AxSYM were above reference values (0.5 and 0.6 μg/L), whereas the Stratus CS yielded negative results (<0.08 μg/L). On the other hand, in contrast to the negative results on AxSYM, values between 0.08 and 0.4 μg/L on the Stratus CS were found in five samples from patients with myocardial damage. One of these patients was in the early phase of AMI (the two methods indicated positive results 2 h later), two had unstable angina, and two others were cardiac surgery patients (early postoperative period). Consequently, for all discordant results, the Stratus CS was in agreement with the clinical diagnosis. There was no discrepancy between the methods for results >2.0 μg/L on the AxSYM.

In conclusion, when performed on samples deriving from two different tubes (whole blood and heparinized plasma) drawn from the same patients at the same time, cTnI values obtained by the Stratus CS in the entire measuring range, including values <0.15 μg/L, were statistically indistinguishable. This attests not only to the excellent precision of the method, as already stated by others (6), particularly in the low concentration range, but also to the quality of the preanalytical phase automatically performed by the system. The agreement of the results allows the system to be used on different specimens in the same patients.

References


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Real-Time PCR Assay with Fluorescent Hybridization Probes for Rapid Genotyping of the CD14 Promoter Polymorphism

To the Editor:
A soluble form of CD14 (1) activates endothelium and smooth muscle (2). CD14 binds lipopolysaccharide, the cell wall component of gram-negative bacteria. Upon lipopolysaccharide binding, monocytes produce pro-inflammatory cytokines and procoagulant activity. In view of the growing evidence for a role of infection with gram-negative bacteria (3), inflammation, and hypercoagulability in the onset of atherosclerosis, two independent studies evaluated the frequency of a genetic polymorphism within the promoter of the CD14 gene in patients with myocardial infarction (MI) (4, 5). This polymorphism consists of a single base exchange (C→T) at position 260 (4) [corresponding to position -159 in study by Unkelbach et al. (5)], with C introducing a HaeIII restriction site. The polymorphic site is located near the Sp 1 binding site of the promoter (4). An increased risk for MI in patients homozygous for the T allele was found (4, 5). Moreover, Unkelbach et al. (5) observed an even stronger association between the TT genotype and the risk for MI in patients without other risk factors such as smoking and hypertension. The odds ratio for MI in normotensive nonsmoking TT homozygotes older than 62 years was 3.8 (5).

Because perioperative MI remains a major complication in surgical patients (6), genotyping for the CD14 promoter polymorphism could become a part of preoperative risk classification of surgical patients.

The techniques reported for CD14 genotyping (restriction fragment length polymorphism and single-strand conformation polymorphism analysis) are time-consuming and require multiple manual steps. Because a high throughput of samples is desirable for future studies, we suggest a rapid-cycle PCR with fluorescently labeled oligonucleotide hybridization probes on the LightCyclerTM instrument (Roche Diagnostics) and subsequent fluorescent probe melting point analysis, which allows genotyping within 60 min.

Genomic DNA samples from 100 healthy blood donors were extracted from whole blood according to standard procedures. The reliability of the proposed assay was confirmed by restriction enzyme digestion with HaeIII.

PCR was performed in disposable capillaries (Roche Diagnostics) in a reaction volume of 10 μL containing 1 μL of DNA (20-80 ng), 0.5 μmol/L each of the primers (sense, 5’-GGTGCACAGATGAGTTCAC-3’; antisense, 5’-CTTGGCTGCTTGAGCAGTT-3’), 1 μL of reaction buffer [LightCycler DNA master hybridization probes 10X buffer (1X = 1.75 mmol/L); Roche Diagnostics], and 0.2 μmol/L each of the probes. The detection probe specific for the T allele (5’-TTCCGTTACGCGCCCT-3’) was labeled at the 3’ end with fluorescein. The anchor probe (5’-GGAGACACAGACCTAGATGCGCTGCA-3’) was labeled with LightCycler Red 640 at the 5’ end.
and modified at the 3' end by phosphorylation to block extension. The PCR conditions were as follows: initial denaturation at 95 °C for 120 s, followed by 60 cycles of denaturation (95 °C for 0 s, 20 °C/s), annealing (55 °C for 10 s), and extension (72 °C for 10 s). The melting curve consisted of 1 cycle at 95 °C for 0 s, 45 °C for 10 s, and then increasing the temperature to 95 °C at a slope of 0.2 °C/s.

The fluorescence signal (F) was monitored continuously during the temperature ramp and then plotted against the temperature (T). These curves were transformed to derivative melting curves \([-\frac{dF}{dT} vs T]\) of the three CD14 genotypes.

Representative results for the three different genotypes (TT, CT, and CC) are given in Fig. 1. In the 100 patient samples, 27% were TT, 41% were CT, and 32% were CC. The proposed technique and the restriction enzyme technique gave identical results. The assay is rapid and accurate and seems especially suited for routine laboratories that process large numbers of samples.

We thank Olfert Landt (TIB MOL-BIOL, Tempelhofer Weg 11-12, 10829 Berlin, Germany) for designing the hybridization probes.

References


Assessment of Vitamin B₁ Status

To the Editor:

The excellent report by Talwar et al. (1) promotes the measurement of thiamin diphosphate (TDP) for the assessment of vitamin B₁ status. My experience with >30 000 people supports this, but only for the investigation of untreated patients.

The TDP assay is more precise than the transketolase activation (ETK) test, and the method described is an important advance for which I thank the authors. In comparing the two methods, Talwar et al. (1) found TDP slightly advantageous in the identification of B₁ deficiency. They and most workers using the ETK test agree that the cutoff point is 25%. I have found it useful to report results in the range 15–25% as borderline. When this is done, there is little to choose between TDP and ETK in terms of clinical usefulness.

Much as I would like to use the more precise TDP assay, there is a problem that surfaces when one wishes to use the laboratory to follow repletion with thiamin. It is very rare for the TDP concentration to remain low after a few days of supplemental B₁, and in many cases, TDP normalizes after a single 100-mg dose. This is not the case for the ETK test. In some cases, several weeks of daily supplementation are needed to normalize the results.

I am in the fortunate position of receiving considerable feedback from the clinicians using our laboratory service and have carefully studied their findings in relation to the laboratory results. In my experience, it is the ETK test that parallels the clinical improvement in supplemented patients.

I support the use of the more precise TDP (HPLC method) in untreated people, but I caution against its use in following supplementation. For this, the ETK test, even with its many limitations, remains the method of choice. A more precise method for measuring this enzyme would be enormously helpful.

I hope this letter will open some further discussion on the use of func-