occurred during the active phase of dermatomyositis.

If the admission cTnT had been used as the sole cardiac marker for the initial clinical assessment, the result would have been misleading. Where Emergency Departments use admission cTnT results to assist initial clinical assessments of chest pain, we suggest that CK markers remain a useful adjunct and that cTnT results require careful interpretation in patients with dermatomyositis.

References


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LightCycler PCR Assay for Genotyping Codon 634 Mutations in the RET Protooncogene

To the Editor:

Mutations of the RET protooncogene are associated with several disorders, including Hirschsprung disease, familial medullary thyroid carcinoma ( FMTC), and multiple endocrine neoplasia type 2A ( MEN 2A) and type 2B ( MEN 2B). More than 85% of the causing mutations in MEN 2A families, TGC (Cys) to TAC (Tyr) and TGC to CGC (Arg) are by far the most prevalent. Mutations in codon 634 are also responsible for 25–30% of patients with FMTC.

Mutation analysis of RET permits
identification of MEN 2A carriers and can reduce morbidity and mortality through early intervention (6).

The methods used to detect RET mutations are time-consuming and require optimization of the PCR to avoid nonspecific PCR products that may interfere with the result. We used real-time PCR and melting curves on a LightCycler (Roche Molecular Biochemicals) to analyze the two most frequent RET mutations, C634Y and C634R, and the less frequent C634S (TGC to TCC).

All subjects had been genotyped previously by sequencing of exon 11 of the RET protooncogene. DNA was isolated from peripheral blood leukocytes by standard methods. The primers used for the amplification were RET 11 Forward (5'-CTCTGGCAGCAGCT-3') and RET 11 Reverse (5'-CTGACCGGAAAGTG-3'), which gave a 217-bp PCR product. The wild-type probe was synthesized from codon 624 to codon 637. The sequence of the anchor probe, labeled with FAM, was 5'-ACGGGCACAGCTC-3', and the sequence of the anchor probe, labeled with LC-Red, was 5'-TCCGACAGTGAATCTTGGTG-3'.

PCR reactions were performed in a total volume of 20 μL in the LightCycler glass capillaries. The reaction mixture contained 9.6 μL of distilled water, 2.5 μL of MgCl₂ (25 mM), 1 μL of each primer (10 μM), 1 μL of each probe (4 μM), 2 μL of DNA-Master Hybridization Probes (Roche Molecular Biochemicals), and 2 μL of genomic DNA (100–500 ng). We used the master mixture for all samples (to reduce sample-to-sample differences) and a control without DNA. PCR conditions were as follows: initial denaturation at 94 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 0 s, annealing at 68 °C for 5 s, and extension at 72 °C for 10 s. After amplification, the melting analysis was performed by denaturation at 94 °C for 0 s, annealing at 50 °C for 0 s, and increasing the temperature to 90 °C with a ramp rate of 0.5 °C/s. The fluorescence emitted was measured during this process, and the melting curves (F/T) were automatically converted to melting peaks (−dF/dT).

The wild-type melting curve showed a single peak at 69.5 °C (Fig. 1), whereas each of the mutation carriers showed two different peaks, one for the wild-type allele at 69.5 °C and a lower one for the mutated allele. The T-to-C transition of the C634R mutation produced a melting peak at 61.5 °C, the G-to-A transition of the C634Y mutation produced a melting peak at 60 °C, and the G-to-C transversion of the C634S mutation produced a melting peak at 58 °C. The assay variation of the melting temperatures was assessed. The interassay variation (CV) was <1.5% and the intraassay variation was <0.5% for the wild-type and the mutant alleles studied.

This method has several advantages over other methods used. The possibility of contamination is reduced because no post-PCR handling is necessary. Nonspecific PCR products will not affect the result because they will not be recognized by the probes. Finally, this method is very rapid and reduces labor and reagent costs. We think it is useful in the screening of RET for the most common mutation in MEN 2A and especially to establish the carrier status in members of families with MEN 2A and FMTC already characterized as having the 634 mutation. All family members can be analyzed simultaneously and in a very short time.

We are grateful to Dr. Josep Oriola (Hospital Clinic, Barcelona, Spain) for providing us with DNA samples from patients affected with MEN 2A and to Dr. Olbert Landt (TIB MOBL, Berlin, Germany) for design of the probes.

References

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S100B Protein Concentrations in Urine Are Correlated with Gestational Age in Healthy Preterm and Term Newborns

To the Editor: S100B is an acidic calcium-binding protein concentrated in the nervous system, where it is located mainly in glial cells (1). Measurements of the protein in cerebrospinal fluid and blood are used to detect brain distress, both in infants and in adults (2, 3), and in the perinatal period are correlated with brain maturation (4). Because collecting urine is a simpler procedure than collecting cerebrospinal fluid or blood, especially in infants in whom anemia of premature infants attributable to blood sampling is common, this study investi-