chemotherapy may be associated with increased concentrations of GAPDH mRNA. Because circulating GAPDH mRNA in cancer patients was thought to originate in apoptotic cancer cells *(2, 5)*, the increased concentrations of GAPDH mRNA detected may be caused by apoptosis in the placental residue in our case.

We conclude that real-time quantitative RT-PCR is a sensitive method to monitor changing mRNA concentrations resulting from apoptotic effects in the placenta and to evaluate invading conditions of the trophoblastic villus.

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Clinical Relevance of Biological Variation of B-Type Natriuretic Peptide

To the Editor:

The cardiac natriuretic hormones (CNHs), atrial (ANP) and B-type natriuretic peptide (BNP), are peptides produced and secreted by the atrial and ventricular cardiomyocytes, respectively. ANP and BNP share potent diuretic, natriuretic, and vascular smooth-muscle-relaxing effects and interact with the hormonal and nervous systems *(1–3)*. Stimulation by hemodynamic overload, as well as by several neuroendocrine (including α-adrenergic agonists, angiotensin II, endothelins, and vasopressin) and immunologic agents and by growth factors, modulates the production of CNHs by the human heart *(1, 2)*. CNHs and their respective counter-regulatory neurohormonal systems (including the adrenergic, endothelin, and renin-angiotensin-aldosterone systems) form a physiologic negative feedback mechanism that regulates cardiac output by controlling blood pressure and heart rate as well as by fluid and electrolyte homeostasis *(1, 2)*.

The clinical relevance of measurement of BNP and its related peptides as diagnostic tools and prognostic markers in patients with cardiovascular diseases has been confirmed recently *(2, 3)*. To achieve correct interpretation of serial test results that are collected for follow-up or for tailored treatment of heart failure patients, several studies *(4–8)* recently evaluated the biological variation of BNP and its related peptides in both healthy persons and cardiac patients.

Because of secretory bursts and its rapid turnover (half-life ~15–20 min), it is not surprising that intraindividual biological variations of plasma BNP concentrations were found to be very large in both healthy persons and in patients with heart failure (ranging from 30% to 50%) *(4, 6, 8)*. Assuming a random variation around a homeostatic set point, the calculated reference change values at 95% confidence ranged from 99% to 130% for BNP in healthy persons and patients *(4, 6–8)*. According to this estimated confidence interval, only a decrease >50% or an increase greater than twofold in plasma BNP should be assumed to be statistically significant in an individual patient.

In contrast to this assumption, a recent clinical trial *(9)* has suggested that a BNP decrease less than this calculated reference change could be clinically relevant in patients with heart failure. In that study, only the group of patients treated with the beta-blocker agent carvedilol, who responded on average with a decrease of only 38% in plasma BNP, showed a clinical improvement *(9)*. Furthermore, several studies have demonstrated that cardiovascular risk (mortality or major cardiovascular events) increases continuously and progressively throughout the whole range of BNP concentrations in patients with cardiovascular diseases (i.e., an absolute threshold for risk cannot be determined) *(2)*.

To explain these conflicting findings, it should be noted that BNP secretion is closely regulated by specific pathophysiologic mechanisms. Thus, changes in plasma hormone concentrations cannot be interpreted as random variations around a set point, but as strictly determined by the activation point of the counter-regulatory system and by changes in

hemodynamics. Clinicians should look at intraindividual variations in BNP as a mirror of variation in neuroendocrine network activity.

According to this hypothesis, we suggest that all changes in BNP concentration should potentially be considered as clinically relevant, even when they are narrower than the calculated intraindividual biological variation, because these variations reflect changes in activation of the neuroendocrine system as a result of specific pathophysiologic mechanisms. In other words, BNP variations should be interpreted and considered by physicians, in the same manner as variations in heart rate and blood pressure, by taking into account clinical history and examination, including response to specific treatments, as well as laboratory and instrumental test findings.

There is another important practical consequence of this approach. At an intraindividual biological variation of ~30%, the estimated imprecision goal for BNP immunoassays should be 15% (i.e., equal to one half of the intraindividual variation) *(10, 11)*. On the contrary, we suggest that all measured variations of plasma BNP >3 times the analytical imprecision of the assay used (i.e., 3 SD of the assay variability) should potentially be considered as clinically relevant. According to this approach, the assay imprecision of BNP immunoassays should be as low as possible. A recent study from our laboratory evaluated the analytical imprecision of several commercial BNP immunoassays *(12)*. On average, this study demonstrated that analytical performance, as well as reference and decision values, varied greatly among the BNP assay methods; in particular, the imprecision of these immunoassays varied, on average, from ~15% (the worst) to 5% (the best) *(12)*. Evidently, the immunoassay with the best imprecision is able to detect significantly narrower changes in BNP concentrations (i.e., variations of ~15% for the method with the lowest imprecision).

We suggest that only clinical criteria should be used to evaluate the pathophysiologic relevance of the measured variation in BNP concentration (defined as greater than 3 SD of assay variability) in an individual patient. Of course, the large number of pathophysiologic mechanisms affecting the CNH system may make it difficult for clinicians to recognize the cause(s) of variations in that system's activity. However, we believe that BNP assays should be considered as an intellectual stimulus in the search for explanations of the variations in hormone concentrations and their relationship to pathophysiologic stimuli and/or pharmacologic interventions. In this sense, assessment of the time course of BNP concentrations may be a novel, meaningful diagnostic tool for the follow-up of patients with cardiac diseases.

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N-Terminal pro-B-Type Natriuretic Peptide Concentrations in Mothers just before Delivery, in Cord Blood, and in Newborns

To the Editor:

The role of the heart as an endocrine organ was established in 1981 by de Bold et al. *(1)*. Usually, in adults, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are secreted mainly by the cardiac atria and ventricles, respectively *(2)*. Plasma concentrations of these peptides, particularly that of BNP, have been shown to reflect cardiac dysfunction and volume overload in adults and children *(3–8)*.

There is evidence that these peptides have possible roles during fetal life in the regulation of organogenesis of the heart and the cardiovascular system, in the regulation of blood pressure and water balance in the developing embryo, and in the transition from fetal to extra-uterine life *(9)*. BNP may also have an important role in the regulation of amniotic fluid volume *(10)*.

Sparse data exist regarding N-terminal proBNP (NT-proBNP) concentrations in umbilical cord blood and in the newborn during the first days