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Zero-stress state of intra- and extraparenchymal airways from human, pig, rabbit, and sheep lung

KAREN O. MCKAY,¹ BARRY R. WIGGS,¹ PETER D. PARÉ,¹ AND ROGER D. KAMM²

¹*University of British Columbia Pulmonary Research Laboratory, St Paul's Hospital, Vancouver, British Columbia, Canada V6Z 1Y6; and* ²*Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

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McKay, Karen O., Barry R. Wiggs, Peter D. Paré, and Roger D. Kamm. Zero-stress state of intra- and extraparenchymal airways from human, pig, rabbit, and sheep lung. *J Appl Physiol* 92: 1261–1266, 2002; 10.1152/jappphysiol.00131.2001.—Alterations in airway wall anatomic properties and the consequential effects on airway narrowing have been assessed by use of computational models. In these models, it is generally assumed that at zero transmural pressure the airway wall exists in a zero-stress state. Many studies have shown that this is often not the case, as evidenced by a nonzero opening angle. In this study, we measured the opening angle of airway rings at zero transmural pressure to test this assumption. The airway tree was dissected from human, pig, sheep, and rabbit lungs. Airways were excised from the tree, and the opening angle was measured. There were obvious species and regional differences in opening angle. Rabbit airways from both extraparenchymal and intraparenchymal sites exhibited marked opening angles (7–82°). Extraparenchymal airways from sheep had large opening angles (up to 50°), but ovine intraparenchymal airways had small opening angles. Measurable opening angles were rarely observed in human and porcine airways of any size. The assumption of a stable zero-stress state at zero transmural pressure is therefore valid for human and porcine, but not rabbit and sheep, airways.

computational modeling; zero transmural pressure; airway wall mechanics; species differences; trachea; bronchus

AIRWAY LUMINAL NARROWING CAUSED by smooth muscle contraction is a normal response that is exaggerated in conditions such as chronic obstructive pulmonary disease (COPD) and asthma. Although many theories have been advanced to explain this phenomenon, there is still considerable debate as to the basic mechanism responsible for the exaggerated airway narrowing. Computerized models of the airway wall have been developed in an attempt to investigate this mechanism. In the model of Moreno et al. (15), thickening of the airway submucosa was shown to have a profound effect on the degree of airway luminal narrowing resulting from smooth muscle contraction. This model was enhanced by incorporating the morphometric di-

mensions of airways from patients with asthma and COPD, as well as from subjects free from respiratory disease (22). This newer model showed that differences in pattern of bronchoconstrictor responsiveness of patients with asthma and COPD and subjects without pulmonary impairment could be explained, in part, by differences in the airway submucosal thickness. These models have used only anatomic considerations to account for the reduction in luminal area after smooth muscle contraction and have neglected the mechanical properties and states of the various types of tissue comprising the airway wall.

Computational modeling has been used to predict the effects of changes in the mechanical properties of airway wall materials, as well as alterations in the anatomy of these structures, on airway wall mechanics (23). In the present model, the zero-stress state of the airway (all internal stresses equal to zero) is assumed to occur when the transmural pressure is zero. It is now well-established that this is not typically the case for arteries (13), veins (24), nor the gastrointestinal tract (5), so there is reason to suspect that airways may also have nonzero internal stresses at zero transmural pressure. The zero-stress state of the tissue influences the stability of the airway wall as it is exposed to compressive and tensile forces during smooth muscle contraction and relaxation and is, therefore, a parameter required for further refinement of computational models of airway narrowing.

If an airway ring is in the zero-stress state at zero transmural pressure, then there will be no alteration to the geometry of the airway when the cut is made to the airway wall. Conversely, if either the ring opens up or the ends overlap, the stresses in the airway wall must have been nonzero before the cut was made (1, 2, 7, 16). Gunst and Lai-Fook (6) have suggested that, in canine trachea, a negative cartilage force tending to spring outward creates a load that opposes the tendency of the muscle to shorten. This implies, as suggested by James et al. (9) for pig trachea, that if the trachealis muscle was cut, some opening angle would exist, and therefore these trachea are not in a zero-

Address for reprint requests and other correspondence: K. O. McKay, Dept. of Respiratory Medicine, The Children's Hospital at Westmead, Locked Bag 4001, Westmead NSW 2145, Australia (E-mail: karenm8@chw.edu.au).

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stress state at a transmural pressure of zero. Okazawa et al. (16) have also commented on the potential preload provided by tracheal cartilage as a result of their observations of a marked opening angle after transection of the trachealis muscle in canine tracheal rings.

To date, no systematic investigation of the zero-stress state of both extraparenchymal and intraparenchymal airways has been reported in any species. Because of the importance of the zero-stress state in the complete understanding of the forces that oppose smooth muscle contraction, and to allow us to verify or refute our model assumption of a zero-stress state at a transmural pressure of zero, we have systematically measured the opening angle of airways from a wide range of thoracic locations from a number of species.

METHODS

Human lungs were obtained from five adult donors (see Table 1) at the time of organ retrieval for transplantation. The lungs were in good condition but were not used for implantation into recipients because of bacterial colonization, the age of the donor, or logistical reasons. In each case, permission for the use of the lungs for research was obtained from the next-of-kin by the transplant coordinators. Lungs and trachea were also removed en bloc from four pigs (~25 kg weight), four New Zealand White rabbits (~3.5 kg weight), and one sheep (~30 kg weight) after anesthesia and death. All elements of the study had received appropriate ethical approval.

Extraparenchymal and intraparenchymal airways of the right lung were isolated by dissection with fine iris scissors as soon as the lungs were excised. All preparations and measurements were completed within 9 h of excision. Any extraneous connective tissue was gently removed and the airway tree arranged on an acetate sheet and covered with saline-soaked gauze. By use of this dissection technique, up to third-generation airways were isolated from the rabbit lung, up to sixth-generation airways from the human and sheep lung, and up to seventh-generation airways in pig lung (the main bronchus being the first generation according to Weibel, Ref. 21). Airways of different luminal caliber were marked and numbered (Fig. 1) and the airway tree photographed as a record of airway location. Rings (3 mm long) were cut at each marked point by making parallel cuts across the airway with a scalpel blade. Each ring was then suspended in a petri dish filled with saline, arranged so that the lumen was visible from above and photographed. A scale was included in each photograph to allow measurement of the luminal diameter of each airway from the photographic prints. A radial cut was then made through airway wall without removing the ring from the petri dish. After 10 min, the rings were rephotographed from above with no change in camera position. All tracheal rings were cut through the

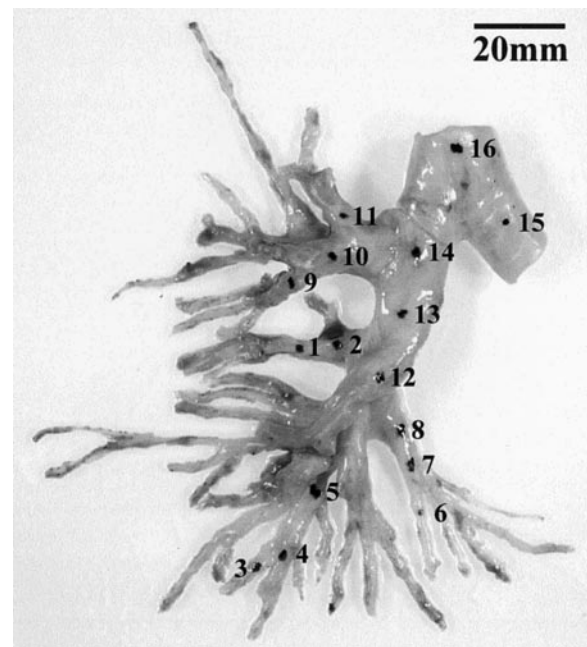


Fig. 1. Airway tree of the right lung from a 45-yr-old female organ donor showing points at which transverse rings were cut to assess the opening angle and zero-stress state of the airway.

trachealis muscle and all bronchial rings through the wall opposite the marked point.

Photographic prints of intact and radially cut rings were compared. The internal diameter of each airway ring was measured from the print of the intact ring. The opening angle and zero-stress state of each airway ring were then calculated from the print of the cut ring by using the method of Han and Fung (7). According to this method, the opening angle is the angle formed between the two radii joining the midpoint of the inner wall and the tips of the inner wall (Fig. 2).

RESULTS

There were marked species differences in the opening angle of airway rings at zero transmural pressure (Fig. 3). The anatomic location within the airway tree from which the airway rings were cut also influenced the magnitude of the opening angle, because intrapa-

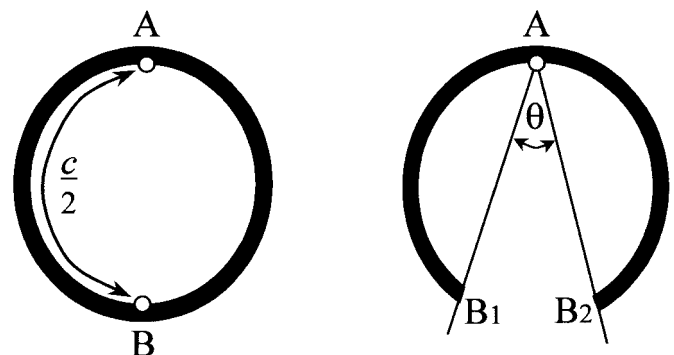


Fig. 2. Measurement of the opening angle according to the method of Han and Fung (4). A and B are points equidistant from one another along the inner circumference (c) of the airway wall. The opening angle was the angle (θ) formed between the two radii joining the midpoint (A) of the inner wall to the tips (B_1 and B_2) of the inner wall.

Table 1. Details of lung donors

Gender	Age	Height	Weight	Cause of Death	Duration of Ventilation
Male	18 yr	178 cm	77 kg	Gunshot	3 days
Male	24 yr	191 cm	83 kg	SAH	3 days
Male	31 yr	178 cm	75 kg	MVA	<24 h
Female	45 yr	158 cm	63 kg	SAH	<24 h
Female	59 yr	177 cm	63 kg	SAH	2 days

SAH, subarachnoid hemorrhage; MVA, motor vehicle accident.

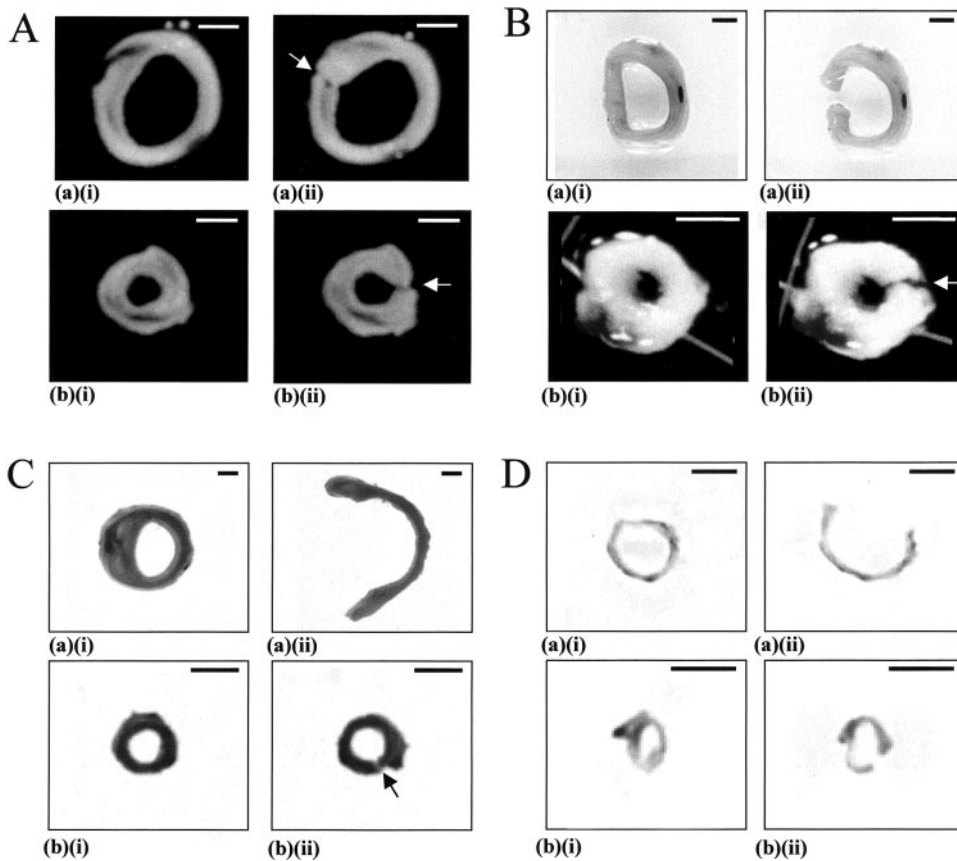


Fig. 3. Examples of opening angles in pig (A), human (B), sheep (C), and rabbit (D) extraparenchymal (a) and intraparenchymal (b) airways. Airways were photographed before (i) and after (ii) a radial cut was made through the airway wall. Arrows indicate the position of the cut where no opening angle is apparent. Bars indicate 5 mm.

renchymal airways from more distal locations within the lung generally had smaller opening angles than tracheal rings (Table 2, Fig. 4).

Rabbit airways from both extraparenchymal and intraparenchymal locations exhibited the greatest open-

ing angles among the four species studied. Opening angles ranging from 27 to 82° were seen in freshly excised tracheal rings from the four rabbits studied (Fig. 4A). Eight of the eleven intraparenchymal airways from the rabbit lungs also produced large opening angles when cut (Fig. 4B).

Extraparenchymal airways from sheep also exhibited marked opening angles, the largest angle of 50° being observed in a tracheal ring (Fig. 4A). Four of the eight intraparenchymal rings studied also had an opening angle (Fig. 4B); these angles ranged from 1 to 6° and were therefore of a lesser magnitude than the angles seen in intraparenchymal airways of the rabbit.

In the human airway tree, in both extraparenchymal and intraparenchymal airway rings of all sizes, a measurable opening angle was rarely observed. In one of the six extraparenchymal airways studied, an opening angle was measured, but in the remaining five rings no opening angle was seen (Fig. 4A). Opening angles in intraparenchymal airways were even less common, with an angle of 8° in one and an angle of 12° in a second ring being measured and another 69 rings having no opening angle (Fig. 4B).

Similar results were seen in airways dissected from the lungs of four pigs. In the extraparenchymal airways, neither cervical tracheal rings nor thoracic tracheal rings exhibited opening angles >5° (Fig. 4A). In large bronchi and in the more distal airways where only very little cartilage was visible, no open-

Table 2. Mean opening angles of intraparenchymal and extraparenchymal airways excised from sheep, pig, human, and rabbit lungs

	Sheep	Pig	Human	Rabbit
<i>Intraparenchymal</i>				
<i>n</i>	8	22	71	11
Internal diameter, mm				
Mean	4.09	2.30	2.95	3.02
Range	2.43–7.03	0.42–6.67	0.56–13.75	2.25–4.49
Opening angle, degrees				
Mean	1.63	0.05	0.28	16.18
Range	0–6	0–1	0–12	0–47
<i>Extraparenchymal</i>				
<i>n</i>	3	3	6	7
Internal diameter, mm				
Mean	14.41	11.22	12.35	5.84
Range	9.73–17.84	11.0–11.67	7.5–14.62	5.31–6.12
Opening angle, degrees				
Mean	33	2	2.17	55.14
Range	6–50	0–6	0–13	27–82

n, Number of airway rings studied.

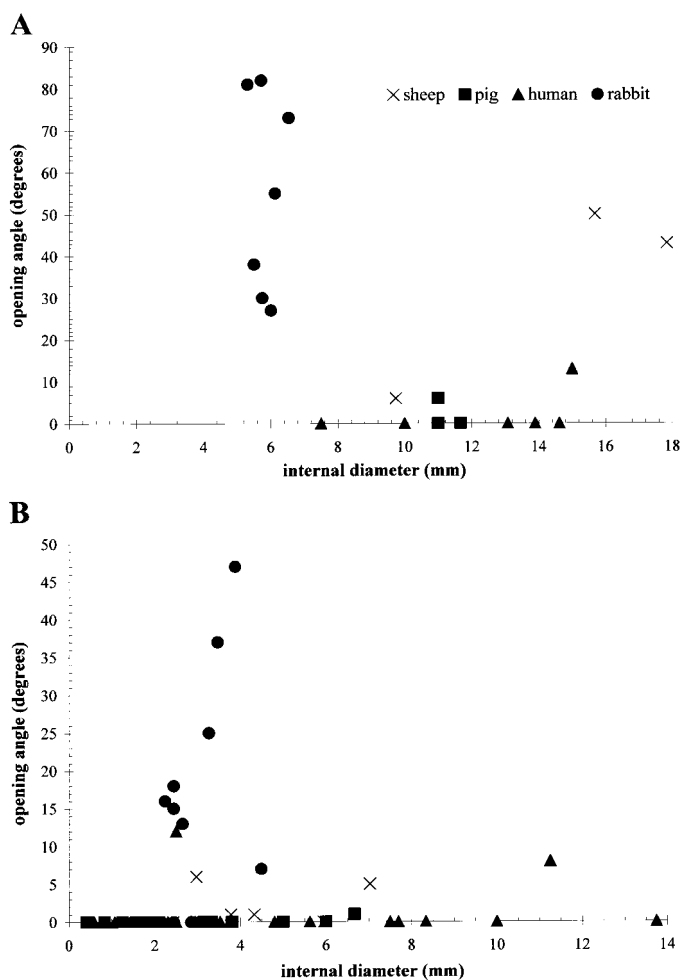


Fig. 4. Distribution of opening angles in pig, human, sheep, and rabbit extraparenchymal (A) and intraparenchymal (B) airways.

ing angles were observed in the 22 airway rings examined (Fig. 4B).

DISCUSSION

The results presented in this study indicate that freshly excised human and pig airways are in a stable zero-stress state, that is, no measurable opening angle is observed at zero transmural pressure. We have also shown that the extraparenchymal airways of sheep and airways from all locations within rabbit lung are not in a zero-stress state at zero transmural pressure. Thus there are marked species differences in the residual stresses within the airway wall as well as differences in residual stresses present in the wall of extraparenchymal and intraparenchymal airways.

The single previous report of the opening angle in human airways was anecdotal in nature and pertained solely to the trachea. De Kock (2) commented that in the "mechanically ideal, normal trachea . . . by cutting through the membrane . . . will be seen to increase the gap between the tips by as much as 70%." We have been unable to observe an opening angle of $>10^\circ$ in tracheas removed from five adult human organ donors. Furthermore, in both smaller caliber extraparenchymal

mal airways and intraparenchymal airways of various sizes from these lungs, we were unable to measure an opening angle of any significance. There is no apparent reason for the discrepancy between the observations made in our study and those of De Kock; however, no details were provided for the "mechanically ideal, normal trachea" in the report by De Kock. In our study, the lungs were all obtained from patients who had died suddenly with no history of respiratory disease and who were of a wide, but relatively young, age range (18–59 yr). Because tissues from the adjacent lung were used for pharmacological studies of airway smooth muscle (unrelated to the present report), we can confirm that loss of tissue viability would be unlikely to explain a lack of opening angle. Thus our results lead to the conclusion that human airways are actually at a zero-stress state at zero transmural pressure.

Apart from the descriptive studies of opening angles in animal airways (6, 9, 16), Han and Fung (7) have been the only investigators to carry out a systematic study of the opening angle within pig and dog trachea. It is therefore difficult, and for most of our results impossible, to compare our findings with those of other investigators. In the study by Han and Fung, the opening angle in porcine trachea measured only $5\text{--}15^\circ$. Such a small angle is reasonably consistent with the extremely small angles ($<6^\circ$) we measured in extraparenchymal airways of the pig and the absence of opening angle in intraparenchymal airways from this species.

Our observation of differences in the magnitude of the opening angle and thus the zero-stress state in intraparenchymal and extraparenchymal airways of the same species, although a novel finding in airways, is not unprecedented for biological tissues. Aortic opening angles have been shown to vary with longitudinal location (8). Regional differences have also been observed in other vascular tissues. For example, the thoracic portion of the rodent inferior vena cava had an average opening angle of 90° , whereas the corresponding value in the abdominal portion was 105° , and that in the superior vena cava was $\sim 75^\circ$ (24). These differences were attributed, in part, to the absence of uniform vessel structure. Structural differences are also likely to explain, in part, the differences in opening angle in intraparenchymal and extraparenchymal airways of the sheep and rabbit, observed in the present study. Extraparenchymal airway walls consist of a cartilage ring interrupted at only one point in the circumference by a membranous portion composed largely of smooth muscle. Conversely, the intraparenchymal airway wall is more heterogeneous in composition, with circumferential interlacing bands of smooth muscle either with or without multiple small cartilage plates depending on airway generation. Thus the vastly different anatomic structure of the extraparenchymal and intraparenchymal airway and the consequent differences in stress distribution within the airway walls provide one likely explanation for the

observed regional differences in opening angle in this study.

In addition to the marked regional differences in opening angle, the results of our study provide evidence for marked species differences in opening angle and thus the zero-stress state of the airways. Although airways from the rabbit and sheep had appreciable opening angles, airways from human and porcine lung had very small or indiscernible opening angles. Species differences in zero-stress state also exist in blood vessels. For instance, the opening angle of the thoracic aorta in pig is 60° whereas in the rat it is only 10° (8). The scientific literature is replete with reports of species differences relating to the structure and function of the lung. There are major species differences in the subgross anatomy (e.g., airway length, airway diameter, airway shape, angle of branching, number of airway generations) of the lung and airways (1, 12, 17, 20). Moreover, there is a large body of evidence demonstrating differences in airway substructure (e.g., volume and distribution of submucosal glands, epithelial cell type and number) between the species (10, 14, 17, 19). In a detailed study of the structure of rabbit trachea, Johnson and colleagues (11) described extensive folds and grooves formed by the tracheal mucosa and that the folding was accentuated when the transpulmonary pressure was reduced to zero. The folds seen in porcine and human airways were not as extensive as those in the rabbit. This observation both supports and provides an explanation for our findings because it suggests that the residual stress within the airway wall, which would be compressive at the inner (mucosal) aspect of the wall for positive opening angles, may be greater in the rabbit than in other species. Species and regional differences in the opening angle of blood vessels have been attributed to variations in the wall structure, wall materials, and mechanical properties of the materials in the vessel wall (4). It seems reasonable therefore to infer that a similar explanation may hold for the regional and species differences in airway opening angle and zero-stress state observed in the present study.

Several factors might contribute to the relative absence of an opening angle in airways compared with arteries. It is thought that residual stress in arteries develops so that, under normal transmural pressure, the circumferential stress is uniformly distributed through the vessel wall (18). Because arteries function at much higher transmural pressures than airways, the level of residual stress needed to accomplish this is much lower in an airway than in an artery. This, in combination with the fact that the wall thickness of an airway is much less than that of an artery, ensures that the range of residual stresses will be considerably reduced. Counteracting this effect, however, is the tendency for airways to exhibit a lower elastic modulus compared with arteries. For a given level of residual stress, the residual strains would consequently be higher.

Blood vessels have been the object of the most detailed studies of the zero-stress state of biological tis-

sues. The results of such studies have indicated that the magnitude of the opening angle, and thus the residual stress within a vessel wall, is likely dependent on six factors. These factors are the region of the vessel studied; the animal species studied; the time after cutting; the position of the cut; the physical, chemical, and biological environment; and the homeostatic condition of the tissue (8). The present study was designed to address only two of these factors in relation to the residual stress within the airway wall: the existence of any regional and species differences in opening angle. Preliminary investigations and previous studies (3, 5, 7) indicated that the remaining four factors were not likely determinants of the zero-stress state of the airways. They were thus not incorporated into the present investigation; however, the possibility remains that one or all of these other factors may influence the airway opening angle to some extent.

The results of this study therefore establish that there are significant species and regional differences in the magnitude of the opening angle and thus the residual stresses and zero-stress state of the airway wall. At zero transmural pressure, human airways (including intraparenchymal airways, the site of airway remodeling in asthma and COPD) exist at a stable zero-stress state. Future modeling to assess the load against which the airway smooth muscle must contract can incorporate these results to assign an initial condition of zero stress in the human airway wall at a transmural pressure of zero. Studies of the effect of perturbations to the stresses within the airway wall and thus the zero-stress state that may occur with the tissue remodeling characteristic of airway disease will further our understanding of airway wall mechanics in both health and disease.

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Present address of K. O. McKay: Dept. of Respiratory Medicine, The Children's Hospital at Westmead (Royal Alexandra Hospital for Children), and Dept. of Paediatrics and Child Health, The University of Sydney, Westmead, NSW 2145, Australia.

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