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. After performing four measurements of cortisol, 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 corticosteroid treatment, as in most studies published to date. However, prednisolone and its metabolites are known to be chemically similar to serum cortisol and might therefore interfere with cortisol measurements by RIA. Analytical methods involving chromatographic separation of cortisol from prednisolone and its metabolites, such as high performance liquid chromatography (HPLC), circumvent this problem of interference.

We compared serum cortisol measurements by both conventional RIA and by HPLC in the same study, which was of a double blind, double dummy, three arm parallel group design. Patients received either oral prednisolone (30 mg/day), fluticasone propionate 1000 µg twice daily (FP2000), or fluticasone propionate 250 µg twice daily (FP500), both by Diskhaler dry powder inhalation. Measurements at the start of the study and after 2 weeks of treatment were performed at the same time in the morning.

The Gilson ASTED (automated sequential trace enrichment of dialsates) system was used followed by separation with HPLC and detection by UV absorbancy. 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 +6°C. 100 µg/1000 µg high (1000 Bq/100 µl) cortisol solution was added to all serum samples after which 0.2 ml of a polyclonal 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 samples available for both RIA and HPLC was 18 for FP2000 and 23 for oral prednisolone, and 18 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 (–72%) was significantly larger than with the RIA method (–34%). As expected, the difference between the cortisol levels measured by RIA and HPLC increased with higher serum prednisone concentrations (data not shown). The difference was fully explained by the fact that serum prednisone 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) used, 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.

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References

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 as to 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 abstention 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 healthcare message 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...
References


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Authors’ reply

Lorna Dunn and Aileen Ogilvie make an important point that the confounding effect of tobacco consumption on 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 FEV0.75 during the first 15 years of abstinence 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 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 FEV0.75 during the first 15 years was significantly less 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 FEV0.75 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 al1 on the prevalence of fibrosing alveolitis (FA) diagnosed by HRCT scanning in rheumatoid arthritis (RA). The 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 22% 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 al3 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 studies4 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 RA 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 in our institute 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 prevalent in RA patients with ILD. The presence of rheumatoid factor appears to be protective against honeycombing in both groups. These differences are to be expected in clinical and HRCT features may be important predictors of outcome.

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Authors’ reply

We are pleased to receive the letter from Saravanan and Kelly in response to our recent publication in Thorax.1 They posed the question concerning the duration of periods of smoking after periods of abstention rather than by the periods of abstention per se. The study by Cottet et al2 showed no significant association between smoking and ILD in RA. In the study by Cottet et al2 68 patients with RA were prospectively studied with HRCT scanning and 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 shown linking smoking to 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 CT and, in addition, were smoking ex-smokers (4 no pneumo, bialar clacrles, restrictive pulmonary function tests, and chest radiographic changes of FA. We are sure this will provide very valuable information that 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. One 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 al6 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 al2 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 RA. We agree with the authors that further work on the natural progression of RA 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 in our institute with a cohort of patients with cryptogenic fibrosing alveolitis (CFA) matched for age, sex, smoking, and respiratory symptoms. 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 are to be expected in clinical and HRCT features may be important predictors of outcome.

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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 an animal model of asthma. 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. 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. The interpretation of the various staining patterns is controversial. In addition, at least two methods should be used to confirm apoptosis, 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 and this must be borne in mind when interpreting these results. Additionally, BAL granulocytes from horses were isolated by use of a density gradient. Density gradients 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+, PI+ and it is surprising to find 40% apoptotic (AV+) cells in a population where nearly all the cells (>90%) are TB–.

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