CLINICAL STUDY

Serum cytokeratin 19 fragments: a dedifferentiation marker in advanced thyroid cancer

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Abstract

Background: This study was undertaken to evaluate serum cytokeratin 19 fragment (Cyfra 21.1) expressions in patients with advanced thyroid carcinoma and to explore the relationship between serum Cyfra 21.1 and the degree of radioiodine (131I) avidity of thyroid carcinoma cells.

Methods: Enrolled were 76 consecutive patients with advanced thyroid carcinoma submitted to high-activity 131I treatment. In each patient, serum thyroglobulin (Tg) and Cyfra 21.1 were measured before 131I administration and compared with the posttreatment whole-body scan results.

Results: Thirty-one (41%) of 76 patients had iodine-avid and 45 (59%) had iodine-refractory diseases respectively. Significantly higher serum Cyfra 21.1, but not Tg, levels were found in patients with 131I-refractory disease compared with patients with iodine-avid disease (P<0.01).

Conclusions: This is the first report describing the potential role of serum Cyfra 21.1 as marker of dedifferentiation and resistance to 131I therapy in patients with advanced thyroid carcinoma.

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Introduction

Differentiated thyroid carcinoma (DTC) comprises 80% of all thyroid cancer cases (1). Distant metastases are rare at the time of diagnosis with ~5% of all DTC patients being affected; recurrent disease occurs in another 10–15% of the cases (2, 3). Approximately, half of these cases can be cured with conventional radioiodine (131I) therapy and/or additional surgical procedures. The remaining patients mostly have dedifferentiated thyroid carcinoma (de-DTC), which has lost the ability to take up 131I; as a consequence, these patients have a poor survival (4). Chemotherapy has shown limited success at best in patients with de-DTC (5, 6). Recently, several tyrosine-kinase inhibitors (TKIs) have been tested in phase II and phase III trials; the majority of de-DTC patients achieved stable disease or partial response (7, 8, 9). Serum thyroglobulin (Tg) decreases in most patients receiving TKIs. However, neither baseline Tg nor Tg changes consistently correlate with the degree or duration of objective response (10, 11, 12). Consequently, changes in the Tg levels in this setting of treatment must be interpreted with caution and could always be confirmed by imaging (9). The cytokeratin 19 (CK19) is an acidic protein of 40 kDa that is part of the cytoskeleton of epithelial cells. Tissue CK19 is highly expressed in DTC, mainly those with papillary histotype (PTC) (13, 14). The CK19 is specifically recognized by two MABs KS 19-1 and BM 19-21; CK19 soluble fragments (Cyfra 21.1) can be measured by employing these antibodies in a specific immunoradiometric ‘sandwich’ assay (15). Increased preoperative serum Cyfra 21.1 levels were found by this assay in patients with localized aggressive DTC histotypes (16). However, data on patients with advanced DTC are scarce and mainly focused on patients with iodine-avid (i.e. differentiated) disease (17). This study was then undertaken to evaluate serum Cyfra 21.1 expression in patients with advanced DTC. Additionally, relationships between tumor histotype, 131I avidity, and serum Cyfra 21.1 levels were explored.

Materials and methods

Between January 2006 and April 2012 enrolled were 76 consecutive patients (males 26, females 50; age 46.4 ± 25.5 years; further characteristics in Table 1) with distant metastases (i.e. lung and/or bone metastases) from histologically proven primary DTC. Histology classification was based on the original surgical
Table 1 Demographic and clinical variables among patients with 131I-avid and 131I-refractory metastatic DTC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>131I-avid (n=31)</th>
<th>131I-refractory (n=45)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n, %)</td>
<td>19 (62%)</td>
<td>31 (68%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.7±24.5</td>
<td>47.3±27.9</td>
<td>NS</td>
</tr>
<tr>
<td>131I activity (GBq)</td>
<td>5.2±2.2</td>
<td>5.5±1.9</td>
<td>NS</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>96.7±36.5</td>
<td>112±47.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ioduria (µg/l)</td>
<td>75±55</td>
<td>81±60</td>
<td>NS</td>
</tr>
<tr>
<td>DTC</td>
<td>29</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>PTC (incl. follicular variants)</td>
<td>24</td>
<td>26</td>
<td>NS</td>
</tr>
<tr>
<td>Mi-FTC (incl. Hürthle cell variant)</td>
<td>5</td>
<td>3</td>
<td>NS</td>
</tr>
</tbody>
</table>

DTC, differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma; Mi, minimally invasive; FTC, follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma.

pathology report; DTC included classical and follicular variants of PTC and minimally invasive follicular thyroid carcinoma (FTC) (including the Hürthle cell variant). The histology was classified as poorly DTC (PDTC) if the primary tumor contained significant areas of aggressive variants of PTC (i.e. tall cell PTC), invasive FTC (including Hürthle cells variant), or insular carcinoma. All patients were initially treated by thyroidectomy and radioiodine ablation (mean administered activity 3 GBq, range 1.1–5.5 GBq) and presented with a basal or stimulated serum Tg > 1.00 ng/ml after 0.7–12 years of follow-up. In all cases, neck ultrasound examination was negative and patients underwent computed tomography (CT) examination of the lungs and magnetic resonance (MR) examination of the neck and mediastinum. Lung metastases were found in 57 patients (seven with coexisting mediastinal metastasis and one with coexisting retro-pharyngeal metastases). Bone metastases were found in nine patients with coexisting lung metastases and in one additional patient. Both CT and MR examinations tested negatively in the remaining 13 patients. According to our clinical protocol, patients with lung metastases received 5.5 GBq, patients with bone metastases (with or without concurrent lung metastases) received 7.4 GBq, and patients with negative CT and MR received 3.7 GBq of 131I sodium iodide. In all cases, a posttreatment whole-body scan (PT-WBS) was obtained and allowed us to detect one additional patient with a CT-negative lung metastasis and five patients with bone metastases. An additional whole-body 18-fluorodeoxyglucose (18FDG) PET/CT scan was done if no abnormal findings were seen on PT-WBS in patients with previous negative CT and MR and previously undetected bone metastases were found in 12 patients. In a limited number of cases, cytological (n=3) or histological (n=1) diagnosis was obtained. In particular, histological confirmation of metastatic DTC was obtained in one female patient (age 43 years) with a 131I-negative, MR-positive, left retro-pharyngeal lymph-node metastasis coexisting with multiple lung metastases (with a mixed pattern at 131I-PT-WBS). In this case, resection was performed due to compressive symptoms. Additional inclusion criteria were as follows: age > 18 years, measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), negative serum Tg autoantibodies (i.e. < 60 IU/ml by TgDYNoTest anti-TGn assay; BRAHMS Diagnostica GmbH, Berlin, Germany), life expectancy > 3 months, leukocyte count ≥ 3000/µl, absolute neutrophil count ≥ 1500/µl, platelets ≥ 100 000/µl, hemoglobin ≥ 9 g/dl, creatinine clearance (according to the Cockroft–Gault equation) ≥ 60 ml/min; no previous therapies with cytotoxic chemotherapy and/or TKIs.

Radioidine therapy and PT-WBS

Levothyroxine (l-T4) therapy was withdrawn for 4–6 weeks before 131I-therapy; TSH levels > 30 mIU/ml were obtained in all cases. Additionally, all patients were placed on a low-iodine diet 2 weeks before 131I treatment; urinary iodine excretion (UIE) was routinely measured and patients with UIE values > 150 µmol/l (n = 3) were rescheduled and only treated once when the UIE was low enough. Premenopausal women were required to have a negative pregnancy test, and all patients of childbearing potential were required to use contraception; 5–7 days after 131I administration a PT-WBS was performed, as described previously (18). Scan images were centrally reviewed and compared with available morphological imaging by two experienced board-certified nuclear medicine physicians (L G and L C). Patients who showed at least one measurable 131I-negative lesion were defined as having 131I-refractory disease. Patients with 131I uptake in all measurable lesions were defined as having 131I-avid disease.

Tg and Cyfra 21.1 measurements

Serum Tg and Cyfra 21.1 were measured both before (onT4) and 4–6 weeks after l-T4 withdrawal (offT4) in each patient. Serum Tg and Cyfra 21.1 were measured in duplicate using the radiometric immunoassays DYNoTest Tg-plus (BRAHMS Diagnostica GmbH) and ELSA-Cyfra 21.1 (CisBio, Gil-sur-Yvette, France) according to the manufacturers’ instructions. Quality control was ensured by assaying two levels of control sera in each series, by reassessing all sera showing a coefficient of variation exceeding 10% and by a bimonthly participation in the European inter-laboratory control Oncocheck.
Statistical analysis

Statistical analysis was performed using Analyse-it version 2.20 for Microsoft Excel (http://www.analyse-it.com). Normally distributed data are expressed as mean ± S.D. The normality of the Tg and Cyfra 21.1 distribution was assessed using the Shapiro–Wilks test. As serum Tg and Cyfra 21.1 values were not normally distributed, the statistical analyses were performed using nonparametric tests. The t-test (normalized variables) and the Mann–Whitney U test (not normally distributed variables) were applied to compare the distribution of variance in different groups. The \( \chi^2 \) test was applied to compare two categorical variables. A \( P \) value <0.05 was considered to indicate statistical significance.

Results

As assessed by PT-WBS, 31 of 76 patients (41%) had iodine-avid and 45 (59%) had iodine-refractory diseases respectively. No differences between the two groups were found in sex, age, pretreatment TSH levels, and UIE. While serum Tg significantly increased after \( t_{T4} \) withdrawal, as expected, neither on\( T4 \)-Tg nor off\( T4 \)-Tg levels differed significantly between patients with \( ^{131} \)I-avid and \( ^{131} \)I-refractory diseases. Serum Cyfra 21.1 was not affected by \( t_{T4} \) therapy but was significantly higher in patients with \( ^{131} \)I-refractory disease compared with patients with iodine-avid disease (\( P < 0.001 \); Table 2). \( ^{131} \)I-refractory disease was prevalent in patients with primary PTC histotypes; however, higher Cyfra 21.1 levels were confirmed in patients with \( ^{131} \)I-refractory even after the exclusion of such cases from the statistical analysis (Table 3).

Discussion

Iodide trapping is a TSH-regulated mechanism involving an energy-dependent transport mediated by the sodium/iodide symporter (NIS) at the basolateral surface of the thyrocyte (19). A significant NIS gene expression is typically found in metastases with absent \( ^{131} \)I uptake in comparison with either primary cancers or metastases with a positive \( ^{131} \)I scan (20). In turn, there is a large body of information demonstrating that patients whose metastases concentrate \( ^{131} \)I have a higher survival rate and thus a better prognosis than patients with \( ^{131} \)I-refractory metastases (3, 4, 5, 9). In our series, patients with \( ^{131} \)I-refractory metastases had significantly higher Cyfra 21.1 levels than patients with \( ^{131} \)I-avid ones. This conforms with previous data as increased Cyfra 21.1 levels were found in patients with primary aggressive DTC but not primary and metastatic classical DTC histotypes (16, 17, 21). Such differences argue that \( ^{131} \)I-refractory thyroid cancer cells (i.e. dedifferentiated cells) are likely the source of the increased serum Cyfra 21.1. Histological diagnosis was obtained in only one metastasis from a patient with multiple metastases and, consequently, no data are available on the relationship between serum and tissue Cyfra 21.1 expression. Anyway, increased serum Cyfra 21.1 levels were previously reported in patients with primary aggressive thyroid carcinomas despite low or absent CK19 immunostaining in corresponding tumor tissues (13, 14, 21). Previous studies in human lung and liver cancer cell lines showed that among CK19-producing cells, only those with caspase-3 (an enzyme involved in apoptosis phenomena) expression induced high Cyfra 21.1 levels in culture supernatants (22, 23, 24). In line with our present results, serum caspase-3 enzyme activity is detectable in patients with metastatic \( ^{131} \)I-refractory thyroid cancer (25). Globally, thyroid tumors with high proliferation rate, diffuse apoptosis, and necrosis are like to release Cyfra 21.1 via caspase-3 action. The fast processing of CK19 molecules explains the coexistence of a negative tissue CK19 staining with high levels of CK19-soluble fragments in serum of patients with such aggressive thyroid tumors (16, 21). Vice versa, low proliferation rate and absent of apoptosis phenomena explain low serum levels of Cyfra 21.1 in patients with classical DTC (25, 26). Interestingly, high Cyfra 21.1 levels were found in \( ^{131} \)I-refractory patients even after exclusion of those patients with primary aggressive thyroid carcinomas. This is in line with previous reported differences between primary thyroid

### Table 2 Serum Tg and Cyfra 21.1 distribution in patients with \( ^{131} \)I-avid and \( ^{131} \)I-refractory metastatic DTC.

<table>
<thead>
<tr>
<th></th>
<th>( ^{131} )I-avid (n = 31)</th>
<th>( ^{131} )I-refractory (n = 45)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>off( T4 )-Tg (ng/ml)</td>
<td>764.7 (15.6–2569.5)</td>
<td>824.8 (4.9–2385.3)</td>
<td>NS</td>
</tr>
<tr>
<td>on( T4 )-Tg (ng/ml)</td>
<td>126.8 (0.6–475.2)</td>
<td>146.4 (1.5–585.9)</td>
<td>NS</td>
</tr>
<tr>
<td>off( T4 )-Cyfra 21.1 (ng/ml)</td>
<td>1.1 (0.1–2.6)</td>
<td>3.15 (0.6–15.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>on( T4 )-Cyfra 21.1 (ng/ml)</td>
<td>1.0 (0.1–2.7)</td>
<td>2.96 (0.5–16.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Tg, thyroglobulin; off\( T4 \), after thyroxine therapy withdrawal; on\( T4 \), during thyroxine therapy.

### Table 3 Distribution of serum Cyfra 21.1 (on\( T4 \)) according to tumor histology and \( ^{131} \)I uptake.

<table>
<thead>
<tr>
<th></th>
<th>( ^{131} )I-avid (n = 31)</th>
<th>( ^{131} )I-refractory (n = 45)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTC</td>
<td>1.1 (0.3–2.7)</td>
<td>2.3 (0.3–16.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PDTC</td>
<td>1.1 (0.1–1.6)</td>
<td>2.7 (0.5–12.9)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

DTC, differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma; MI, minimally invasive; FTC, follicular thyroid carcinoma; HC, Hürthle cell; PDTC, poorly differentiated thyroid carcinoma.
carcinomas and their metastases at the genetic level, as the number of chromosomal abnormalities increases as thyroid carcinomas progress (27). Then, although the majority of primary thyroid carcinomas leading to $^{131}$I-refractory disease were aggressive histotypes, primarily well-differentiated tumors were also responsible for $^{131}$I-resistance and increased Cyfra 21.1 in our series. In contrast to previous studies, Tg levels were similar in patients with $^{131}$I-positive and $^{131}$I-negative advanced DTC in our series. As a positive relationship exists between $^{131}$I uptake and Tg secretion, such differences are probably due to the different criteria used to define $^{131}$I-refractory disease; in fact, while patients with almost one $^{131}$I-positive lesion were previously defined as $^{131}$I-positive, patients with almost one $^{131}$I-negative lesion are now classified as $^{131}$I-refractory (9). Growth phenomena are typically independent of TSH in aggressive and de-DTCs; accordingly, no associations were found between Cyfra 21.1 and TSH levels in our series. In summary, our data show that serum Cyfra 21.1 is significantly elevated in patients with $^{131}$I-refractory metastatic differentiated thyroid cancer but not in patients with $^{131}$I-avid metastases. The $^{131}$I scan is the yardstick to define the disease as $^{131}$I-refractory and to select patients for trials with new drugs as TKIs. As previously remarked, changes in serum Tg should always be confirmed by imaging in the setting of TKIs. However, conventional imaging criteria (i.e. RECIST) may also have their own limitations when determining the effects of TKIs on tumor volume (28). Therefore, new circulating biomarkers are warranted to help identify patients most likely to benefit from these therapies. Further studies will be necessary to validate our preliminary results; in particular, larger prospective randomized studies will be designed to independently validate the predictive and/or prognostic value of Cyfra 21.1 and to determine the most appropriate time point(s) for assessment. In conclusion, serum Cyfra 21.1 may serve as a marker for recurrent $^{131}$I-refractory thyroid cancer and is an important potential monitoring tool for alternative treatment approaches.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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