Conventional RIA underestimates cortisol suppression in the presence of prednisolone

Concerns about suppression of the hypothalamic pituitary adrenal (HPA) axis by systemic steroids as well as by inhaled corticosteroids have been widely held since their introduction. Several studies have suggested that inhaled corticosteroids can replace oral corticosteroids during exacerbations of asthma and in severe asthma. We have recently published a study in which treatment of unstable asthmatic patients for 2 weeks with high doses of inhaled fluticasone resulted in a greater improvement in airway hyperresponsiveness than oral prednisolone.

Additionally—and to our surprise—we found a comparable decrease in serum cortisol levels with fluticasone 1000 µg twice daily and oral prednisolone 30 mg/day. A radioimmunoassay (RIA) method was used to determine serum cortisol suppression in blood with high doses of inhaled fluticasone resulting in high levels of cortisol by RIA severely underestimates cortisol in the presence of prednisolone. Consequently, we believe that RIA is not appropriate for monitoring systemic activity.

The upper and lower limits of measurement were found to be 688 and 6.9 nmol/l, respectively, and the coefficient of variation ranged from 5.6% to 7.0%.

For RIA analysis samples were homogenised and diluted at +60°C. 100 µg of 1000 µg H’ (1000 Bq/100 µl) cortisol solution was added to all serum samples after which 0.2 ml of a pooled normal rabbit antiserum was added. The sensitivity of the assay was 15 nmol/l and the coefficient of variation ranged from 5% to 8%.

The number of patients with cortisol levels (fig 1) was 28 for FP2000, 33 for oral prednisolone, and 33 for FP500. There were no significant differences at baseline between the groups or between the methods of cortisol measurement. Both treatment with FP2000 and with oral prednisolone significantly reduced serum cortisol levels (fig 1), but suppression of serum cortisol in the oral prednisolone group using the HPLC method (–34%, fig 1) was significantly larger than with the RIA method (–34%, fig 1). As expected, the difference between the cortisol levels measured by RIA and HPLC increased with higher serum prednisolone concentrations (data not shown). The difference is fully explained by the fact that serum prednisolone levels were not separately identified from cortisol by the RIA method.

This crossreactivity of prednisolone with cortisol can differ considerably between laboratories and with the RIA method (monoclonal or polyclonal), but is always present and ranges from 10% to 100%. There were no significant differences in the change in serum cortisol levels between the HPLC and RIA methods in the inhaled fluticasone groups (FP2000 and FP500).

We conclude that determination of serum cortisol by RIA severely underestimates serum cortisol suppression over a range of 6.9–690 nmol/l serum cortisol in the presence of prednisolone. Our study shows that cortisol suppression in the presence of prednisolone should not be assessed by conventional RIA.


Smoking cessation

We welcome the study by Pelkonen et al as a further contribution to our knowledge base on smoking cessation and its effects on pulmonary function and mortality. We feel, however, that some shortcomings in the methodology may bring into question the magnitude of the results.

Our main concern relates to the difficulties in quantifying levels of tobacco exposure. Since tobacco consumption is a continuous variable, confounding factors may occur within each group when categorised too broadly. More information about duration and levels of smoking would help to avoid this problem. No information is given about whether intermittent quitters returned to original habits or resumed smoking at reduced levels. Beneficial effects described in this group could therefore be due to extended periods of decreased tobacco consumption rather than a period of abstinence.

There are no data provided on smoking status from 1974 to 1989. If large numbers of those classed as intermittent quitters had permanently stopped smoking by this time, the value of temporary quitting would be overestimated. Furthermore, no data exist on the duration of periods of abstinence among intermittent quitters. If a significant proportion of this group exhibited prolonged periods of smoking cessation, the relevance of this study to short term quitters is debatable.

Even accepting the beneficial effects of intermittent quitting, we question the importance of this finding in a public health setting. Surely the main benefit of smoking cessation must remain the same: permanent smoking cessation should remain the goal and is superior to intermittent quitting. However, we recognise that this finding could provide encouragement to those who have relapsed following
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.

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References
2 Leon DA. Failed or misleading adjustment for confounding. Lancet 1993;342:479–81.

Authors’ reply
Lorna Dunn and Alleen Ogilvie make an important point that the confounding effect of intermittent quitting 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 abstinence rather than by the periods of abstinence 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 abstinence.

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 3 years. Intermittent quitters were either baseline past smokers who smoked at least once in the previous week or 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 or both examinations (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 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 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 abstinence.

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. Moreover, 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 abstinence, although we agree that the main goal should be permanent smoking cessation.

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Fibrosing alveolitis in patients with RA

We read with interest the paper by Dawson et al. 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 a prevalence of 11% in never smokers compared with current smokers at 20% in unselected patients with RA not studied with HRCT scanning. Cigarette smoking is associated with ILD and smoking, as shown by others, is not significantly different between RA associated ILD and smoking.

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 3 years. Intermittent quitters were either baseline past smokers who smoked at least once in the previous week or 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 or both examinations (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).

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

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References

Authors’ reply
We are pleased to receive the letter from Saravan and Kelly in response to our recent publication in Thorax. 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 study and no HRCT based study has shown a statistically significant association between RA associated FA and smoking. In the study by Cortet et al. 68 patients with RA were prospectively studied with HRCT scanning and 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 with idiopathic interstitial lung disease (ILD) and a prevalence of 20% of ILD (17% ground glass pattern and 2.9% reticulon pattern) was still found. It is true that in our study the absolute risk of ever smoking cannot be excluded as a factor for RA 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. 1 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 RA. We are sure this will provide very helpful information about the progression of RA 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. 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-Leissl et al. 2 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. 3 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 4 and without RA. 5 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 honeycomb.

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References

Measuring granulocyte apoptosis in airway inflammation

We read with interest the paper by Turlej et al describing enhanced survival of lung granulocytes in a 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. 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 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 function5 and this must be borne in mind when interpreting these results. Additionally, BAL granulocytes from both 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 equine 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.

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References

Authors’ reply

We thank Dr Kelly and colleagues for their interest in our paper. In the past equine neutrophils were isolated by use of a density gradient. Density gradient methods are 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.1 The interpretation of the various staining patterns is controversial. In addition, at least two methods should be used to confirm apoptosis,2 and only one is used in the study.

It is clear that equine heaves is very close to atopic hyper-responsiveness, and the late asthmatic response.

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References
Smoking cessation

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