogen elimination pattern ("distribution of ventilation") analysis. The method has been tested on a group of healthy newborn infants and on a mechanical lung model. TGV, calculated according to DuBois *et al.* (5), was used as reference in the studied infants. The reproducibility of the measurements was tested in infants and on a mechanical lung model. This lung model was also used to test the accuracy of the FRC calculation method.

METHOD AND THEORY

The system is illustrated in Figures 1 and 2. It consists of a face-out volume displacement body plethysmograph (Fig. 1), a minicomputer, and equipment for measurement of ventilatory air flow and breathing gas nitrogen concentration (Fig. 2). The plethysmograph is constructed from transparent plexiglas and has previously been described in detail (10). The ventilatory air flow is measured by a pneumotachometer, Fleish No. 1, connected to a small aperture in the plethysmograph wall, and a differential pressure transducer (Elema-Schönander EMT 32). The breathing gas nitrogen concentration is measured by a Hewlett-Packard 47302A nitrogen analyzer having a rise time of 30 ms and a delay time to the 10% point of less than 50 ms. The analyzer probe and a Rendell-Baker face mask are connected to a T-tube, through which a constant gas flow is maintained. The face mask is partly filled with grease (Macrogol) in order to diminish the dead space and to make the system air-tight. The dead space of the whole system is approximately 2 ml. A polygraph (Mingograph, Siemens-Elema) is used for monitoring the ventilatory flow and nitrogen concentration signals.

An examination is performed with the baby lying in the plethysmograph, and the face mask gently placed over the resting or sleeping baby's nose and mouth. During an expiration, the gas flow in the mask is changed to 100% oxygen and a nitrogen washout test is performed. When the nitrogen concentration in the expired air stays below 2%, the gas flow is returned to the original breathing air composition and the test is finished. The ventilatory air flow and the nitrogen concentration signals (Fig. 3) are sampled by the computer throughout the test and stored on disk for the calculations which follow.

The sampling frequency is 250 Hz, corresponding to a sample interval of 4 ms, and the signals are filtered by a fourth order

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Fig. 1. The plethysmograph, where *a* indicates the position of the baby's face, and *b* is the pneumotachometer connection.

Fig. 2. The test arrangement, where *a* is the pneumotachometer and *b* is the face-mask.

Fig. 3. A representation of the air flow and the nitrogen concentration during a washout test.

Butterworth low-pass filter with a cut-off frequency of 60 Hz before the sampling. The minicomputer is a PDP 11/40 and the software is written in FORTRAN. During the computer calculations, the instantaneous product of the sampled flow and nitrogen concentration is digitally integrated during each expiration and inspiration in order to obtain the volumes of expired and inspired nitrogen (Fig. 4). The nitrogen volumes expired during the washout are added until the breath after which the end-expiratory nitrogen concentration stays below 2%, and the sum is finally divided by the end-expiratory nitrogen concentration prior to the oxygen breathing. This concentration approximates the initial alveolar concentration. The result is the FRC volume given in body temperature and pressure, saturated with water vapor. Besides the expired nitrogen volumes, the breath-by-breath end-tidal nitrogen concentrations, expired and inspired

Fig. 4. The nitrogen volume calculation principle.

tidal volumes, are calculated and stored on disk, where they are accessible for resimulation of tests and other programs.

Before multiplication, the two sampled signals are adjusted by the computer with respect to nitrogen analyzer delay and plethysmograph characteristics. The nitrogen analyzer delay is approximately 75 ms and was determined by introducing a flow of 100% oxygen into a tube, to which the gas analyzer probe and the pneumotachometer were attached. The delay was calculated from the resulting flow and concentration signals, both by computer analysis of the sampled signals and through visual inspection of an analog plot. The low-pass filtering influence of the plethysmograph, which introduces a delay in the flow signal and thereby reduces the total delay between the signals, is compensated for by using the derivative of the recorded air flow and the time constant of the plethysmograph, according to Ref. 10.

$$V_t' = V_{pn}' + RC V_{pn}'' \quad (1)$$

where V_t' is the flow initiated by the thorax movement, V_{pn}' is the pneumotachometer flow, RC is the time constant of the plethysmograph, and V_{pn}'' is the time derivative of V_{pn}' . By using an appropriate RC value in this compensation, the desired 75-ms total delay between the sampled signals is obtained. The RC value has previously been determined as 30 ms (10). The filtering compensation of the air flow is made continuously in the computer programs, before the multiplication with the correctly delayed concentration value is performed. The delay value is 76 ms and is chosen in order to correspond with the sample interval of 4 ms. No correction is made in the calculations for the corresponding time constant of the respiratory system, since it is smaller than, and well separated from, the plethysmograph constant and therefore assumed not to affect the results (10).

A flow and a nitrogen concentration calibration signal are generated on every test occasion. The flow signal is obtained from a calibrated rotameter, and the concentration signal originates from the nitrogen analyzer, sampling room air. The calibration signals are sampled by the computer and used for calibration in the calculation routines. No corrections are made in the calculations for the elimination of tissue-solved nitrogen. The method and the calculations, including the software, are described in detail elsewhere (19).

In order to evaluate the method during predetermined conditions, a mechanical lung model, according to the plan in Figure 5, has been constructed. This model consists of three Plexiglas disks, 0.5 mm thick, attached to each other, and a loudspeaker for generation of the ventilatory oscillations. The mixing chamber, *i.e.* the lung volume, consists of a 160-mm wide hole in the middle disk, which is limited by a membrane attached above a corresponding hole in the bottom disk and a top disk with a gas outlet-inlet. The membrane, made of latex rubber, performs the ventilatory oscillations by using mechanical transmission from the loudspeaker. The total lung model volume is 107 ml. The lung model can be ventilated with varying tidal volumes and frequencies by using a signal generator connected to the loudspeaker.

The mechanical model has been used to test the reproducibility and the accuracy of the calculated volumes. In these tests, the complete construction was fitted into the plethysmograph using the same test arrangement as in Figure 2, and thereby simulating the clinical measuring situation (Fig. 6). Ten washout tests were

Fig. 5. A cross-section of lung model, where *a* is the loudspeaker, *b* is the mechanical transmission, *c* is the mixing chamber, *d* are air-channels, *e* is the latex membrane, and *f* is the gas inlet-outlet.

Fig. 6. The lung model fitted into the plethysmograph, where *a* is the plethysmograph, *b* is the pneumotachometer connection in the plethysmograph wall, *c* is the lung model, and *d* is the gas inlet-outlet of the mixing chamber.

performed. The frequency and the tidal volume were held at approximately constant values, the frequency was 50 breaths/min, and the mean tidal volume was 18.6 (SD = 0.3) ml. The theoretical total lung volume at midexpiration was 107.3 ml, and the theoretical FRC value was thus 98.0 ml, *i.e.* the total volume minus half the tidal volume. The FRC volumes were calculated by using all the expired nitrogen volumes until the concentration was well below 1%, typically 0.5 to 0.7%, and appropriate delay compensation.

The reproducibility was further tested in a group of nine infants by repeating the test twice with an interval ranging from 15 to 35 min between the measurements. The accuracy of the method was also evaluated in newborn infants by comparing the calculated FRC volumes with simultaneously measured TGV. The TGV measurement was performed with the baby still resting in the plethysmograph. The cheek and submandibular tissues were supported by a stiff cuff (10). A rigid face mask connected by a rigid tube to a pressure transducer (Elema-Schönander, EMT 34) was placed over the infant's nose and mouth. At the end of an expiration, the port of the mask was closed manually. The mouth pressure and body volume variations were recorded on the polygraph during three to five consecutive breaths. At least three successful determinations with pressure and volume signals in phase during airway closure were made in each infant. The mean TGV was then calculated according to DuBois *et al.*

After parental consent, 40 healthy newborn infants were examined on 64 occasions, at ages between 1.5 and 144 h. They were delivered vaginally or by cesarian section. Sixteen of these infants had recovered from respiratory disease. The median birth

weight was 3.36 kg (range, 1.85–4.30 kg) and the median gestational age was 39 wk (range, 33–42 wk).

RESULTS

The reproducibility and the accuracy of the method were tested by using the mechanical lung model and resulted in a mean value of the calculated FRC of 96.2 ml, a standard deviation of 2.8 ml, and a coefficient of variation of 2.9%. This corresponds to a 95% confidence interval from 94.2 to 98.2 ml. The theoretical FRC was 98.0 ml.

The reproducibility was also tested in nine newborn infants. The results are presented in Figure 7. The correlation coefficient was $r = 0.92$ ($p < 0.001$) and the mean difference between the examinations was 3.6 ml, with a 95% confidence interval from -2.2 to 9.4 ml.

The calculated mean FRC in the group of 40 infants, 64 measurements, was 67.2 (SD = 18.1) ml, and the corresponding TGV was 84.9 (SD = 19.6) ml. The mean difference between TGV and FRC was thus 17.7 (SD = 16.7) ml. A paired *t* test showed that the difference between FRC and TGV was statistically significant ($p < 0.001$). A comparison of the FRC and TGV values can be seen in Figure 8. The correlation coefficient was $r = 0.69$ ($p < 0.001$).

DISCUSSION

Several problems are involved when gas technique methods are designed for lung volume measurements in neonates. The amount of gas to be measured is often very small, and even a minor leak will consequently cause a great error. It is also important to keep the "dead space" of the measuring system as small as possible in order to minimize the influence on the respiration. The general problems of pneumotachometer variation and compensation according to temperature and gas composition (21) also have to be taken into account, as well as the impossibility of getting any cooperation from the baby during the test. In the method presented in this paper, these problems have been carefully considered and the method has a number of advantages, although the FRC calculation principle (Fig. 4), based on the sampled signals, is similar to previously described methods (3, 15, 17, 18).

The use of a plethysmograph for ventilatory air flow measurement implies that the flow through the pneumotachometer con-

Fig. 7. Relationship between two consecutive calculations of FRC in nine newborn infants ($FRC(2) = 9.5 + 0.9 \times FRC(1)$).

Fig. 8. Relationship between calculated FRC and TGV in 64 simultaneous measurements ($FRC = 13.3 + 0.6 \times TGV$).

sists of gas with approximately constant temperature and composition, and therefore no temperature or viscosity compensation to the recorded air flow signal is necessary. These variations may be as large as 10 to 15% during a test if the pneumotachometer is placed in the breathing air gas stream (21). The use of the plethysmograph also implies that the calculated volumes are automatically given in body temperature and pressure (saturated) and that TGV and basal ventilation can be measured during the same session. Furthermore, the plethysmograph and the face-mask modifications have reduced the equipment dead space, and thereby reduced its influence on the ventilatory pattern. By using 100% oxygen for the test, air contamination due to leaks and inappropriate washout starts are easily detected from the nitrogen concentration signal. When applying the method, however, it is necessary to consider the time constant of the plethysmograph. The time constant is approximately 30 ms and introduces phase distortion in the flow signal in the actual frequency domain, *i.e.* around 1 Hz. This distortion is compensated for by using the previously presented Equation 1. By using the initial end-expiratory nitrogen concentration in the FRC calculations, the method becomes independent of oxygen therapy given to the test subject, whereas the calculations are always based on actual breathing air concentration. Finally, the use of a computer, data reduction, and storing of the resulting breath-by-breath end-expiratory nitrogen concentrations, expired nitrogen volumes, expired and inspired tidal volumes, make it possible to analyze further and recapitulate the washout test. It is also worth noticing that if, for a certain project, the advantages of the plethysmograph and the reduced dead space can be neglected, the flow signal for the calculations can as well be measured by a pneumotachometer in the mask system.

No corrections have been made in the calculations for the elimination of tissue nitrogen that takes place during the 100% oxygen breathing. If studies of tissue nitrogen elimination during pure oxygen breathing in adults and animals (7, 12, 16) can be applied to newborn infants, the calculated FRC volume is overestimated by less than 1 ml/kg body weight. The overestimation of FRC is probably further reduced while the calculations are stopped at the 2% level, where all the initial nitrogen is not eliminated.

The tests on the mechanical lung model indicate that the reproducibility and the accuracy of the method are good. Ten consecutive measurements gave a 95% confidence interval which included the true FRC volume. As the washout tests were stopped

Table 1. FRC (N_2) in comparison with previous studies: FRC values are adjusted to 3.4 kg body weight

	Body weight (kg)	Age (h)	FRC (ml)
Nelson <i>et al.</i> (13)	1.3–4.1	16–1704	72.9
Strang <i>et al.</i> (20)	2.2–3.9	5–144	52.4
Prod'homme <i>et al.</i> (14)	2.3–4.3	24	83.0
Hanson <i>et al.</i> (8)	1.9–4.3	1.75–128	57.8
Present study	1.9–4.3	1.5–144	67.2

when the "end tidal nitrogen concentrations" were approximately 0.5% the remaining nitrogen in the "lung" would further reduce the difference between the estimated and the true mechanical "lung" volume.

The reproducibility test in infants indicates some of the problems involved in this kind of measurement. The results show a good correlation between the two measurements, but there is some individual variation. The two FRC measurements were performed with the baby at quiet sleep, as judged from the breathing pattern, and at times 15–35 min apart, in order to allow equilibration between alveolar gas and breathing air. However, it is known from adults that cyclical variations in FRC exist during normal breathing (11), and it has also been suggested that such variations can occur in infants as a consequence of different sleep stages (9). During the time between the measurements, it is thus possible that a change in lung volume may have occurred, resulting in the observed individual variation.

The TGV, measured according to DuBois *et al.*, was used as reference method. There was a significant correlation between FRC and TGV ($r = 0.69$, $p < 0.001$). However, as in previous studies (13), we found considerable differences between FRC and TGV. This phenomenon has often been attributed to the presence of trapped gas within the infant's lung. Whether this is true or whether this difference can be caused methodologically has recently been questioned (1). The justification for using pure oxygen as diluting gas during FRC measurements has also been questioned (6). However, since the method measures the nitrogen content of the lung at the onset of the test, the calculated FRC represents the correct lung volume, as long as no parts of the lung become completely isolated, *i.e.* their gas transport becomes completely inhibited, due to the oxygen breathing. In a recent report, it has been proposed that pure oxygen breathing for about 3 min has little or no effect on the FRC (4). A comparison with previously published FRC values determined by means of nitrogen methods are presented in Table 1. Our results are in the same range, and the discrepancies could be due to methodological differences and differences between the studied infants. Estimation of FRC using helium dilution has also given lung volumes in healthy full term infants in the same range as those obtained by nitrogen dilution (2, 4). Furthermore, Boon *et al.* (4) found no difference in FRC determined by helium dilution at various breathing air oxygen concentrations.

In summary, the design of the method and the use of a computer bring about accurate and fast results, and enable further analysis of the obtained data. The method has also been found easy to use and is therefore suitable for clinical application.

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Ontogeny of Protection of Neonatal Mice from Lethal Herpes Simplex Virus Infection by Human Leukocytes, Antiviral Antibody, and Recombinant α -Interferon

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ABSTRACT. We have studied the peripheral blood leukocytes from human infants in an assay involving the protection of neonatal mice from herpes simplex virus (HSV) infection by human antibody, interferon, and leukocytes. Recombinant DNA α -interferon (IFLrA), antibody, and Ficoll-Hypaque-purified mononuclear cells (MC) from human adults administered intraperitoneally protected neonatal mice from a lethal HSV challenge 1 day later (73.6% survival). MC obtained from human infants less than 130 days old in combination with IFLrA and antibody afforded no protection (15.2% survival; $p < 0.0005$ compared to survival with adults' MC). MC from infants over 130 days protected the neonatal mice [60% survival; not significantly different from survival using adult cells, but significantly ($p < 0.0005$) different than survival using MC from younger infants]. The ontogeny of MC protection parallels the clinical development of resistance of infants to HSV infection. (*Pediatr Res* 18:1164-1167, 1984)

Abbreviations

HSV, herpes simplex virus
IFLrA, recombinant DNA human α -interferon
MC, human mononuclear leukocytes
PFU, plaque-forming units
IP, intraperitoneal

Neonatal humans are unusually susceptible to severe and often fatal HSV infections (1). The immunologic defect that explains their susceptibility to this common virus in the first months of life with development of resistance in the second half of the first year of life remains to be elucidated (1, 2). As in humans, neonatal mice are extremely susceptible to fatal HSV infection (3). We have developed a murine model of neonatal HSV infection in which infant mice can be nearly completely protected from an otherwise lethal HSV infection by a combination of human antiviral antibody, human natural or recombinant DNA α -interferon, and human mononuclear leukocytes (4-6). None of these factors by itself was protective. While leukocytes from adult humans could confer this protection, those obtained from umbilical cord blood were unable to protect in combination with either antibody (7) or antibody and interferon (4). We have now

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