an attempt to quit smoking and reassure them that their efforts have not been in vain. This could provide the motivation needed for a second and possibly successful attempt to quit.

L Dunn, A Ogilvie
Department of Epidemiology and Public Health, University of Newcastle Medical School, Newcastle upon Tyne NE2 4HA, UK

References

1 **Pelkonen M**, Notkola I-L, Tukiainen H, *et al*. Smoking cessation, decline in pulmonary function and total mortality: a 30 year follow up study among Finnish cohorts of the Seven Countries Study. *Thorax* 2001;**56**:703–7.
2 **Leon DA**. Failed or misleading adjustment for confounding. *Lancet* 1993;**342**:479–81.

Authors' reply

Lorna Dunn and Aileen Ogilvie make an important point that the confounding effect of tobacco consumption on the decline in pulmonary function may occur when the levels of tobacco exposure are categorised too broadly. They think that the benefit of intermittent quitting on the decline in $FEV_{0.75}$ in our study might be explained by decreased tobacco consumption after periods of abstention rather than by the periods of abstention per se. They also point out that, if a considerable proportion of intermittent quitters stopped smoking permanently between 1974 and 1989, it would have led to overestimation of the value of temporary quitting. The third question concerns the duration of periods of abstention.

In our study the data on smoking habits were recorded at baseline and in subsequent re-examinations by a standard questionnaire. The interval between examinations was usually 5 years. Intermittent quitters were either baseline past smokers who smoked in at least one of the subsequent re-examinations or baseline smokers who were quitters in one or more re-examinations but relapsed back to smoking later. To be recorded as a quitter in an examination a subject had to have given up smoking more than a year previously. During the first 15 years, 27 of 75 intermittent quitters were recorded as quitters in one examination (corresponding to at least 1 year of abstinence), 32 were recorded as quitters in two examinations (corresponding to at least 2 years of abstinence), and 16 were recorded as quitters in three examinations (corresponding to at least 3 years of abstinence).

During the first 15 years intermittent quitters reduced the number of cigarettes smoked daily compared with continuous smokers, although not significantly. To measure tobacco consumption more precisely, a new variable was constructed by computing the mean reported daily cigarette consumption at each examination point. For intermittent quitters only, the data from the examinations when they reported smoking were used in making up this variable. When we then additionally adjusted our analyses for this new variable, the decline in $FEV_{0.75}$ during the first 15 years was still significantly less among intermittent quitters than in continuous smokers (data available from the authors on request). The benefit of intermittent quitting on the decline in pulmonary function therefore also seems to be mediated through periods of abstention.

Among both intermittent quitters and continuous smokers there were study subjects who stopped smoking permanently between 1974 and 1989. The proportion of such study subjects was greater among intermittent quitters than among continuous smokers. However, when we made additional adjustments for both the mean daily tobacco consumption during the first half of the follow up period and for quitting smoking during the latter half of the follow up period, intermittent quitters still lost less $FEV_{0.75}$ during the whole 30 years than continuous smokers (data available from the authors on request).

In conclusion, it seems that some protection may be gained from periods of abstention, although we agree that the main goal should be permanent smoking cessation.

M Pelkonen, I-L Notkola, H Tukiainen, M Tervahauta, J Tuomilehto, A Nissinen
Department of Pulmonary Diseases, Kuopio University Hospital and Department of Public Health and General Practice, University of Kuopio, P O Box 1627, FIN-70211 Kuopio, Finland; Margit.Pelkonen@uku.fi

Fibrosing alveolitis in patients with RA

We read with interest the paper by Dawson *et al*[1] on the prevalence of fibrosing alveolitis (FA) diagnosed by HRCT scanning in rheumatoid arthritis (RA). This well designed cross sectional study estimates the prevalence of FA at 19% in patients with RA irrespective of respiratory symptoms. This is in keeping with current literature and our earlier report of 20% in unselected patients with RA not suspected of having interstitial lung disease (ILD).[2]

However, neither of these studies has been sufficiently powered to assess a possible association of smoking with ILD. Smoking may adversely affect the outcome of ILD in RA and Saag *et al*[3] suggested that smoking was the most consistent independent predictor of ILD patterns in lung function tests and chest radiographs in RA. One of our previous studies[4] reported a prevalence of ILD of only 5% on HRCT scanning in a cohort of 20 never smokers with RA, while Dawson *et al* reported a prevalence of 11% in never smokers compared with 22% in smokers. There is therefore evidence of a trend towards an association between ILD and smoking which could be explored in a larger study. However, a sample size of 450 patients would be needed to test the hypothesis that smokers are twice as likely to develop ILD in RA than never smokers (95% confidence; power = 80%; smoker/never smoker ratio 2:1).

We agree with the authors that further work on the natural progression of FA diagnosed by HRCT scanning in RA is due. We have commenced a longitudinal prospective study of 18 RA patients with ILD diagnosed by HRCT scanning compared with a cohort of patients with cryptogenic fibrosing alveolitis (CFA) matched for age, sex, smoking, and respiratory symptoms.[5] There are significant baseline differences in clinical and radiological features between these two groups. Clubbing and honeycomb appearance on the HRCT scan is more common in patients with CFA while ground glass appearance is more common in RA patients with ILD. The presence of rheumatoid factor appears to be protective against honeycombing in both groups. These differences in clinical and HRCT features may be important predictors of outcome.

V Saravanan, C A Kelly
Queen Elizabeth Hospital, Gateshead NE9 6SX, UK

References

1 **Dawson JK**, Fewins HE, Desmond J, *et al*. Fibrosing alveolitis in patients with rheumatoid arthritis as assessed by high resolution computed tomography, chest radiography, and pulmonary function tests. *Thorax* 2001;**56**:622–7.
2 **McDonagh J**, Greaves M, Wright AR, *et al*. High resolution computed tomography of the lungs in patients with rheumatoid arthritis and interstitial lung disease. *Br J Rheumatol* 1994;**33**:118–22.
3 **Saag KG**, Kolluri S, Scwartz DA, *et al*. Rheumatoid arthritis lung disease. Determinants of radiographic and physiological abnormalities. *Arthritis Rheum* 1996;**39**:1711–9.
4 **Hassan WU**, Keaney NP, Holland CD, *et al*. High resolution tomography of the lung in lifelong non-smoking patients with rheumatoid arthritis. *Ann Rheum Dis* 1995;**54**:308–10.
5 **Rajasekaran BA**, Shovlin D, Lord P, *et al*. Interstitial lung disease in patients with rheumatoid arthritis: comparison with cryptogenic fibrosing alveolitis. *Rheumatology* 2001;**40**:1022–5.

Authors' reply

We are pleased to receive the letter from Saravanan and Kelly in response to our recent publication in *Thorax*.[1] The relationship between smoking and RA associated FA is an interesting one. There is no consistent finding in the literature of smoking and RA associated FA and, as far as we are aware, no prospective HRCT based study has shown a statistically significant association between RA associated FA and smoking. In the study by Cortet *et al*[2] 68 patients with RA were prospectively studied with HRCT scanning. Cigarette smoking was less prevalent than in the North of England and the ratio of smokers to non-smokers was 1:3. No statistical association was seen linking smoking and interstitial lung disease (ILD) and a prevalence of 20% of ILD (17% ground glass pattern and 2.9% reticular pattern) was still found. It is true that in our study the absolute risk of ever smoking cannot be excluded as a risk factor for FA as the number of lifelong non-smokers is small; however, the pack year data are adequately powered to show no statistically significant difference.

With regard to the paper by Rajasekaran *et al*,[3] we feel it necessary to point out that the patients in their study with FA and RA had the diagnosis confirmed by HRCT scanning and, in addition, were symptomatic with dyspnoea, bibasal crackles, restrictive pulmonary function tests, and chest radiographic changes of FA. We are sure this will provide very valuable information about the progression of FA in patients with RA but it will not add to our knowledge on the outcome of HRCT changes detected at a subclinical stage.

Rajasekaran *et al* found honeycombing on the HRCT scan in three of 18 patients with RA associated ILD and in four of 18 patients with CFA; this difference is not statistically significant.[3] None of these patients was rheumatoid factor positive, which has led the authors to postulate that rheumatoid factor may be protective against honeycombing in ILD. These findings are in direct contrast to those of Muller-Leisse *et al*[4] who found higher levels of rheumatoid factor to be associated with ground glass changes and honeycombing on the HRCT scan, and also to McDonagh *et al* [5] who reported that at least five of 16 patients (31%) had honeycombing and were rheumatoid factor positive. This finding is particularly interesting given that there is evidence in the literature of smoking being associated with seropositivity for rheumatoid factor in patients with[6] and without RA.[7 8] We

would suggest that larger studies need to be undertaken and explored for confounding factors such as smoking before a statement can be made that rheumatoid factor is protective against honeycombing.

J K Dawson, J Desmond, M P Lynch, D R Graham
Department of Rheumatology, St Helen's Hospital, St Helen's, Merseyside, UK; twodocs@doctors.org.uk

References

1 **Dawson JK**, Fewins HE, Desmond J, *et al*. Fibrosing alveolitis in patients with rheumatoid arthritis as assessed by high resolution computed tomography, chest radiography, and pulmonary function tests. *Thorax* 2001;**56**:622–7.
2 **Cortet B**, Perez T, Roux N, *et al*. Pulmonary function tests and high resolution computed tomography of the lungs in patients with rheumatoid arthritis. *Ann Rheum Dis* 1997;**56**:596–600.
3 **Rajasekaran BA**, Shovlin D, Lord P, *et al*. Interstitial lung disease in patients with rheumatoid arthritis: a comparison with cryptogenic fibrosing alveolitis. *Rheumatology* 2001;**40**:1022–5.
4 **Muller-Leisse C**, Bussmann A, Meyer O, *et al*. Pulmonary manifestations in rheumatoid arthritis: high-resolution computed tomography in correlation with the skeletal changes and the laboratory chemical changes. *Rofo Fortschr Geb Rontgenstr Neuen Bildgeb Verfahr* 1996;**165**:438–44.
5 **McDonagh J**, Greaves M, Wright AR, *et al*. High resolution computed tomography of the lungs in patients with rheumatoid arthritis and interstitial lung disease. *Br J Rheumatol* 1994;**33**:118–22.
6 **Wolfe F**. The effect of smoking on clinical, laboratory, and radiographic status in rheumatoid arthritis. *J Rheumatol* 2000;**27**:630–7.
7 **Tuomi T**, Heliovaara M, Palosuo T, *et al*. Smoking, lung function, and rheumatoid factors. *Ann Rheum Dis* 1990;**49**:753–6.
8 **Jonsson T**, Thorsteinsson J, Valdimarsson H. Does smoking stimulate rheumatoid factor production in non-rheumatic individuals? *APMIS* 1998;**106**:970–4.

Measuring granulocyte apoptosis in airway inflammation

We read with interest the paper by Turlej *et al* describing enhanced survival of lung granulocytes in an animal model of asthma.[1] As discussed by the authors, modulation of immune cell apoptosis is likely to be important in controlling inflammatory processes, and the paper enhances our understanding of this.

However, we feel that there are some methodological problems with the study. Firstly, the animal model they describe, though having some similarities with asthma, is closer to chronic obstructive pulmonary disease (COPD). Neutrophils are the predominant inflammatory cells in this model. This condition is often known as COPD in horses.[2]

Secondly, although the authors refer to the use of annexin V (AV) and propidium iodide (PI), they do not describe the methodology used or how they interpreted the staining with AV and PI. This is important because there are controversies surrounding the interpretation of this method of assessing apoptosis.[3] The interpretation of the various staining patterns is controversial. In addition, at least two methods should be used to confirm apoptosis,[4 5] and only one is used in the study.

It is noted that the blood granulocytes are isolated by use of a density gradient. Density gradients may interfere with some neutrophil functions[6] and this must be borne in mind when interpreting these results. Additionally, BAL granulocytes from healthy horses were isolated by use of a density gradient, whereas this was not used for the diseased horses. This difference of methods introduces a potential bias into the study. We have previously attempted to isolate neutrophils from human BAL fluid with no success (unpublished observations) and would be interested to know if the authors achieved this separation easily. We are also surprised at the viability of >90%. Cell viability is likely to diminish with increasing rates of apoptosis, and it is notable that the BAL granulocytes from healthy horses have apoptotic rates of around 40%.

This study is interesting, but the methodological issues raised must be considered in interpreting the results.

M G Kelly, J S Elborn
Respiratory Research Group, Department of Respiratory Medicine, Belfast City Hospital, Belfast, UK

M G Kelly, V Brown, M Ennis
Department of Clinical Biochemistry, Centre for Inflammation, Infection and Repair, Queens University, Belfast, UK

Correspondence to: Dr M Kelly, Department of Respiratory Medicine, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB, UK; m.g.kelly@qub.ac.uk

References

1 **Turlej RK**, Fievez L, Sandersen CF, *et al*. Enhanced survival of lung granulocytes in an animal model of asthma: evidence for a role of GM-CSF activated STAT5 signalling pathway. *Thorax* 2001;**56**:696–702.
2 **Raulo SM**, Sorsa T, Tervahartiala T, *et al*. MMP-9 as a marker of inflammation in tracheal epithelial lining fluid (TELF) and in bronchoalveolar fluid (BALF) of COPD horses. *Equine Vet J* 2001;**33**:113–5.
3 http://www.cyto.purdue.edu/hmarchiv/2000/0536.htm (accessed 6 October 2001).
4 **Anon**. *Apoptosis. Applied reagents and techniques. Instruction manual*, 2nd ed. San Diego: Pharmingen, 1998.
5 http://www.cyto.purdue.edu/hmarchiv/2000/0382.htm (accessed 6 October 2001).
6 **Glasser L**, Fiederlein MT. The effect of various cell separation procedures on assays of neutrophil function. *Am J Clin Pathol* 1990;**93**:662–9.

Authors' reply

We thank Dr Kelly and colleagues for their interest in our paper. In the past equine heaves was called COPD but, because equine heaves is completely different from human COPD, specialists in the field have recommended avoiding the erroneous term "COPD" for designating this disease.[1] Indeed, it is now clear that equine heaves is very close to atopic asthma and these diseases share important characteristic features including hypersensitivity to aeroallergens, Th2 type immune response, chronic airway inflammation, reversible airway obstruction, non-specific airway hyperresponsiveness, and production of allergen specific IgE.[2 3] It is correct that neutrophils are the predominant inflammatory cells in equine heaves, but this does not exclude the use of this model in asthma studies. Indeed, neutrophils are known to play an important role in asthma whereas recent studies have questioned the importance of eosinophils in this disease.[4 5]

In our study only small amounts of granulocytes were recovered from the lung of the horses so we were only able to use one method to assay these cells for apoptosis. We chose the method that has been found to be the most sensitive marker of granulocyte apoptosis—the annexin V (AV)/propidium iodide (PI) method.[6] The results obtained with this method were interpreted as recommended: AV–/PI– cells were considered alive, AV+/PI– cells were considered apoptotic, and AV+/PI+ cells were considered necrotic. This is the first time we have heard of controversy surrounding the interpretation of the results obtained with this method, probably because they have not been published in scientific journals. According to the archives we have read using the web addresses provided by Dr Kelly and colleagues, it appears that this controversy exclusively concerns the status of AV–/PI+ cells. Such cells are uncommon and were not observed in our study.

We agree that density centrifugation may interfere with neutrophil function. To the best of our knowledge there is no other way of separating granulocytes from other cell types. As mentioned in the Methods section of our paper, cell viability of freshly isolated granulocytes was evaluated by trypan blue (TB) exclusion. The cells were then cultured for different times and assayed for apoptosis using AV/PI. Cells in an early state of apoptosis are AV+ and TB–. It is therefore not surprising to find 40% apoptotic (AV+) cells in a population where nearly all the cells (>90%) are TB–.

F Bureau
Department of Physiology, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège, Belgium

References

1 **Robinson NE**. International Workshop on Equine Chronic Airway Disease. Michigan State University 16–18 June 2000. *Equine Vet J* 2001;**33**:5–19.
2 **Eder C**, Crameri R, Mayer C, *et al*. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet Immunol Immunopathol* 2000;**73**:241–53.
3 **Lavoie JP**, Maghni K, Desnoyers M, *et al*. Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. *Am J Respir Crit Care Med* 2001;**164**:1410–3.
4 **Bryan SA**, O'Connor BJ, Matti S, *et al*. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;**356**:2149–53.
5 **Leckie MJ**, ten Brinke A, Khan J, *et al*. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;**356**:2144–8.
6 **Walsh GM**, Dewson G, Wardlaw AJ, *et al*. A comparative study of different methods for the assessment of apoptosis and necrosis in human eosinophils. *J Immunol Methods* 1998;**217**:153–63.

CORRECTION

In the Programme and Abstracts of the British Thoracic Society Winter Meeting 2001 published in *Thorax* 2001;**56**(Supplement III), an error occurred in abstract S130 "Management of pneumothorax in a district general hospital: compliance with the BTS guidelines" by Al-Aloul M, *et al* which appeared on page iii40. The name of the second author which appeared as K U Torrey should have been K U **Toori**.