Supplemental Data

Materials and Methods

Chromogranin A assay details.

Calibrators, controls, and patient samples are incubated overnight with beads coated with an anti-human CGA mouse monoclonal antibody (clone LK2H10, Lab Vision, CA), followed by incubation with a second, acridinium ester-labeled anti-human CGA mouse monoclonal antibody (clone PHE5, Lab Vision). The resulting relative light units are directly proportional to the CGA concentrations. The assay is calibrated against the culture supernatant of a human neuroblastoma cell line, which in turn is standardized against human recombinant CGA (CIS-Schering, France).

The assay has an imprecision of <10% CV across a CGA concentration range of 13-194 ng/mL. The functional sensitivity of the assay was determined using low patient sample pools and found to be ~5.0 ng/mL (15.4% CV at 5.3 ng/mL). The linear range was established to be 5-400 ng/mL. All values >400 ng/mL are repeated at a dilution. The reference range of the assay was established by testing samples from 177 healthy adults (22-81 y), 144 children (0.027-18 y) and 86 pregnant women (gestation weeks 14-22). Based on this cohort, the reference range was set at $\leq$225 ng/mL for all populations. Because clearance of CGA occurs via hepatic metabolism and renal excretion, we also tested 74 samples from patients with renal failure (serum creatinine 2.1 – 9.9 mg/dL) and 137 samples from patients with liver disease (serum alpha fetoprotein 5.5 – 80,800 ng/mL). These patients had very high CGA levels (mean concentrations: 2741 ng/mL and 886 ng/mL, respectively), indicating that in these patients CGA measurements are uninterpretable.