

Correspondence

Consideration of Important Preanalytical Conditions for the Assessment of Circulating Matrix Metalloproteinase-9

To the Editor:

We read with interest the recent article of Tazaki and colleagues (1) describing increased activity of matrix metalloproteinase-9 (MMP-9) in blood of patients with obstructive sleep apnea syndrome (OSAS). The significance of blood collection as an important preanalytical determinant has not been taken into account by the authors since there is evidence that blood sampling markedly determines the concentration of circulating MMP-9. These facts have previously been discussed in analytical journals (2, 3).

Studies from our own laboratory demonstrate the importance of a standard procedure for the collection of specimens for MMP measurement in blood. A report of our own results on the effect of different blood sampling tubes on MMP-9 measurement is shown in Figure 1. Briefly, venous blood samples from eight healthy volunteers were simultaneously collected in different devices for preparation of serum samples. The tubes were centrifuged within 30 minutes after venipuncture at $1,600 \times g$ for 15 minutes at room temperature. The MMP-9 concentration was measured in the supernatants using commercially available ELISA kits (Medac Diagnostika, Wedel, Germany). MMP-9 concentrations in serum samples collected in tubes with clot activator were about threefold higher than in pure serum samples and also higher than the concentrations found in plasma samples (Figure 1).

As platelets and leukocytes contain high concentrations of MMP-9, the varying release of MMP-9 from blood cells during the sampling process could cause these differences (3). These important conditions should be considered in the interpretation of increased MMP levels. Tazaki and coworkers did not clearly distinguish between serum or plasma samples and that may lead to misinterpretation of their results. Recently, the use of blood samples collected with sodium citrate was suggested to avoid the detrimental effect of other anticoagulants or serum and to optimize the diagnostic validity of MMPs in peripheral blood (4).

Conflict of Interest Statement: M.J. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.J. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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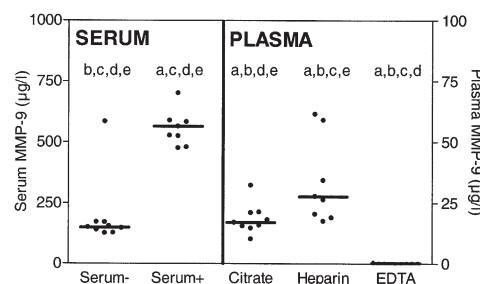


Figure 1. MMP-9 concentrations in serum and plasma. Median values of MMP-9 in samples prepared from blood of eight healthy adults. *Left (serum):* serum–, pure serum prepared in Monovette tubes without additive; serum+, serum prepared in tubes containing kaolin-coated granulate as clot activator. *Right (plasma):* Citrate, heparin, and EDTA, plasma prepared in tubes coated with sodium citrate, lithium heparin, or K-EDTA. Significant differences of at least $p < 0.05$ (ANOVA, repeated measures; posterior test according to Tukey) between the samples are indicated by the following symbols: (a), from serum–; (b), from serum+; (c), from plasma-citrate; (d), from plasma-heparin; (e) from plasma-EDTA.

From the Author:

We thank Drs. John and Jung for their comments on our article (1). We agree that the significance of blood collection is an important preanalytical determinant for the measurement of the concentration of circulating matrix metalloproteinase (MMP)-9. In our study we measured the serum levels of MMP-9 and compared levels between obese control subjects and patients with obstructive sleep apnea syndrome. Serum samples were collected using tubes without clot activator from all subjects. Serum levels of MMP-9 were measured with an ELISA kit obtained from Amersham Biosciences that can detect the pro-MMP-9 and pro-MMP-9-TIMP-1 complexes. The MMP-9 detection kit had < 3% cross-reactivity with pro-MMP-1, pro-MMP-2, and pro-MMP-3. We agree that MMP-9 levels may differ significantly between serum and plasma samples. In addition, it will be important to investigate the effect of different blood sampling techniques on the activity of circulating MMP-9.

Conflict of Interest Statement: K.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this letter.

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GOAL—Asthma Control, but at What Cost?

To the Editor:

The method chosen by the investigators in the Gaining Optimal Asthma Control (GOAL) study (1) to measure systemic side effects of mostly high dosages (< 1,000 µg/day) of the inhaled

steroid, fluticasone, 24-hour urinary cortisol, is insensitive, unreliable, and not reassuring. Unfortunately, it has already been demonstrated in a study sponsored by the manufacturers of fluticasone that there is no dose/response relationship for this drug and 24-hour urinary cortisol or 0800 hours cortisol (2), the two methods nearly always used in the "extensive safety data."

When a sensitive measure of hypothalamic-pituitary-adrenal (HPA) axis function is used, the picture is entirely different. For example, in a study for the National Heart Lung and Blood Institute's Asthma Clinical Research Network, using plasma cortisol AUC, a dosage of fluticasone of only 352 µg/day produced 50% adrenal suppression, a considerably greater degree than the equipotent dosage of beclomethasone, leaving the authors to conclude that "increasing the dose beyond this point of maximum efficacy ... resulted in increase in systemic effect especially with fluticasone metered dose inhaler with its CFC propellant" (3). Any clinical improvement at higher doses could well be due to the systemic rather than the local effect of this inhaled corticosteroid. Remember that the systemic effect of 1,000 µg/day of fluticasone has been estimated as equivalent to 11 mg of oral prednisolone (4).

The clinical significance of such findings is confirmed by our report that fluticasone was associated with nearly all cases of adrenal crisis due to inhaled corticosteroids in the UK, despite it being the least prescribed (5), and in the Health Canada database, where all nine cases of adrenal insufficiency (including two with adrenal crisis) reported between 1996 and 2002 were associated with the use of fluticasone (6).

Failure to demonstrate "clinical evidence of adrenal suppression" after 1 year's study is neither convincing nor reassuring. Except in some children, the clinical features of adrenal insufficiency are of insidious onset, nonspecific, and difficult to recognize. Also, in our study, 75% of patients developed adrenal crisis more than 1 year after starting fluticasone (5), longer than the duration of this study.

It should cause considerable concern not only that this study involves a large majority of patients ending up on doses of fluticasone double the probable maximum effective dose, but also, once again, the tests used to measure systemic effect are insensitive and unreliable.

Conflict of Interest Statement: G.R.G.T. received lecture fees totaling \$12,000 from AstraZeneca in 2002 and he has had no financial relationship with any commercial entity that has an interest in the subject of this letter since then.

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From the Authors:

Dr. Todd's letter focuses on suppression of the HPA axis caused by high doses of inhaled corticosteroids. His concerns, summarized in the last paragraph of his letter, are of limited relevance to the GOAL study (1).

They suggest that patients ended up on fluticasone propionate at "double the probable maximum effective dose," and cite evidence from dose-response studies in which single measures (usually FEV₁) serve as the endpoint (2). In GOAL, the dose was individualized (increased at intervals) to obtain the maximal clinical response, and a composite measure, which is more discriminating of control, was used (3). The dose was not excessive as it was increased according to clinical need. The results confirm that some patients only achieve control with the highest dose, at times only after 3 to 6 months. These results raise questions about conventional methods of assessing dose-response relationships of corticosteroids in chronic uncontrolled asthma.

GOAL examined a fundamental question—whether asthma control can be achieved. Practical limitations of design prevented inclusion of dose-minimization strategies (e.g., step-down routines). However, the highest dose of corticosteroids employed was one approved and widely used in adults. The results provide useful evidence on the superiority and steroid-sparing effects of combination treatment. We recognize that the question of how hard to try in individual patients to achieve control (with respect to dose and duration of treatment) remains a challenge. The decision is based upon patient needs and expectations, cost, and potential adverse effects.

A second concern of Dr. Todd is the "insensitive and unreliable" tests of HPA axis function. We accept that 24-hour urinary cortisol are less sensitive than the ACTH stimulation test, but the latter was not a practical option in a large scale outpatient study, and is not without risk. The 24-hour urinary cortisol method is superior to single point measurement of blood cortisol, widely used and accepted by regulatory authorities (4, 5).

The references on adrenal suppression and crisis cited by Dr. Todd are not relevant to GOAL. The second is of unusual design, two involved use of a pressurized metered dose inhaler (MDI) that results in higher systemic exposure to corticosteroid than the Diskus device, and one concerned mainly children. Of the four adults with adrenal crisis, all were on doses higher than used in GOAL. The clinical relevance of the reductions in urinary cortisol in some patients in the GOAL study remains controversial, and must be weighed against the risk of exacerbations, and the "cost" of continued uncontrolled asthma.

Conflict of Interest Statement: E.D.B. received honoraria for speaking at scientific meetings and courses organized and financed by AstraZeneca, Boehringer Ingelheim and GlaxoSmithKline and served on Advisory Boards for the following companies: AstraZeneca, Boehringer Ingelheim, Hoffman le Roche and GlaxoSmithKline. He performed consultancy services for AstraZeneca, Aventis, Altana, Hoffman le Roche and Kyowa Hakko.

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Penh Is Not a Validated Technique for Measuring Airway Function in Mice

To the Editor:

We read with interest the recent article by Kumar and colleagues outlining an important benefit of blocking proinflammatory cytokines in a mouse model of chronic asthma (1). The clinical experience with drugs that block Th-2 cytokines, particularly interleukin (IL)-5, has been disappointing, to the point that clinical development of these drugs has essentially been dropped (2). Chronic asthma is essentially a developmental disease that begins in childhood. We have argued that the development of asthma should be considered a two-phase process consisting of an initiation stage, which essentially occurs in childhood and during which allergen-induced Th-2-mediated inflammation is the major driving process, followed by a consolidation phase in which chronic inflammation induces a self-sustaining cycle of airway remodeling, heightened responsiveness, and airway inflammation that results in chronic asthma in adulthood (2). Viewed in this light, the impressive results obtained by Kumar and coworkers (1) are not surprising, in that while the chronicity of exposure in their model (3) is a significant improvement over the relatively acute exposure of many other models, this is still likely to represent the Th-2-driven initiation phase of asthma in childhood.

There is, however, one aspect of Kumar and colleagues' report (1) that is potentially open to misinterpretation and on which we wish to comment. They used enhanced pause (Penh) to approximate lung function and bronchial responsiveness in their mice. Penh has fallen into disrepute as a measure of lung function, as elaborated by a group of 22 internationally respected respiratory physiologists (4). Kumar and coworkers do highlight this issue but go on to state: "In our chronic model, we have previously shown that the observed changes in Penh closely correlate with increased specific airway resistance as measured by the forced oscillation technique . . . (19)." We would like to point out that the previous study referred to as their Reference 19 (see Reference 5 herein) did not use unrestrained plethysmography and hence did not compare Penh with direct measurements of

airway function, using forced oscillations. While that study (5) did show that when inflammation was largely specific to the airways it did result in heightened responsiveness that was confined to the airways, the fact that the two studies (1, 5) both reported similar phenomena cannot, in any way, be taken as supporting the use of Penh as a measure of airway function or bronchial responsiveness. Penh will be influenced by any factor that alters breathing pattern. While this includes changes in airway function, it can also include many other factors, including change in respiratory drive (4). While not detracting in any way from the findings presented by Kumar and coworkers (1), it would be wrong for readers of the *Journal*, especially those not trained in respiratory physiology, to conclude from their article that Penh was validated by a sophisticated and specific measurement of airway resistance and, consequently, could be used to represent airway function in mouse models of asthma.

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Dr. Kumar was given the opportunity to respond to this letter but declined to do so.