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13 **IMPACT OF PREGNANCY ON PROGNOSIS OF DIFFERENTIATED**
14 **THYROID CANCER: CLINICAL AND MOLECULAR FEATURES**

15

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29 pregnancy, thyroid cancer, estrogen receptor, DTC outcome

30

31 Manuscript: 3739 words

32

33 **ABSTRACT**

34 **Objective:** Differentiated Thyroid Cancer (DTC) commonly occurs in women of child-
35 bearing age and represents the second most frequent tumor diagnosed during pregnancy
36 only behind breast cancer. It is possible that associated physiological changes could
37 favour tumor development and growth. However, few data are available about the
38 outcome of DTC related to pregnancy, leading to conflicting results. **Methods:** 340
39 patients with DTC <45 years old were retrospectively studied. Patients were divided into
40 three groups according to the time of tumor diagnosis respect of pregnancy. Group 1:
41 diagnosis of DTC at least 2 years after delivery, Group 2: diagnosis during pregnancy or
42 within the second year after delivery, Group 3: nulliparous patients at the time of diagnosis.
43 We evaluated clinical outcome and immunohistochemical expression of estrogen receptor
44 α (ER α), estrogen receptor β (ER β), progesterone receptor (PGR) and aromatase. We
45 also analysed the gene expression of NIS and the prevalence of BRAF V600E mutations.
46 **Results:** Persistence/recurrence of disease was significantly higher in group 2 patients
47 than control groups (p: 0.023). No significant differences were observed in other clinical
48 parameters. Furthermore no difference among the groups were recorded about ER
49 pattern, NIS expression and BRAF mutations. **Conclusions:** Persistence/recurrence of
50 DTC is significantly higher in pregnant patients, suggesting that pregnancy could really
51 exert a negative prognostic role in patients with DTC. The underlying mechanisms are not
52 yet clarified and further studies are required. Our results suggest that a more careful follow
53 up is needed when diagnosis of DTC occurs during pregnancy or shortly after.

54

55 **Abbreviations**

56 DTC: differentiated thyroid cancer; rhTSH: Recombinant human thyroid stimulating
57 hormone; Tg: tireoglobuline; S-Tg: tireoglobuline measured under suppressive therapy
58 with I-T4; A-Tg: tireoglobuline measured at ablation with I-131; rhTSH-Tg: tireoglobuline
59 measured after stimulation with rhTSH; AbTg: Anti-Tireoglobuline Antibody; MTS:
60 metastasis; ER: estrogen receptor; AR: androgen receptor; PGR: progesterone receptor;
61 RAI; radioiodine ablations with I-131; NIS: Na-I symporter

62

63 **INTRODUCTION**

64 Differentiated Thyroid Cancer (DTC) is a relatively rare neoplasia. It represents 3.6% of all
65 malignant tumors in the United States (SEER Cancer Statistics Review, National Cancer
66 Institute Surveillance, Epidemiology, and End Results; 1975–2005. Available from
67 <http://seer.cancer.gov>) and it is generally characterized by good prognosis. Consequently,
68 studies evaluating the prognosis of this tumor have to consider a wide number of cases
69 and a long-term follow-up to highlight differences in survival or disease recurrence rate.
70 The majority of relapses usually occurs within 5 years from the initial treatment, and only
71 sporadic cases have been subsequently documented (1, 2). Despite the low incidence, in
72 the USA DTC represents the second most frequent diagnosed tumor during pregnancy,
73 only after breast cancer. In women of child-bearing age about 10% of thyroid carcinomas
74 is diagnosed during pregnancy or early after delivery (SEER Cancer Statistics Review,
75 National Cancer Institute Surveillance, Epidemiology, and End Results; 1975–2005.
76 Available from <http://seer.cancer.gov>). These findings have led to hypothesize that during
77 this period the presence of several physiologic changes, such as hormonal secretion,
78 growth factors and negative iodine balance, could create a favourable environment for the
79 development and growth of tumors.

80 However, only few studies about the outcome of DTC related to pregnancy have been
81 published. A recent review (3) reports that pregnancy is not generally described in
82 literature as a determining condition for prognosis of DTC, neither in terms of DTC-related
83 death (4), nor of overall survival (5, 6) .

84 Nevertheless, these findings are in contrast with the more recent study published by
85 Vannucchi et al. (7), who reported that DTC in pregnant women had a significant increase
86 of persistent/recurrent disease than those in non pregnant patients. Since the parameters
87 and the methodology in each study were very different, their results were not easily
88 comparable. In fact, the studies conducted by Yasmeen et al. (5) and Herzon et al. (6),
89 focused mainly on the overall survival, while the study of Moosa and Mazzaferri (4) had
90 DTC-related death and disease recurrence, evaluated by biopsy or by ¹³¹I uptake in
91 distant sites, as primary focus. On the contrary, Vannucchi et al. (7) have evaluated
92 persistent/recurrent DTC through more sensitive tests, such as basal and stimulated Tg
93 levels after exogenous TSH injection (rhTSH, Thyrogen[®], Genzyme Corporation, Sanofi
94 Company, Cambridge, MA) which have not been used in the other studies and may - at
95 least partially - explain the different conclusions.

96 Moreover, Vannucchi et al. (7) observed a significantly higher immunohistochemical
97 expression of the Estrogen Receptor α (ER α) in tumors from pregnant women compared
98 to the control groups. With special reference to hormone receptor expression in thyroid
99 tumors, a recent study (8) on the immunohistochemical expression of estrogen and
100 androgen receptors (AR) in DTC showed that ER α was acquired or increased in tumor
101 samples as compared to the corresponding normal tissue, whereas AR and ER β
102 expression was decreased in tumors compared with the surrounding normal tissue. These
103 patterns appeared also to be associated with the clinical behaviour, being the high

104 expression of ER α and AR and the low expression of Er β associated with a more
105 aggressive phenotype.

106 In such a controversial situation, we therefore designed the present study to characterize
107 at clinical, phenotypical and molecular levels DTC cases in pregnancy as compared to
108 matched control groups.

109

110 **MATERIAL AND METHODS**

111 **Patients**

112 We retrospectively evaluated more than 1200 medical records of patients with DTC treated
113 and followed up from 2001 to 2011 at the Nuclear Medicine Department of Mauriziano
114 Hospital, which covers up to 80% of all radioiodine ablations with I-131 (RAI) performed in
115 the Piedmont region. This allowed us to obtain an extremely homogeneous population,
116 representative of DTC epidemiology in the fertile women population.

117 Among them, 340 women were selected according to the following inclusion criteria:

- 118 • Age \leq 45 years at the time of surgery
- 119 • Total thyroidectomy
- 120 • I-131 radioiodine ablation
- 121 • L-T4 TSH-suppressive therapy (TSH \leq 0.1 mU/l) (9)
- 122 • Follow up \geq 1 year
- 123 • rhTSH test during follow up or persistent disease (S-Tg > 2 ng/ml)

124

125 Patients were divided into three groups according to the time of diagnosis of DTC respect
126 to pregnancy. Group 1 included women (n=152, median age 40, range 25-45) with DTC

127 diagnosis at least 2 years after delivery. Group 2 included women (n=38, median age 35,
128 range 26-41) with diagnosis of DTC during pregnancy or within two years after delivery.
129 Group 3 included nulliparous patients at the time of diagnosis (n=150, median age 30,
130 range 15-45).

131 Tumors were classified following the World Health Organization classification (10), staged
132 according to the 6th edition of TNM staging (American Joint Committee on Cancer, AJCC).
133 (11) and classified as Low and High Risk according to the European Consensus
134 Statement criteria (9). ETA guidelines divide patients into three groups: Very Low, Low and
135 High Risk but the first group was not represented in our series because it includes patients
136 with no indications for RAI .

137 Remission or persistent/recurrent disease were defined according to the European and
138 American guidelines for the management of DTC (9, 12):

- 139 ▪ REMISSION: S-Tg and rh-TSH-Tg < 0,6 mcg/l, negative AbTg and
140 normal neck ultrasound.
- 141 ▪ PERSISTENT/RECURRENT disease at least one of the following criteria:
 - 142 - S-Tg > 2 µg/L
 - 143 - rh-TSH-Tg > 2 µg/L
 - 144 - Persistence of AbTg > 4 years with a trend to increase (an increasing
145 antibody production or newly antibodies appearance as a consequence of an
146 increased of autoantigen production) (13, 14)
 - 147 - Neck or distant MTS
 - 148 - Radioiodine uptake outside thyroid bed

149

150 Serum Tg levels were measured during L-T4 withdrawal, immediately before RAI and after
151 12 months of L-T4 suppressive therapy. Then, patients received one injection of rh-TSH
152 (0.9 mg i.m., Thyrogen[®], Genzyme Corporation, Sanofi Company, Cambridge, MA) for two
153 consecutive days; serum samples for TSH and Tg measurements were collected on days
154 0 (before first rh-TSH administration), 3 and 4. Neck ultrasonography was performed 6 and
155 12 months after RAI. TSH levels were evaluated using a chemiluminescent immunoassay
156 (Access Immunoassay Systems, Beckman Coulter[®], Inc.), Tg levels were determined
157 using chemiluminescent immunoassay (Access Immunoassay Systems, Beckman
158 Coulter[®]), with a functional sensitivity of 0.6 µg/L; Ab-Tg antibodies were detected with
159 chemiluminescent immunoassay (Access Immunoassay Systems, Beckman Coulter[®],
160 Inc.). Based on Tg assay, we considered 0.6 µg/L as the cut-off value between
161 undetectable and measurable Tg levels, according to Mazzaferri *et al.* (1)

162

163 **Immunohistochemical analysis of hormone receptors and aromatase in tumor** 164 **tissues**

165 Immunohistochemical evaluation of estrogen receptor α (ER α), estrogen receptor β (ER β),
166 progesterone receptor (PGR) and aromatase were performed in 37 histological specimens
167 selected from the three different groups (12 of group 1, 10 of group 2, 15 of group 3).
168 Cases for immunohistochemical analysis were blinded selected to obtain 3 groups
169 homogenous in terms of age and stage, regardless of the outcome.

170 Immunohistochemical analyses were carried out on paraffin-embedded tissue sections of
171 5 µm, after dewaxing, dehydration in alcohol and rehydration in PBS pH 7,5. Endogenous
172 peroxidase block was performed by immersion of the slides in 0,3% solution of methanol
173 and hydrogen peroxide for 15 minutes. Then sections were incubated with the following
174 monoclonal primary antibodies: ER α (clone 1D5, dilution 1:300, Dako, Glostrup, DK), ER β

175 (clone PPG5/10, dilution 1:50, Dako), PGR (clone 636, dilution 1:300, Dako), and
176 Aromatase (clone mca2077s, dilution 1:50, Serotec, Kidlington, UK). A biotin-free, dextran
177 chain-based detection system (EnVysion, Dako) and diaminobenzidine as the chromogen
178 were used according to standard protocols. All markers were assessed in tumoral and
179 peritumoral tissue using H-score evaluation, which takes into account both quantitative
180 and qualitative expression with a 0-300 range scale.

181

182 **Molecular analysis**

183 ***Nucleic acids isolation.*** Genomic DNA was isolated from formalin-fixed, paraffin-
184 embedded tissues using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). RNA was
185 isolated from paraffin embedded material using the high pure RNA paraffin kit (Roche,
186 Mannheim, Germany) following the manufacturer's instructions. The quantity of isolated
187 DNA and RNA was assessed using a Biophotometer (Eppendorf, Hamburg, Germany).

188 ***BRAF point mutation analysis.*** The presence of *BRAF* point mutation (V600E) was
189 analysed using pyrosequencing and PCR primers following previously published protocols
190 (15). PCR amplification for the pyrosequencing assay was performed according to
191 standard protocols. The amplicons were mixed with sequencing primers and sequencing
192 was performed using a PyroGold reagent kit (Biotage AB) according to the manufacturer's
193 protocol. Results were analyzed using the PSQ-96 MA 2.0.2 software (Biotage).

194 ***Quantitative real time PCR for the sodium/iodide symporter.***

195 Relative cDNA quantitation of the sodium/iodide symporter (NIS) and an internal reference
196 gene (β -actin) were done using a fluorescence-based real-time detection method [ABI
197 PRISM 7900 Sequence Detection System (Taqman); Applied Biosystems/Life
198 technologies, Foster City, CA]. Beta-actin primers and probe were previously published
199 (16), whereas for NIS the TaqMan gene expression assay 20X (SLC5A5

200 Hs00166567_m1, Applied Biosystems) was used according to manufacturer's instructions.
201 The PCR reaction mixture consisted 1,200 nmol/L of each primer, 200 nmol/L probe, 200
202 nmol/L each of dATP, dCTP, dGTP, dTTP, 3.5 mmol/L MgCl₂, and 1x Taqman Universal
203 PCR Master mix to a final volume of 20 μL (all reagents were from PE Applied
204 Biosystems,). Cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes, followed
205 by 46 cycles at 95°C for 15 seconds and 60°C for 1 minute. To analyze target gene
206 expression in individual tumors, the relative gene expression levels were expressed as
207 ratios (differences between the C_t values) between 2 absolute measurements (genes of
208 interest/internal reference gene). Then, the ΔΔC_t values were calculated subtracting ΔC_t
209 values of each case to the value of the normal sample expression, and converting the ratio
210 by the $2^{-\Delta\Delta C_t}$ formula; cases were considered of low or high expression according to the
211 median expression level obtained.

212

213 **Statistical analysis**

214 The clinical (age, outcome, number of treatments, ablation-Tg levels, High/Low risk
215 classification) and pathological/molecular features (histology, pTNM stage, hormone
216 receptor expression, NIS gene expression and BRAF mutation status) were compared
217 among the three groups of patients by using the χ^2 test for dichotomic variables and the
218 Mann Whitney and Kruskal-Wallis tests for continuous variables, as appropriate. The
219 reciprocal correlation among immunohistochemical markers was evaluated using the
220 Spearman's test. Statistical significance was defined as p<0,05.

221 A logistic multivariable analysis was performed. Dependent dichotomous variable was
222 tumor persistence/recurrence (1) or remission (0). Age, T, N and multifocality of primary
223 tumor and pregnancy (DTC diagnosis during pregnancy or within 2 years after delivery: 1;

224 Other groups: 0) were the independent variables. All these analyses were performed using
225 STATISTICA for Windows Ver. 8.0.

226

227

228 **RESULTS**

229 Clinical, biochemical, histopathological and molecular parameters in the three groups are
230 reported in Table 1.

No significant differences were noticed in the number of treatments for achieving clinical remission, in the tumor size or extrathyroidal invasion, in the lymphnodal metastatic involvement at diagnosis, in histology and in High Risk/Low Risk classification of patients according to the ETA guidelines (9).

231 Clinical remission was obtained in 150/152 patients (98.7%) of group 1, 34/38 patients
232 (89.5%) of group 2 and in 143/150 patients (95.3%) of group 3. Persistent/recurrent
233 disease was observed in 2/152 patients (1.3%) of group 1, 4/38 patients (10.5%) of group
234 2 and in 7/150 patients (4.7%) of group 3. Our results showed a significant difference (χ^2 :
235 7.532, P= 0.023) in the outcome among the three groups, with a greater percentage of
236 persistent disease in group 2 than in group 1 and 3. Group 1 and 3 did not show any
237 significant difference. Only 4/38 patients in group 2 had cytological diagnosis while
238 pregnant. They underwent thyroidectomy in the early postpartum period, achieving clinical
239 remission, showing that the surgical delay of few months was not a factor that could
240 influence the worst outcome of group 2.

241 As regards the expression of hormone receptors (Figure 1), the percentage of intratumoral
242 and peritumoral expression of ER α in the 37 histological samples were globally low, with
243 no detection of significant differences between the groups (P=0.96). ER β showed a high
244 expression in the peritumoral tissue in a large number of cases, while in tumoral tissue its

245 expression was quite variable, similarly in the three groups ($P=0.82$). PGR expression was
246 mostly negative in peritumoral tissue, while it was quite variable in tumoral tissue, in a
247 similar way in the three groups ($P=0.41$). A significant correlation was observed in tumor
248 tissue between $ER\alpha$ and PGR (Spearman's R value: $R=0.49$; $P=0.002$). Aromatase was
249 negative both on peritumoral and tumoral tissue in all the samples analyzed. BRAF V600E
250 mutation, known as a negative prognostic factor (18), was detected in 25% in group 1,
251 44,4% in group 2 and 60% in group 3 (average of whole samples= 43%). The difference
252 was not statistically significant ($P=0.191$) showing that the worst outcome observed in
253 patients of group 2 is independent from BRAF mutation. However, BRAF was mutated in
254 100% of patients with persistence of disease and in 37,5% of patients in remission,
255 irrespective of the group.

256 NIS gene expression levels were also not different in the three groups ($P=0.82$) nor
257 associated to BRAF mutation status ($P=0.55$).

258 Logistic multivariable analysis performed on the whole population (thus excluding
259 molecular analyses) showed pregnancy (Group 2) as the unique independent variable for
260 persistent/recurrent DTC prediction. The relative risk (RR) was 1.12 (95% CI 1.02-1.22;
261 $p=0.02$). Age, T, N and multifocality of the primary tumor did not enter the model.

262 In the 37 patients with molecular and immunohistochemical data available, BRAF mutation
263 and low NIS expression were strong independent predictors of persistence/recurrence of
264 DTC (Table 2), whereas $ER\alpha$ and PGR did not enter the model. Pregnancy and $ER\beta$
265 positivity were of borderline statistical significance. .

266 Power analysis was performed grouping the entire population into patients with DTC
267 during or within 2 years after delivery (38 subjects) vs all other patients (302 subjects), with
268 values of 79% and 87%,. by two-sided and one-sided tests, respectively.

269

270 **DISCUSSION**

271 Thyroid cancer discovered during pregnancy represents a challenge for the clinicians
272 because, at present, there are still no reliable data available supporting a specific
273 management of pregnancy-associated DTCs. Currently pregnant patients with a
274 cytologically suspicious thyroid nodule for DTC do not require surgery during pregnancy
275 except in cases of rapid nodular growth and/or the appearance of lymph node metastases
276 (19).

277 Most studies showed that pregnancy did not worsen the prognosis of DTC. In four studies,
278 the prognosis of women with DTC diagnosed either during pregnancy or within the first
279 postpartum period was compared to that of women diagnosed at another time as controls.
280 In three of these works (4-6), no difference was found in DTC prognosis between pregnant
281 women and control groups. However, in the fourth study (7), Vannucchi et al. reported a
282 significant worse outcome in pregnant patients. As a matter of fact, they observed 60% of
283 recurrent/persistent disease in pregnant women (group 2) vs 4.2% in women with DTC
284 diagnosed more than 1 year after delivery (group 1) and 13.1% in nulliparous patients
285 (group 3). Moreover, a higher expression of ER α in tumor samples of pregnant women
286 was reported.

287 In order to verify these conflicting results, we selected a homogeneous population, dividing
288 patients into three groups according to the criteria adopted by Vannucchi et al.. We
289 extended group 2 to women with DTC diagnosis within 2 years after delivery instead of 1
290 year, arbitrarily assuming that in tumors with low biological aggressiveness, such as DTC,
291 pregnancy-induced hyperestrogenism may exert its tissue activity in a longer period. At our
292 knowledge no published data are available on this issue. Moreover, in our population the
293 rate of persistent/recurrent disease in patients diagnosed within 1 year or between 1 and 2
294 years after delivery was very similar (9,5% - 2/21 cases - and 11,7% - 2/17 cases –
295 respectively). On the contrary, all the patients (14/14) diagnosed between 2 and 3 years

296 after delivery displayed clinical remission.

297 Consistent with the data reported by Vannucchi et al, we confirmed a significant correlation
298 between pregnancy and a worse outcome of DTC ($p= 0,023$), representing the unique
299 independent variable for persistent/recurrent disease prediction.

300 Indeed, thyroid cancer diagnosed during pregnancy (group 2) was found to be significantly
301 associated with persistence or relapse of DTC compared to those diagnosed more than 2
302 years after delivery (group 1) or before pregnancy (group 3).

303 Taken together, recent evidence supports the hypothesis that pregnancy may negatively
304 affects the prognosis of DTC. The discrepancy with previous studies could be attributed to
305 the different criteria used for the outcome evaluation, as suggested elsewhere (3).

306 Previous papers used the overall survival, DTC-related death and disease recurrence
307 (evaluated by biopsy or whole body scan) as outcome criteria, which were probably not
308 appropriate for a long survival disease with frequent indolent course. In the present study,
309 according to Vannucchi et al., the persistence/recurrence of disease was investigated
310 using more sensitive and precocious markers such as basal and rhTSH stimulated
311 thyroglobulin and neck ultrasonography, as suggested by European and American
312 guidelines (9, 12).

313 Nevertheless, the worst outcome in patients of group 2 cannot be referred to a higher
314 prevalence of a worse staging at the time of diagnosis or to a more aggressive histological
315 phenotype because, in our study, no significant differences in the examined clinical and
316 morphological parameters were observed.

317 The mechanisms by which pregnancy could affect the DTC outcome are not easily
318 explainable. In order to verify whether molecular and/or phenotypical features were
319 influencing the results above, we tested the protein expression of sex hormone receptors,
320 as well as the gene expression of NIS and the prevalence of BRAF mutations in the three
321 groups. Indeed, we cannot support the negative prognostic role of estrogens, as previously

322 suggested (7), considering that our results did not show any significant expression of ER α
323 and no differences among the three groups were observed. The discrepancy between
324 these results has to be clarified, but a difference in the methodological approach could be
325 considered. For example different antibody dilutions were used in the two works (1:300 vs
326 1:100 dilution). However, it has to be noted that the good correlation between the low
327 expression of ER α and PGR justifies the reliability of our findings. The
328 immunohistochemical analysis was performed also for the detection of ER β , showing a
329 variable expression without any significant difference among the three groups of patients.
330 Furthermore, aromatase expression resulted generally very low, leading us to keep out its
331 potential pathophysiological role.

332 In the multivariable logistic regression analysis, BRAF^{V600E} mutations are associated with a
333 worse prognosis, but their similar distribution among the groups excludes a
334 pathophysiological role on the poorer outcome of group 2 patients .

335 We hypothesized that the worse outcome of group 2 could be explained by a lower
336 response to radioiodine therapy. As resulted by the multivariable logistic regression
337 analysis, NIS lower expression is associated with a higher persistence/recurrence of DTC,
338 but its distribution was not different among the three groups, excluding a role in affecting
339 the outcome of group 2.

340 In conclusion, our results, obtained in a large homogeneous population, confirm that
341 pregnancy could really exert a negative prognostic role, at least in terms of risk of
342 persistent disease or recurrence, in patients with differentiated thyroid cancer. Further
343 studies are needed to clarify the pathophysiological mechanisms. At the present state of
344 our knowledge, a more careful follow up is needed when diagnosis of DTC occurs during
345 pregnancy or shortly after. However, the impact on DTC prognosis is not so heavy to justify
346 the reconsideration of the American guidelines for the management of thyroid cancer
347 during pregnancy (19).

348

349 **DECLARATION OF INTERESTS**

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

352

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356

357 **AUTHOR CONTRIBUTIONS**

358 Ilaria Messuti and Stefania Corvisieri equally contributed to the drafting of this work.

359

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363

364

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439 **FIGURE LEGENDS**

440 **Figure 1.** Immunohistochemical analysis of hormone receptors. A case of multifocal
441 papillary carcinoma (group 3) (**a**; H&E, original magnification 40x), with the predominant
442 nodule of the follicular variant (**b**; H&E, original magnification 200x), and high expression

443 of ER α (c), ER β (d) and PGR (e) (c-d-e: immunoperoxidase, original magnification 200x);
 444 f: scatter plot graphs of the distribution of hormone receptors in the three groups of DTC.
 445

446 **TABLES**

447 Table 1. Clinical, histological and molecular characteristics of patients with a DTC
 448 diagnosis at least 2 years after delivery (Group 1), during pregnancy or within two years
 449 after delivery (Group 2), or before pregnancy/nulliparous (Group 3)

450 * Group 2 was significantly different as compared to both group 1 and group 3.

451 ** The ablation-HTG cut-off was defined according to Webb et al. (17)

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453

454 Table 2: Logistic regression analysis for persistence/recurrence of DTC

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456

457 **Table 1**

458

	Group 1	Group 2	Group 3	P value
Age at diagnosis (years): Median (range)	40 (25-45)	35 (26-41)	30 (15-45)	<0.001
Duration of follow up (years): Median (range)	5 (1-27)	6 (1-10)	6 (1-20)	0.31
Remission	150/152 (98.7%)	34/38 (89.5%)	143/150(95.3%)	0,023*
Persistence/recurrence	2/152 (1.3%)	4/38 (10.5%)	7/15 (4.7%)	
Number of treatments	1,19	1,21	1,28	0,22

(average)				
Ablation-HTG <10 ng/ml **	127/152 (83,5%)	27/38 (71%)	110/150 (73,3%)	0,060
Ablation-HTG >10 ng/ml **	25/152 (16,5%)	11/38 (29%)	40/150 (26,7)	
High Risk	68/152 (44.7%)	19/38 (50%)	79/150 (52,7%)	0,38
Low Risk	84/152 (55.3%)	19/38 (50%)	71/150 (47,3%)	
TNM (T<3)	105/152 (69%)	26/38 (68.4%)	95/150 (63.3%)	0,85
TNM (T>3)	47/152 (31%)	12/38 (31.6%)	55/150 (36.7%)	
TNM (N -)	114/152 (75%)	29/38 (76.3%)	102/150 (68%)	0,54
TNM (N +)	38/152 (25%)	9/38 (23.6%)	48/150 (32%)	
Histology (High Risk)	54/152 (35.5%)	16/38 (42.1%)	62/150 (41.3%)	0,85
Histology (Low Risk)	98/152 (64.5%)	22/38 (57.9%)	88/150 (58.7%)	
ER α tumor expression	3/12 (25 %)	3/10 (30 %)	4/14 (28.6 %)	0.96
ER β tumor expression	5/12 (41.7%)	5/10 (50%)	7/15 (46.7%)	0.92
PGR tumor expression	4/12 (33.3%)	3/10 (30%)	8/15 (53.3%)	0.419
BRAF V600E mutation	3/12 (25%)	4/9 (44.4%)	9/15 (60%)	0,19
NIS fold change <1	8/12 (66,6%)	5/8 (62,5%)	9/13 (69,2%)	0,9

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Table 2

Variable	RR (95% CI)	P value
Pregnancy	1.26 (0.97-1.55)	0.09
Er β positive staining	0.69 (0.38-1.00)	0.06

Presence of BRAF mutation	1.46 (1.16-1.77)	0.005
High NIS expression	0.66 (0.36-0.96)	0.03

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