

Mast cell densities in bronchial biopsies and small airways are related

M L Carroll,^{1,2} N G Carroll,¹ H J Gundersen,³ A L James^{1,2}

¹West Australian Sleep Disorders Research Institute/Department of Pulmonary Physiology, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

²School of Medicine and Pharmacology, University of Western Australia, Crawley, Australia

³Stereological Research Laboratory, University of Aarhus, Aarhus, Denmark

Correspondence to

Alan James, West Australian Sleep Disorders Research Institute/Department of Pulmonary Physiology, Sir Charles Gairdner Hospital, Hospital Avenue, Nedlands, WA 6009, Australia; alan.james.scgh@health.wa.gov.au

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ABSTRACT

Introduction The study sought to determine whether mast cell counts in endobronchial biopsies of large airways are related to mast cell counts in the small airways.

Methods Lungs, obtained postmortem from 10 subjects who had died of non-respiratory causes, were fixed in inflation. Mast cell densities (cells/mm³), determined with an optical disector, were compared on histological sections (30 µm thick) of biopsies and small airways stained with human anti-mast cell tryptase.

Results Mean mast cell density over the inner airway wall in biopsies was significantly related to mean mast cell density over the total airway wall in the small airways ($r = \sim 0.80$, $p < 0.01$). A minimum of three biopsies per case was required to demonstrate this relationship. Within relevant count areas, mast cell density was about 1.6-fold higher in the small airways than in the biopsies.

Conclusions These findings suggest that when mean counts from at least three biopsies per case are used, intersubject comparisons of mast cell density in the inner airway wall in endobronchial biopsies reflect intersubject comparisons of mast cell density over the total airway wall in small airways. This is despite the observation that mast cell densities are generally higher in the small airways.

INTRODUCTION

Endobronchial biopsy has been described as the 'gold standard' for assessment of airway inflammation in asthma^{1,2} and has been used frequently in the research setting.^{3–10} However, concerns remain regarding the ability of biopsies to represent airway inflammation throughout the bronchial tree,^{3,7,11,12} the limited size and depth of endobronchial biopsies,^{3,7,11} and the fact that they are usually taken from carinal sites.¹² Endobronchial biopsies are generally limited to sampling of the large airways,¹¹ whereas the small and large airways are known to be involved in asthma.^{13–22} The distribution of inflammatory cells in the small airways may differ from that in the large airways.^{15,16} In large airways, most of the mast cells are found in the inner airway wall (WAI), which is the compartment sampled by bronchoscopic endobronchial biopsy¹⁶ (figure 1A,B). In small airways, fewer mast cells are confined to the WAI and many more are spread over the total airway wall (WAT), particularly from the outer border of the smooth muscle to the parenchyma¹⁶ (figure 1C,D). Since the density of cells, such as mast cells, may be increased in the small airways relative to the large airways,¹⁶ a high density of cells cannot necessarily be regarded as evidence of inflammation.

A systematic study, using unbiased stereological methods, of the density of mast cells in the large and small airways from the same lung has not been undertaken previously.

Recently, we have shown that endobronchial biopsies can be used to represent mast cell density in the WAI in large airways²³ provided that the mean count from at least four biopsies (from different sites) per case is used. The aim of the present study was therefore to investigate whether mast cell density in endobronchial biopsies from the large airways is related to mast cell density in the small airways.

METHODS

Whole lungs (eight left, two right) were obtained at postmortem from 10 subjects (all men, aged 14–44 years) who had died from non-respiratory causes. Consent for the study of these tissues was obtained from the next of kin and approval was obtained from the Sir Charles Gairdner Hospital Human Research Ethics Committee. A medical history was available for only four subjects, and in each case it excluded the diagnosis of asthma. Lungs were fixed at inflation with 10% neutral buffered formalin at a pressure of 25 cm H₂O.

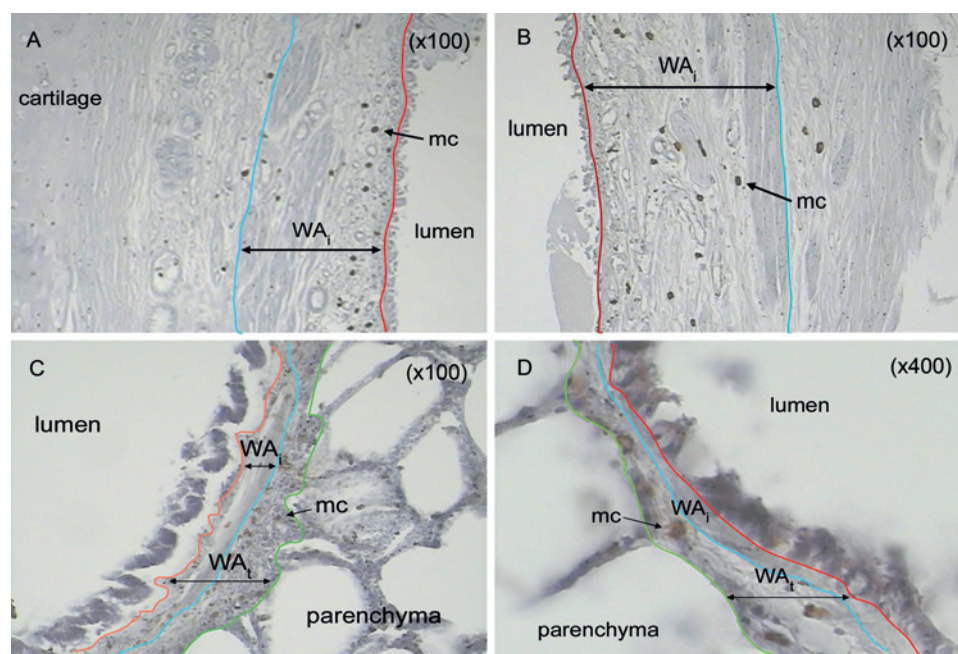
Biopsies were taken, using standard cupped forceps, from large airways down to the 5th or 6th generation, in a manner described previously.²³ For each subject, six biopsies were selected at random from those collected. The upper and lower lobes of the lungs were then separated and each lobe was embedded randomly in a block of agar. Thereafter, slices (5 mm thick) were cut at uniform intervals from a random start point (figure 2). Using a sampling template, uniform random cores were taken from the slices.

These tissue specimens were embedded separately in paraffin wax, sectioned at 30 µm for brightfield microscopy and stained with anti-mast cell tryptase monoclonal antibody AA1 (Dako, Campbellfield, Victoria, Australia) using the immunoperoxidase technique. Sections with preparation artefacts were discarded or replaced, and this resulted in an average of five usable biopsies per case.

All small airways that appeared in transverse section of cores of parenchymal tissue were identified and included in the analysis if their long diameter to short diameter ratio was 3 or less,¹⁹ and basement membrane perimeter was less than 8 mm. These measurements were conducted on a Computer Assisted Stereology Toolbox (CAST) grid system (Olympus, Ballerup, Denmark)²³ at $\times 12.5$ magnification. An average of 11 small airways per case, with a mean basement membrane perimeter of 2.49 mm (range 0.51–7.62 mm), were analysed.

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Figure 1 Mast cell distribution in large and small airways. Airway compartments are delineated by coloured lines. Mast cells (mc), with examples arrowed, are stained brown. (A) Field within a transverse section of a large airway. (B) Field within an endobronchial biopsy from the large airways showing numerous mast cells in the inner airway wall (WA_i). (C,D) Fields within small airways showing mast cells widely distributed across the total airway wall (WA_t). Relatively few mast cells are seen in the inner airway wall.



Stereological cell counts

Three-dimensional (3D) counts of mast cells were conducted with an optical disector on CAST, using an oil immersion lens at $\times 1000$ magnification, to give density in cells per cubic millimetre (cells/mm³).²³ Cells were counted using a uniform random sample of fields comprising 17–34% of each area described below. In biopsies, the reference areas (see figure 1) were the WA_i, from the epithelial basement membrane to outer border of the airway smooth muscle layer, where definable,²⁴ and the area of the airway wall to a depth of 100 μ m below the basement membrane, similar to that used in previous studies.^{3 4 6 10} In small airways, the reference areas were the WA_i, where definable, and the WA_t, from the basement membrane to the adventitial border.²⁴

Data analysis

Pearson's correlation coefficient was used to test the relationships between mast cell densities in biopsies and the small airways. The analysis was conducted first using the mean values of all biopsies and all small airway sections. A bootstrap method using one, two, three, four or five biopsies (selected randomly, without replacement) was then employed to determine the minimum number of biopsies required to represent the relationship between mast cell density in biopsies and mast cell density in the small airways. Analyses (for each of the 1–5

biopsies) were carried out four times and the resulting four Pearson correlations were averaged. Mean *r* values were reported as Fisher Z weighted means.²⁵ Probability values less than 0.05 were considered significant.

RESULTS

Mean mast cell densities in biopsies and small airways

Table 1 shows the mean values of all airways combined for mast cell densities for biopsies and small airways in different airway compartments. The mean 3D mast cell density in the WA_i or 100 μ m deep to the basement membrane of the small airways was similar to that in the biopsies, with values approximately 1×10^4 cells/mm³, but in the WA_t in the small airways, mast cell density was, on average, approximately 1.4–1.6-fold higher than that seen in the biopsies.

Relationships between mast cell density in biopsies and small airways

Figure 3 shows plots of mean mast cell densities in endobronchial biopsies of large airways versus transverse sections of small airways, using all available biopsies per case (mean=5) and all measured small airway transverse sections per case (mean=11). Counts over the WA_i on biopsies were significantly correlated with counts over the WA_t in small airway transverse sections ($r=0.80$, $p<0.01$) and reflected more than 60% of the variance in

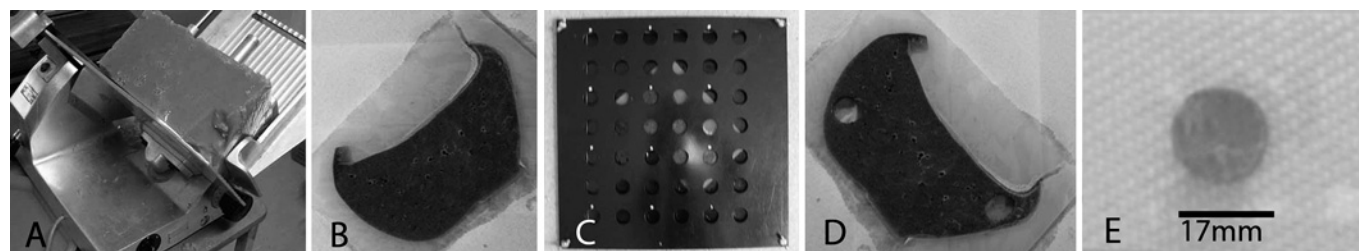


Figure 2 Random sampling of the lung. (A) Lobe, orientated at random in a block of agar, is sliced at uniform intervals from a random start to obtain slices (5 mm thick). (B) Uniform, random slice from a lung (5 mm thick). (C) Lung slice orientated at random under a sampling template. (D) Cores (17 mm diameter) were cut from every 2nd hole in every 2nd row. (E) Core (5 mm thick, 17 mm diameter) of lung tissue.

Table 1 Mast cell densities (mean of case means) in endobronchial biopsies and small airways

	Count area	Mean mast cell density (cells/mm ³)
Biopsies	WAI	9.7 (± 3.3) $\times 10^3$
	100 μ m deep	11.2 (± 4.5) $\times 10^3$
Small airways	WAI	10.9 (± 3.4) $\times 10^3$
	WAt	15.3 (± 4.1) $\times 10^3$

WAI, inner wall area; WAt, total wall area; 100 μ m deep, to a depth of 100 μ m below the basement membrane.

the small airway counts (figure 3A). However, the correlation between counts to a depth of 100 μ m below the basement membrane on biopsies and counts over WAt in small airway transverse sections were lower and not significant (figure 3B). There were no significant relationships between mean mast cell densities in the WAI in biopsies and those in the WAI in transverse sections of small airways (figure 3C,D).

Effect of number of biopsies

The case means based on all transverse sections of small airways were compared with counts based on one, two, three or four randomly selected biopsies per case and all biopsies per case. When 3D counts over the WAI in biopsies were used, a mean of only two biopsies was required to show a significant relationship with mast cell counts in the WAt in the small airways (table 2). However, for biopsy counts to reflect more than 50% of the variance in mast cells counts in the small airways, a mean of at least three biopsies was required. Counts to a depth of 100 μ m below the basement membrane on biopsies were not

significantly related to counts in the small airways regardless of the number of biopsies used (figure 3D).

DISCUSSION

The present study showed that mast cell density, measured in 3D, was about 1.6-fold greater in the WAt in small airways than it was in the WAI in biopsies. Despite this difference, mean mast cell density in the WAI on biopsies was strongly related to mean mast cell density in the WAt in small airways. When at least three biopsies were used, mean 3D mast cell density within the WAI in biopsies reflected about 60% of the intersubject variance in mean 3D mast cell density within the WAt in small airways. A significant, but more modest, relationship between counts in biopsies and the small airways was evident when using the average of two biopsies only, but, as with findings for the large airways,²³ mast cell counts from a single biopsy were not related to mean counts in the small airways.

The findings in this study suggest that even though mast cell density is systematically higher in the WAt of small airways than it is in the WAI of large airways, the differences between subjects in mast cell density over the WAt in their small airways are broadly similar to the differences between subjects that can be observed in mast cell density over the WAI of their endobronchial biopsies.

Endobronchial biopsies of the large airways and the WAI of the small airways

While mast cell counts in the WAI in biopsies reflected over 60% of intersubject variation in counts over the WAt in small

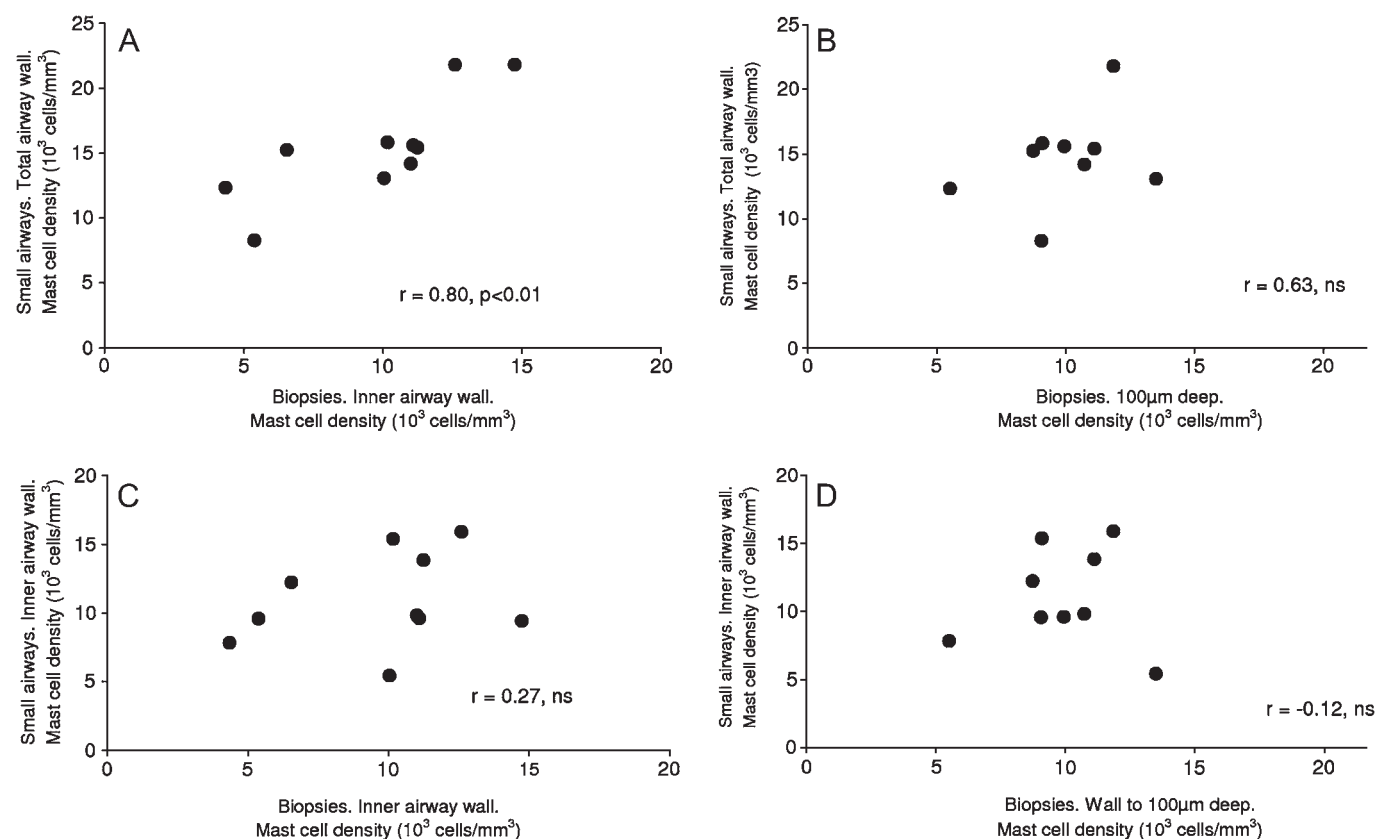


Figure 3 Mean mast cell density in transverse sections of small airways versus mean mast cell density in endobronchial biopsies of large airways. (A) Small airways, total airway wall versus biopsies, inner airway wall. (B) Small airways, total airway wall versus biopsies, 100 μ m deep. (C) Small airways, inner airway wall versus biopsies, inner airway wall. (D) Small airways, inner airway wall versus biopsies, 100 μ m deep. 100 μ m below the basement membrane; r , Pearson correlation coefficient; ns, not significant.

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Table 2 Correlations between mast cell densities in biopsies and the total airway wall in transverse sections of small airways: effect of number of biopsies

Biopsies		Small airways	
No. used	Count area	Count area	r†
1	WAI	WAt	0.46
2	WAI	WAt	0.65*
3	WAI	WAt	0.82***
4	WAI	WAt	0.77**
All	WAI	WAt	0.80**
1	100 µm deep	WAt	0.35
2	100 µm deep	WAt	0.41
3	100 µm deep	WAt	0.55
4	100 µm deep	WAt	0.40
All	100 µm deep	WAt	0.63

*p<0.05; **p<0.01; ***p<0.001.

†Correlations were with the mean of all small airways per case. Fisher Z weighted average of four trials.

WAI, inner wall area; WAt, total wall area; 100 µm deep, to a depth of 100 µm below the basement membrane.

airways, they reflected only 7% of intersubject variation in counts over the WAI in small airways. This lack of correlation is almost certainly due to the very small volume of the WAI in small airways and the differing distributions of mast cells across the airway wall in small and large airways (figure 1).

The findings of the present study are consistent with the study in smokers by Battaglia *et al*²⁶ that reported a wide range of mast cell densities in the lamina propria of small airways, considerably overlapping that in the lamina propria of large airways. Similarly, a study by Hamid and colleagues²⁷ found that mast cell density in small airways varied more widely in the 'inner' region, between the epithelium and the inner border of the smooth muscle layer, than it did in the 'outer' region, from the outer border of the smooth muscle layer to the adjacent parenchyma. Balzar *et al*²⁸ suggested that in small airways the distribution of mast cells in the WAI may be functionally less important than that in the outer airway wall, at least in respect to lung function in asthma, as mast cell density in the outer region only, but not the inner region, was related to measures of lung function.

Mast cell counts in the small airways and counts to a depth of 100 µm below the basement membrane on biopsies of the large airways

A number of biopsy studies that have reported the density of airway inflammatory cells, including mast cells, have confined their cell counts to a depth of approximately 100 µm or less below the basement membrane.^{3 4 6 10} In the present study, intercase comparisons of mean mast cell density based on such a reference area in biopsies did not represent intercase comparisons of mast cell density over the WAI or the WAt in the small airways. Mean mast cell density to a depth of 100 µm below the basement membrane on biopsies reflected, on average, only about 40% of intersubject variation in mast cell density in the WAt and virtually none of the intersubject variation in the WAI in small airways. We have previously reported similar findings in relation to mast cell densities in the large airways.²³

Generalisability of the findings

This study compared the densities of mast cells in the large and small airways only. Carroll *et al*¹⁵ proposed that if a bronchial biopsy is representative of the infiltration of eosinophils and lymphocytes within an airway, then biopsy of the proximal

airways is likely to be representative of this process throughout the bronchial tree in cases of asthma. Although agreeing with the findings of the present study of mast cells, this proposition was based on comparisons of mean eosinophil and lymphocyte densities *between* case groups in the WAI of large and small airways, not between the densities of a given inflammatory cell in the large and small airways *within* cases.

The lung specimens in the present study were fixed using formalin, and blocks were embedded in paraffin. This will have resulted in variable shrinkage compared with the resected lungs or lobes. Shrinkage of tissue volume may have led to an increase in the numerical density of mast cells compared with that in the lungs before fixation and embedding. If there was a systematic difference in shrinkage between large and small airways, a biased comparison of mast cell density between these airways might result. We observed that the mast cell volume density was 1.6 times higher in the WAt in small airways. Shrinkage due to fixation and tissue processing is variable and can be up to 30% in large animal lungs.²⁹ This would not explain all of the 60% difference we observed between the large and the small airways in the current study. Moreover, variability of shrinkage would be expected to reduce the likelihood of observing a significant correlation between the large and the small airways, and therefore is unlikely to have accounted for the results we observed.

In the present study, we did not have recognised case groups. Details regarding clinical history were known for only 4 of the 10 available subjects, and in each instance ruled out asthma. Since the mast cell is a constitutive resident of the bronchi, it seems plausible that mast cell density in the WAI of the large airways and in the WAt of the small airways in cases of asthma would be related in the way that the present study suggests. Comparing mast cell counts between the large and small airways within asthma cases may be confounded by degranulation of mast cells, since the percentage of degranulated mast cells is increased in asthma cases compared with control subjects.¹⁶ In the present study and in many studies of mast cells, specific staining of intracellular granular contents is used for identification. If degranulation differs between the small and large airways, the relation between biopsies of large airways and wall area of small airways that we observed in the present study may not hold. However, the study of Carroll *et al*¹⁶ suggests that this is likely to have only a small effect. That study showed that degranulation of mast cells was significantly greater in WAt in the large airways across all case groups,

Take-home messages

- The density of mast cells in small airways is greater than that in large airways within the same subject. The increased density of mast cells in small airways should not be construed as 'inflammation'.
- The density of mast cells seen in biopsies of the large airways is strongly related to that seen in the small airways. Biopsies of the large airways can therefore provide information about the density of mast cells in other parts of the bronchial tree.
- Three biopsies are required to adequately assess mast cell density in the small airways.
- These observations do not necessarily apply to other cells in the airway wall or to localised pathological processes such as inflammation.

ranging from 1.14-fold higher in non-fatal cases of asthma to about 1.5-fold in control subjects. Despite the fact that the difference between large and small airways was greatest in control cases, we still observed a significant relationship between the large and small airways in the present study.

In conclusion, the present study shows a strong relationship between mast cell density in the WAI on biopsies of the large airways and the WAT in transverse sections of the small airways, although mast cell density tends to be higher in the small airways. Confirmation of the applicability of this relationship to other inflammatory cells and to disease states awaits intrasubject comparisons.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Sir Charles Gairdner Hospital Human Research Ethics Committee.

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