Commentary on: Differences in Nucleotide Hydrolysis Contribute to the Differences between Erythrocyte 6-Thioguanine Nucleotide Concentrations Determined by Two Widely Used Methods

To the Editor:
In their article, Shipkova et al. (1) present evidence to indicate that the concentration of erythrocyte thioguanine nucleotides (TGNs) measured by the method of Lennard (2) differ considerably from those measured by the method of Dervieux and Boulieu (3). The assay method studied by Shipkova et al. was superseded over a decade ago for studies of thiopurine metabolism (4). However, Shipkova et al. have made a valid observation. Both assays (2, 3) are based on the same general principle, hydrolysis of the thionucleotide back to the parent thiopurine before measurement of the liberated thiopurine by HPLC. Shipkova et al. (1) attribute the differences in the TGN concentrations measured, in part, to the hydrolysis protocols of the two methods. However, despite the differences recorded in the TGN concentrations measured, there was an excellent and highly significant correlation between the two methods.

For the optimization of thiopurine therapy, Shipkova et al. (1) make a plea for method-independent therapeutic ranges and the standardization of analytical procedures. However, the former is difficult when the main obstacle is the lack of commercially available, quality-assured thiopurine standards. To investigate thionucleotide hydrolysis, we initially used a commercially available thionosinic acid (6-mercaptopurine riboside 5'-monophosphate) preparation and studied 6-mercaptopurine yield (5). We eventually obtained a small sample of 6-thioinosine 5'-monophosphate, from what was then Burroughs Wellcome, and confirmed the time and conditions of the acid hydrolysis to 6-thioguanine (6). These conditions were maintained in subsequent developments of our assay (2, 4) and reconfirmed in more recent times (7).

That the hydrolysis conditions may differ when the in vivo product of drug metabolism is a mixture of mono-, di-, and triphosphates comes as no surprise.

However, this hydrolysis procedure is not the only step that needs to be considered in the analysis of thionucleotide metabolites. The thiopurine thiol moiety is easily oxidized; thus, the thiopurine metabolite of interest is degraded (8). The spectrum of thiopurine metabolites that can be measured by our assay methodology (thioguanine, 8-hydroxymercaptopurine, mercaptopurine, 8-hydroxythioguanine, thioxanthine, and thiouric acid plus methylthioguanine and the methylmercaptopurine metabolites) are safe from oxidation and stable for several days when back-extracted or reconstituted in 0.1 mol/L HCl. Our reversed-phase isocratic HPLC system (4) shows no deterioration in performance from repeat injections (>1000) of 50 μL of this acid. One of the most important features of our assay methodology, which is also reported in the method of Dervieux and Boulieu (3), is the quantification, in a single injection, of the TGNs and the methylmercaptopurine nucleotide metabolites of 6-mercaptopurine (4). These metabolites are used as compliance indicators in our studies of oral 6-mercaptopurine therapy (9).

To conclude, quantification of TGN can differ among laboratories, and method-specific therapeutic ranges are required for the interpretation of the results generated. The lack of commercially available thiopurine metabolite standards hinders progress in standardization.

References

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To the Editor:
We thank Lynne Lennard for her interest and commentaries on our article (1). We appreciate the support of our effort to draw attention to the method dependency of therapeutic ranges currently used for therapeutic drug monitoring of thiopurine drugs.

We would, however, like to discuss some of Lennard’s comments. Although the assay used in our investigation (2) was superseded by an improved version, the modified method differs only in its capability to detect methylated thiopurine metabolites such as 6-methylmercaptopurine (3). The hydrolysis conditions, which were the focus of our work, are absolutely identical. We chose the basic assay (2) for three reasons: (a) this procedure has been routinely used in our laboratory for many years; (b) there is good comparison between the 6-thioguanine nucleotide (6-TGN) concentrations measured with both procedures (un-
published own observations); and (c) 6-TGNs are considered the active thiopurine metabolites, whereas the role of 6-methyl-mercaptopurine for therapeutic drug monitoring is still a matter of debate (except for compliance).

We agree that oxidation of the thiopurine thiol moiety is also a critical point in the analysis of thionucleotide metabolites. This point certainly requires more attention when method harmonization is intended. Although we concentrated on the hydrolysis step, we addressed the question of oxidation in our investigation by including a set of experiments on the effect of increasing concentrations of the antioxidant dithiothreitol on 6-thioguanine (6-TG) recovery. Higher dithiothreitol concentrations were associated with enhanced 6-TG recovery (1).

The aim of our publication was to draw attention to method-dependent therapeutic ranges that preclude a comparison of results from clinical studies based on different methods. A considerable number of methods for the measurement of 6-TGNs have been published, and new methods or modifications of existing assays are still being generated. As discussed in our report, these methods differ in several analytical steps, including the choice of matrix, the analytical procedure, and the way results are reported. However, these new or modified methods have usually not been compared with an established procedure. In addition, there is unfortunately no external quality-control system available that could provide information about comparability of results. The major problems for a real standardization include the lack of a reference method and an approved and appropriate reference material such as a 6-TGN standard. We also agree that the matter is complicated by the fact that the product of drug metabolism in vivo is a mixture of mono-, di-, and triphosphates. However, according to our experience, many physicians involved in the care of patients receiving thiopurine drugs, as well as laboratory involved in therapeutic drug monitoring of these patients, do not have sufficient information about the method dependency of the reported 6-TGN concentrations and may be misled when comparing individual values with therapeutic ranges derived from the literature. To improve the comparability between the different methods, it is therefore important that efforts be made to find a consensus on the analytical conditions as well as the format of result reporting.

References

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*Natricuretic Peptides (NPs): Automated Electrochemiluminescent Immunoassay for N-Terminal pro-BNP Compared with IRMAs for ANP and BNP in Heart Failure Patients and Healthy Individuals

To the Editor:

We evaluated the performance and diagnostic accuracy of an electrochemiluminescence immunoassay (ECLIA) method for N-terminal pro-B-type natriuretic peptide (NT-proBNP) in healthy persons and in patients with cardiac disease, and then compared the results obtained with the ECLIA method with results from IRMA methods for BNP and atrial natriuretic peptide (ANP).

We studied 58 healthy individuals [mean (SD) age, 58 (8) years; 19 women and 39 men] and 148 consecutive patients [mean age, 64 (13) years; range, 20–80 years; 47 women and 101 men] with cardiomyopathy, admitted to the Department of Cardiovascular Medicine of our Institute. The study was done from November 2001 to October 2002. All healthy participants were nonobese, normotensive, and free from acute diseases, and all denied the use of any drug during the 4 weeks before the study. All had normal values for the main plasma indices and nonpathologic erythrocyte and leukocyte counts and urine analysis. In all of the participants, a complete cardiologic examination, including electrocardiogram and echocardiographic investigation (left ventricular ejection fraction >55%), was performed; in patients >50 years of age, an effort stress test was performed to exclude asymptomatic heart disease. Cardiac morphology and function were assessed in all patients by Doppler echocardiography, radionuclide ventriculography, or cardiac catheterization, when needed. Primary dilated cardiomyopathy was found in 95 patients, whereas the other 53 patients suffered from secondary cardiomyopathy; of these, 38 had ischemic cardiomyopathy. A total of 22 patients were in functional New York Heart Association (NYHA) class I, 72 in class II, and 54 in class III–IV; the mean (SD) left ventricular ejection fraction was 31.4 (9.6)%.

NT-proBNP was measured by a fully automated “sandwich” ECLIA method using an Elecsys® 2010 analyzer (Roche Diagnostics). This ECLIA is based on two polyclonal antibodies: a biotinylated antibody and a ruthenium derivative-labeled antibody. Total duration of assay was 18 min. Plasma ANP and BNP