Chronic lymphocytic thyroiditis is associated with invasive characteristics of differentiated thyroid carcinoma in children and adolescents

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Abstract

**Background.** The association between chronic lymphocytic thyroiditis (CLT) and thyroid cancer is an interesting topic. The aim of the present study was to evaluate if demographic and histological characteristics as well as the long-term outcome of thyroid cancer was different in children and adolescents with and without CLT. **Methods.** The medical records of children and adolescents (≤ 21 years old) were reviewed. The following data were recording: gender, year and age at diagnosis, family history of thyroid cancer, history of external radiation therapy, histological type (papillary and variants, follicular and variants), tumour size, multifocality, infiltration of thyroid parenchyma or surrounding soft tissues, vascular invasion, presence of lymph node and distant metastases. Information about the presence of TgAb and TPOAb was also collected. **Results.** One hundred eight children and adolescents (median age 19.0, interquartile range 4.0 years) were diagnosed with DTC. Thirty one patients (28.7%) presented histological characteristics compatible with CLT. Infiltration of thyroid parenchyma was more frequent in patients with CLT compared to patients without (74.2% versus 48.1% respectively, p=0.024). Familial PTC was more frequent in patients with CLT compared to those without CLT (20.7 vs 2.8% respectively, p=0.009). There was no better outcome with respect to the presence of CLT or not. **Conclusions.** Children and adolescents with CLT present more frequently familial PTC as well as thyroid cancer with invasive characteristics.
**Introduction**

Lymphocytic thyroiditis (LT) is a chronic inflammatory disease of the thyroid due to autoimmune responses. According to International Classification of Diseases (ICD-10-CM Diagnosis Code E06.3) LT is defined as «diffuse infiltration of the thyroid gland with lymphocytes, resulting in progressive destruction of the parenchyma and hypothyroidism». Some physicians consider the presence of serum thyroid autoantibodies, e.g. thyroid peroxidase (TPOAb) and thyroglobulin antibodies (TgAb), as sufficient evidence for the diagnosis of chronic autoimmune thyroiditis. This is based upon the observation that thyroid antibodies correlate well with the presence of lymphocytic infiltrates found during histological examination of the thyroid in individuals without prior history of thyroid failure.

During the last three decades, an increase in the annual frequency of chronic LT (CLT) was observed. In a retrospective study done in the University Hospital of Messina, Italy, from January 1975 to December 2005 it was found that CLT has become ten times more frequent than it was until 1990 and that affected patients were relatively younger compared to the past\(^1\). An increase in the annual frequency of CLT, but to a lesser extent, was observed in Catania, which is located south of the Messina province, between 1995 and 2005\(^2\). Different environmental modified factors could probably explain this marked increase occurred in a relatively short period of time\(^1\).

Regarding the association of CLT with other thyroid diseases it has been observed that the most common condition is papillary thyroid carcinoma (PTC)\(^3\) and this association is being studied since the first report in 1955 by Dailey et al\(^4\). Coexistence of the two diseases ranges between 0.5 to 58%\(^2,3,5,6\). This wide distribution of coexisting CLT and PTC may at least in part be due to differences in histological examination and criteria of autoimmunity.
characterization, patient selection or indications for thyroidectomy, population background and mainly to environmental factors\(^2\). A recent meta-analysis demonstrated that the occurrence rate of CLT was 2.8 times higher in patients with PTC compared to patients with benign thyroid diseases\(^7\) and 2.4 times higher in patients with PTC than in those with other histopathological types\(^7\). Albeit indirectly, these results suggest that patients with CLT might well present a stronger predisposition towards the development of PTC. Furthermore, CLT and PTC share some common epidemiological features, such as the relationship to ionizing radiation exposure and dietary iodine, as well as some molecular features\(^6\).

Regarding the influence of CLT on the evolution of PTC, different studies showed a protective effect of thyroid autoimmunity, i.e. less aggressive disease at presentation and/or better long-term outcome\(^8\)\(^-\)\(^{11}\). However multivariate analysis in other studies revealed that CLT was not an independent predictor of better outcome of DTC\(^12\)\(^-\)\(^{14}\). The above mentioned findings refer to patients regardless of age and there are no published studies focusing on the characteristics and outcome of thyroid cancer in children with and without CLT. However the frequency of PTC in children and adolescents with CLT has been evaluated in two published studies. In the first one, eleven of 365 children and adolescents (3%) with CLT were diagnosed with PTC\(^15\) and in the second study, three of 228 Greek children and adolescents (1.3%) with CLT presented PTC\(^16\).

The aim of the present study was to evaluate if demographic and histological characteristics as well as the long-term outcome of thyroid cancer were different in children and adolescents with and without autoimmune thyroiditis taking into consideration that information in the literature is scarce.

**Materials and Methods**
The medical records of children and adolescents with histologically proven DTC followed in our hospital were reviewed. The following data were recorded: presenting signs or symptoms of the disease, year of diagnosis of thyroid cancer, age at diagnosis, gender, family history of thyroid cancer, history of external radiation therapy to the head and neck, type of surgery, histological type (papillary and variants, follicular and variants), tumour size, number of tumour foci (unifocal/multifocal), thyroid capsule invasion, vascular invasion, infiltration of thyroid parenchyma or surrounding soft tissues presence of lymph node metastases as well distant metastases. Information about the presence of TgAb and TPOAb was also collected. Postoperative staging was done based on the tumour, nodes, metastases (TNM) system as proposed by the American Joint Committee on Cancer (AJCC). Histological criteria used for the diagnosis of lymphocytic thyroiditis were: diffuse lymphocytic infiltration with presence of germinal centres in the stroma, presence of thyroid follicles with oxyphilic change of the follicular epithelium and presence of fibrosis.

Patients were divided in two groups based on the presence (Group A) or not (Group B), of lymphocytic thyroiditis. Data analysis was performed using the statistical package SPSS (version 17.0; SPSS Inc., Chicago, IL). Kolmogorov-Smirnov or Shapiro-Wilk test was used to assess the normality of distribution of continuous variables. Continuous variables not normally distributed are described by median and IQR (interquartile range). Independent samples T-test or Mann-Whitney U-test was used to test for between-groups differences in continuous variables. Data for categorical variables are presented as absolute numbers and percentages. Chi-square test was used to assess between-groups differences in categorical variables. In all cases a p value < 0.05 was considered significant. All data were analyzed anonymously. The study was approved by the Institutional Review Board.
Results

One hundred eight children and adolescents with DTC (82 females, 76%) were identified. Patients first visited their pediatrician or general practicing doctors because of a family history of thyroid diseases or due to a cervical mass accidentally found by themselves or by their parents or by a physician in a routine examination. All had a short follow up of 4-10 months before thyroidectomy which was performed as they were found to harbor a nodule suspicious (FNA and/or ultrasound) for malignancy. No patient had received external radiation therapy in the neck or head for the treatment of another primary malignancy. Preoperatively TgAb and TPOAb were measured in 98 patients and were found to be positive in 27 (27.6%). Near total or total thyroidectomy was performed in all patients with central and/or lateral lymph node dissection in 69 of them.

Histology revealed papillary carcinoma in the majority of the cases (93.5%) (Table 1). Median (IQR) tumour size was 15.5 (21.0) mm (range 5-70 mm). Although not statistically significant, tumour size tended to be larger in males in comparison to females [20.0 (30.0) vs. 13.5 (17.0) mm, p=0.063]. There were no differences between males and females regarding age at diagnosis, familial and sporadic cancer, histological type or other histological characteristics. Classification according to TNM system showed (a) T1: 58, T2: 19, T3: 27 and T4: 4 patients, (b) N0: 50, N1a: 17, N1b: 41 patients and (c) M0: 98, M1: 10 patients.

Thirty one patients (from the 108) (28.7%), presented histological characteristics compatible with CLT and these patients comprised Group A. Among them, thyroid autoantibodies were found to be positive in 25 (80.6%). Seventy seven patients without histological characteristics compatible with CLT comprised group B. Among patients of Group B two were found with positive TgAb but none had TPOAb. The percentage of CLT was similar
in males and females (19.2% and 31.7% respectively, p=0.220) and frequency was also similar in
patients > 18 years-old compared to those ≤ 18 years-old (31.3% and 25.0% respectively,
p=0.481). Thyroid carcinoma presented intrathyroidal invasion more frequently in Group A
compared to Group B (74.2% versus 48.1% respectively, p=0.024). Other clinical and
histological characteristics did not differ between Group A and Group B (Table 1).

Thirty nine patients were found to have tumors ≤ 10 mm, ten of them with CLT. No differences
were found regarding the percentage of CLT in patients with tumour size ≤ 10 mm compared to
those with size > 10 mm. Familial PTC was found more frequent in Group A compared to Group
B (20.7 vs. 2.8% respectively, p=0.009). Median duration of follow up was 54 months (range 7-
324) for Group A and 53 months (range 8-347) for Group B (p=0.732). From the total cohort, 48
patients were followed for five years and 27 of those for at least ten years. 38 (79.2 %) of the
former and 22 (81.5 %) of the later were found free of the disease. No differences were observed
regarded the disease status in patients with or without CLT after five or ten years of follow up (p
= 0.948 and p = 0.738 respectively).

**Discussion**

In the present study we evaluate clinical and histological characteristics in 108 children
and adolescents with DTC according to the presence or not of CLT. Out of the overall cohort,
thirty one patients (28.7%) presented histological characteristics compatible with CLT. We found
that the infiltration of the thyroïd parenchyma and familial PTC was more frequent in patients
with CLT compared to those without. However, no better outcome was observed in regards to the
absence or presence of CLT. To our knowledge, this is the largest (regarding the number of
patients) study in terms of examining thoroughly the relationship between CLT and DTC in children and adolescents.

A significant increase in the number of Hashimoto’s thyroiditis associated with PTC was observed in surgical specimens during the last two decades as reported in a recently published study written to celebrate a century since the identification of Hashimoto’s Thyroiditis. This association remains unexplained as it is difficult to answer if it represents a simple co-occurrence of two relatively common thyroid conditions, or if it indicates a true cause–effect relationship. There have been many studies trying to elucidate this association. One of these supported that the oncogenic RET/PTC rearrangements, a specific genetic alteration in PTC patients, are present in Hashimotos’ patients. RET/PTC mutations are reported in about 68 up to 95% of Hashimotos’ patients. Moreover, it is reported that the RET/PTC1 oncogene, when exogenously expressed in primary normal human thyrocytes, induces the expression of a large set of genes involved in inflammation and tumor invasion. Other studies suggest a role for inflammation in the development of thyroid cancer. In one of these it is reported that patients with Hashimoto’s thyroiditis were three times more likely to have thyroid cancer and they suggested a possible molecular mechanism for thyroid carcinogenesis through the increased expression of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in patients with Hashimoto’s thyroiditis, while in another it was demonstrated that stepwise increments in overexpression of both COX-2 and iNOS were demonstrated in epithelial cells in HT, follicular adenoma, and papillary thyroid cancer while normal thyroid tissue showed little expression.

The frequency of lymphocytic thyroiditis in children with DTC was reported in few published studies, ranging from 6.3% to 43.0%. Nevertheless, these studies do not clarify which histopathological criteria were used by the authors to determine the presence of CLT. In
particular, in an older study of 48 children and adolescents with thyroid cancer only 3 (6.3%) had CLT\textsuperscript{24}. In a more recent study 23 from 54 (43%) children with DTC (48 PTC and 6 FTC) had «background thyroiditis» on histological examination\textsuperscript{25} and in another one 16 from 90 (17.8%) children and adolescents with DTC (78 PTC and 12 FTC) showed «diffuse thyroiditis» on histological examination\textsuperscript{26}. In the present study we found that 28.6% patients presented with CLT. Differences in patient selection might have contributed to this variability that was associated with geographic and ethnic diversity in the prevalence of CLT and DTC.

The prognostic value of thyroid autoimmunity for the outcome of DTC has also been studied; yet, with conflicting results. A number of studies showed a protective effect of thyroid autoimmunity, i.e. less aggressive disease at presentation and/or better long-term outcome\textsuperscript{8-11}. However, multivariate analysis in other studies revealed that CLT was not an independent predictor of better outcome of DTC \textsuperscript{12-14}. Nevertheless, the above-mentioned studies report data on adults only or both adults and children and in the latter case patients are not divided in two groups based on their age; therefore it is impossible to extract information regarding children. To our knowledge, no studies have been carried out in children and adolescent populations regarding the prognostic value of thyroid autoimmunity for the outcome of DTC. On the other hand, some studies report the prevalence of DTC in children with CLT, which was found to be 1.3-3.0\% \textsuperscript{15, 16}. In one of those \textsuperscript{15}, more girls were affected by CLT, but males with CLT had increased risk to develop thyroid cancer (odds ratio [OR], 2.95; 95\% confidence interval [CI], 1.44-6.20).

It has been reported that lymphocytes migrate around a malignant tumour in an effort to restrict disease extension\textsuperscript{27}. This process, known as peritumoral infiltration, is different from that described in CLT as diffuse lymphocytic infiltration. Kamma et al\textsuperscript{28} in a small study with 9
patients aged < 20 years with PTC (all with serum negative antibodies) have found that lymphocytic infiltration was observed in more aggressive cases, predominantly near the tumour and the degree of infiltration was correlated with the extension of carcinoma. They concluded that lymphocytic infiltration around the tumour is an immunologic reaction induced by antigens of the carcinoma itself. Gupta et al \(^\text{29}\) demonstrated that among 48 children and adolescents with thyroid carcinoma (39 PTC and 9 FTC), those who had a high number of proliferating lymphocytes, demonstrated by the proliferating cell nuclear antigen Ki-67, had a better disease-free survival than those with lower proliferating numbers. In the present study, cases with peritumoral lymphocytic infiltration were scarce and therefore not discussed as a third group.

In our study we found that familial PTC is more frequent in children with CLT in comparison to those without CLT. A possible explanation is that children with a positive family history of CLT are more frequently examined for thyroid disease (or more frequently referred to specialists).

It is difficult to answer if children with CLT should be closely monitored for the development of thyroid cancer, as some studies suggested that the prevalence of PTC may be higher in children with CLT \(^\text{16}\). As children with CLT are followed-up due to functional thyroid disorders \(^\text{30}\), the additional examination by ultrasonography could detect nodule(s) with suspicious characteristics.

A limitation of the present study is its retrospective nature. Our study is the largest one focusing on different histological characteristics and evolution of DTC in patients with and without CLT, although it seems to include a relative not so big number of patients. Considering that thyroid cancer is relatively rare in children and that both paediatric and adult
endocrinologists follow these patients, it is evident that a single centre may not have a large number of patients for treatment and follow-up. Multi-centre studies with larger number of patients and long follow-up duration are needed in order to evaluate disease course.

In conclusion, in this retrospective study it was shown that children and adolescents with CLT present more frequently invasive DTC and familial PTC. No better outcome of the cancer was observed between children with CLT in comparison to those without. Multicentric, epidemiological studies are needed to confirm our results. Paediatricians or primary care physicians should be aware of this association when diagnosing or treating children with CLT.

Declaration of interest. There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Figure 1.** Proportion of patients with TPOAb and/or TgAb, TPOAb and TgAb, only TPOAb and only TgAb in serum in the group of patients with measured thyroid antibodies. Proportion of unknown thyroid antibodies status in the entire study population. Group A: patients with histological characteristics compatible with CLT. Group B: patients without histological characteristics compatible with CLT.
**Table 1.** Clinical characteristics of the patients/participants and histological characteristics of their tumours. Group A: patients with histological characteristics compatible with CLT. Group B: patients without histological characteristics compatible with CLT. Data are presented as median (interquartile range in parentheses) or absolute numbers (percentages in parentheses).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A n=31</th>
<th>Group B n=77</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td>20.0 (4.0)</td>
<td>19.0 (5.0)</td>
<td>0.217</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>5 (16.1)</td>
<td>21 (27.3)</td>
<td>0.220</td>
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<tr>
<td>Female</td>
<td>26 (83.9)</td>
<td>56 (72.7)</td>
<td></td>
</tr>
<tr>
<td>Familial</td>
<td>6 (19.4)</td>
<td>2 (2.6)</td>
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</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
<td>0.953</td>
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<tr>
<td>Papillary classic type</td>
<td>19 (61.3)</td>
<td>51 (66.2)</td>
<td></td>
</tr>
<tr>
<td>Papillary-follicular variant</td>
<td>8 (25.8)</td>
<td>16 (20.8)</td>
<td></td>
</tr>
<tr>
<td>Papillary-other variants*</td>
<td>2 (6.5)</td>
<td>5 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>2 (6.5)</td>
<td>5 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>13.0 (22.0)</td>
<td>18.0 (20.0)</td>
<td>0.559</td>
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<tr>
<td>Bilateral</td>
<td>8 (25.8)</td>
<td>17 (24.0)</td>
<td>0.843</td>
</tr>
<tr>
<td>Multifocal</td>
<td>13 (41.9)</td>
<td>25 (32.5)</td>
<td>0.478</td>
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<tr>
<td>Nodule capsule invasion**</td>
<td>5 (100.0)</td>
<td>9 (50.0)</td>
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<tr>
<td>Thyroid parenchyma infiltration</td>
<td>23 (74.2)</td>
<td>37 (48.1)</td>
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<tr>
<td>Thyroid capsule invasion</td>
<td>12 (38.7)</td>
<td>23 (29.9)</td>
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<tr>
<td>Infiltration of surrounding soft tissues</td>
<td>7 (22.6)</td>
<td>17 (22.1)</td>
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</tr>
<tr>
<td>Lymph node metastases***</td>
<td>17 (85.0)</td>
<td>41 (83.7)</td>
<td>1.000</td>
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<tr>
<td>Vascular invasion</td>
<td>7 (22.6)</td>
<td>13 (17.3)</td>
<td>0.722</td>
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<tr>
<td>Lymph vessel invasion</td>
<td>3 (9.7)</td>
<td>8 (10.5)</td>
<td>1.000</td>
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<tr>
<td>Vascular embolus</td>
<td>3 (10.0)</td>
<td>3 (3.9)</td>
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<td>Metastasis</td>
<td>2 (6.5)</td>
<td>8 (10.4)</td>
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<tr>
<td>Number of patients with measured auto-antibodies</td>
<td>29 (93.5)</td>
<td>69 (89.6)</td>
<td>0.523</td>
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Number of patients with positive antibodies: 25 (86.2), 2 (2.9), <0.001

<table>
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<td>Surgery</td>
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<td>0.931</td>
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<tr>
<td>TT</td>
<td>11 (35.5)</td>
<td>28 (36.4)</td>
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<tr>
<td>TT &amp; LND</td>
<td>20 (64.5)</td>
<td>49 (63.6)</td>
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<tr>
<td>RAI</td>
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<tr>
<td>Number of treatments</td>
<td>1 (1)</td>
<td>1 (1)</td>
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<tr>
<td>Total dose (mCi)*****</td>
<td>110 (150)</td>
<td>100 (150)</td>
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<tr>
<td>Duration of follow up (months)</td>
<td>54 (83)</td>
<td>53 (109)</td>
<td>0.732</td>
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<td>Patients’ condition at last follow up</td>
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<tr>
<td>Free of disease</td>
<td>25 (89.0)</td>
<td>50 (77.0)</td>
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<td>Death</td>
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<tr>
<td>Distant metastases</td>
<td>2</td>
<td>8</td>
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</tr>
<tr>
<td>Local disease</td>
<td>1</td>
<td>7</td>
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<tr>
<td>Follow up &lt;10 months</td>
<td>1</td>
<td>8</td>
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<tr>
<td>No follow up</td>
<td>2</td>
<td>4</td>
<td></td>
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</tbody>
</table>

* Variants: trabecular (n = 3), sclerosing (n = 3), solid (n = 1), ** among the 23 patients with nodule capsule, *** among the 69 patients who underwent lymph node dissection, **** among patients with measured auto-antibodies, TT: total (or near total) thyroidectomy, LND: lymph node dissection, RAI: radioactive iodine. *****In both groups patients received 131I, one to five times (maximum dose of 600 mCi)
Figure 1. Proportion of patients with TPOAb and/or TgAb, TPOAb and TgAb, only TPOAb and only TgAb in serum in the group of patients with measured thyroid antibodies. Proportion of unknown thyroid antibodies status in the entire study population.

Group A: patients with histological characteristics compatible with CLT.
Group B: patients without histological characteristics compatible with CLT.