nogenesis (11, 12). In neoplastic cells, some usually unmethylated CpG islands in the promoter region become aberrantly methylated, and this leads to transcriptional silencing of various genes (13). The human LDHA and LDHB genes both have CpG-rich regions in their promoters (14).

In the patient described here, we observed that the promoter region around exon a of LDHA was aberrantly methylated. This might silence expression of the somatic LDHA gene, possibly leading to relative increases in LDHB protein concentrations and LD1 activity. We also found that increased concentrations of electrophoretically fast-moving LD isoenzymes in some types of cancer are the result of transcriptional silencing of LDHA expression as result of aberrant methylation of the LDHA promoter. Thus, as reported previously, enzyme abnormalities in tumors occasionally originate from aberrant methylation. Significant increases in LD1 and LD5 have been reported previously (15, 16). The LD isoenzyme patterns in these patients could be the result of aberrant LDHA or LDHB methylation in cancer cells.

Human testicular germ-cell tumors are typically characterized by overrepresentation of 12p. These tumors were shown to contain striking amplification of a restricted region of 12p that included the K-ras protooncogene. Seminomas with this 12p amplification do not undergo apoptosis, and the tumor cells showed prolonged in vitro survival, as do seminoma cells with a mutated ras gene (17). Indeed, high concentrations of LD1 may yield a better prognostic predictor for the patients with testicular germ cell tumors (5, 18). Amplification of 12p is associated with poor prognosis, whereas methylation of LDHA may indicate a good prognosis. This issue should be addressed by investigating a large sample of patients with high LD1 attributable to amplification of LDHB or methylation of LDHA.

To our knowledge, this is the first reported case in which the specific LD isoenzyme pattern in serum was linked directly to promoter methylation. The next issue is whether methylation of the LDHA promoter is a common mechanism underlying increased LD1 concentrations in germ-cell tumors.

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Reference Intervals for Serum Calcitonin in Men, Women, and Children, Jean-Pierre Basuyau,¹ Eric Mallet,² Marcelle Leroy,² and Philippe Brunelle² (¹Laboratoire de Biologie Clinique et de Radioanalyse, Centre Henri-Becquerel, Rouen, France; ²Service de Pédiatrie, Hôpital Charles-Nicolle, CHU de Rouen, France; *author for correspondence: fax 33-2-32-082950, e-mail jeabas@rouen.fnclcc.fr)

The strategy currently used to investigate thyroid nodules in most cases involves measurement of calcitonin to exclude the possibility of medullary thyroid cancer (MTC). Techniques used to measure calcitonin have become more reliable, sensitive, and easy to perform. Automated nonsotopic techniques now exist, such as the Advantage® system.

We found that some children have particularly high calcitonin. Previous reports also have shown that calcitonin is higher in young children (1–9), but most of these studies are now outdated and are based on immunona-
diometric or even radioimmunologic (RIA) methods, which sometimes involve an extraction step. These techniques are also not very sensitive or specific (interference by procalcitonin). Because most studies addressing this subject investigated specific populations (premature infants, newborns, or hypotrophic or hypocalcemic children), we concluded that it would be useful to determine reference intervals for blood calcitonin in children with the Advantage system. Because the manufacturer (Nichols Institute Diagnostics) found a significant difference between men and women, we also evaluated this finding.

The Advantage is an automated multiparametric random-access system that uses an acridinium ester chemiluminescence technique and a magnetic separation step \((10, 11)\). The monoclonal antibodies used recognize the 11–23 (capture monoclonal antibody) and 21–32 (labeled monoclonal antibody) fragments of human calcitonin. Results are obtained within 45 min.

The measurement is carried out using a serum sample \((150 \mu L)\). The sample is transported on ice and centrifuged at 4 °C. Serum samples can be stored at −20 °C until use. All samples were tested within 5 days of collection. According to the manufacturer, the detection limit of the system is 1 ng/L. The technique is linear up to 1500 ng/L, and there is no hook effect until 500 000 ng/L. There is no cross-reactivity with procalcitonin and calcitonin from other species (pig, salmon, chicken) at concentrations <40 mg/L. The reproducibility (CV) observed in our laboratory over a 17-month period \((n = 160)\) was 11%, 9.8%, and 7.1%, respectively, at concentrations of 5, 20, and 190 ng/L.

We collected samples from 151 children (age range, 4 months to 15 years), hospitalized or consulting at Rouen University Hospital, with normal blood calcium and phosphate. Only leftover biological samples were used to measure blood calcitonin and, when possible, ionized calcium. These samples were treated, transported, and stored in our laboratory in accordance with good laboratory practice guidelines.

The samples used to determine usual values in adults were also leftover biological samples from adults with normal thyroid function. A total of 358 sera (282 from women and 76 from men) were evaluated.

According to the manufacturer, the reference intervals for calcitonin in adults are <11.5 ng/L for men and <4.6 ng/L for women. Our laboratory used a reference interval of <10 ng/L, as defined by GETC (French Calcitonin-Secreting Tumors Study Group). The difference in concentrations between men and women, described by Suzuki \((12)\), was not addressed. Given the distribution obtained in 358 patients (Table 1), this threshold is probably too high for women.

The manufacturer did not supply any reference intervals for children. The results for our population of children are summarized in Fig. 1 and in Table 1: the reference values differed considerably between adults and children, particularly neonates.

The range of calcitonin values in children was so large that it was not possible to identify a significant sex ratio in these children. In addition, we observed no correlation between calcitonin concentration and other biological variables. Sample size was insufficient to measure serum parathyroid hormone, and we were able to measure ionized calcium in only 14 cases (results not shown).

When measured with the Advantage system, calcitonin concentrations are higher in young infants than in adults;
similar results have already been obtained with an isotopic method, which showed that concentrations are particularly high during the first week of life (4, 5), in low-birthweight children (3), and in premature infants (6, 8). The RIA (1, 7) and the immunoradiometric assay (9) produced similar results for healthy infants.

Calcitonin concentrations are highest during the postnatal period, but high concentrations can also be observed in 6-month-old children. The child with the highest value in our series (75 ng/L at the age of 4.5 months) was retested 1 month later, and the value was halved (32.4 ng/L).

The pentagastrin test was also carried out on a child who presented with the clinical signs of MTC at the age of 6 months (persistent diarrhea, calcitonin concentration of 28 ng/L). The results of this test would have been considered suspicious or even abnormal in an adult (basal value, 9 ng/L; peak, 50 ng/L).

These two children had procalcitonin concentrations within the reference interval. This confirms that the substance measured was in fact probably calcitonin, as suggested by the specificity study carried out by the manufacturer, which confirmed no significant interference, in particular by procalcitonin. Although prophylactic thyroidectomy is not performed before the age of 3 years, evaluation of calcitonin in children younger than 6 months of age for MTC diagnosis is of more than casual clinical interest.

The reference interval for serum calcitonin is wider in children, especially newborns, than in adults (1–9). Our results suggest the following reference intervals:

**Children:**
- $<40$ ng/L in children under 6 months of age
- $<15$ ng/L in children between 6 months and 3 years of age
- Over 3 years of age, the values are indistinguishable from those observed in adults

**Adults:**
- $<5$ ng/L for females
- $<12$ ng/L for males

The highly significant difference between men and women was described by Suzuki (12) but ignored in the consensus definition of reference values. It is necessary, however, to exclude the unlikely possibility that this difference is not an artifact of the technique used. Values that would be considered to be highly suggestive of MTC in adults are not in newborns. We propose that high values do not necessarily indicate a need for a pentagastrin test, especially in infants less than 1 year: the basal value should be confirmed several weeks later before performing this type of additional testing.

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### Table 1. Distribution of calcitonin concentrations for 151 infants and 358 adults.

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<tr>
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*UD, undetectable.

References


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