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1 **TPOAb and thyroid function are not associated with breast cancer outcome; evidence**
2 **from a large-scale study using data from the Taxotere as Adjuvant Chemotherapy Trial**
3 **(TACT, CRUK01/001)**

4
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14

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28 **Key words:** Autoimmune thyroid disease; Autoimmunity; Hyperthyroidism; Hypothyroidism;

29 Thyroid function; Thyroid peroxidase antibodies; Breast cancer.

30 **ABSTRACT**

31 **Background:** Small-scale studies correlated the presence of thyroid autoimmunity
32 with both improved or worsened breast cancer outcome.

33 **Objectives:** We aimed to clarify this association in a large cohort using the phase-III
34 randomized controlled “Taxotere as Adjuvant Chemotherapy Trial” (TACT, CRUK01/001).

35 **Methods:** TACT women >18-years-old with node-positive or high risk node-negative
36 early breast cancer (pT1-3a,pN0-1,M0), with stored plasma (n=1974), taken 15.5 [7.0-24.0]
37 months (median [IQR]) after breast surgery were studied. Patients had also received
38 chemotherapy (100%), radiotherapy (1745/1974 [88.4%]), hormonal therapy (1378/1974
39 [69.8%]), or trastuzumab (48/1974 [2.4%]). History of thyroid diseases and/or related
40 treatments was not available.

41 The prognostic significance of autoantibodies to thyroid peroxidase (TPOAb; positive ≥ 6
42 kIU/L), free-thyroxine and thyrotropin (combined: euthyroid, hypothyroid, hyperthyroid) was
43 evaluated for disease-free survival (DFS), overall-survival (OS), time-to-recurrence (TTR),
44 with Cox regression models in univariate and multivariable analyses. The extended median
45 follow-up was 97.5 months.

46 **Results:** No difference in DFS was found by TPOAb status (unadjusted-hazard ratio
47 [HR]: 0.97, 95%CI: 0.78-1.19, P=0.75) and/or thyroid function (unadjusted-HR [hypothyroid
48 versus euthyroid]: 1.15, 95%CI: 0.79-1.68, P=0.46; unadjusted-HR [hyperthyroid versus
49 euthyroid]: 1.14, 95%CI: 0.82-1.61, P=0.44). Similar results were obtained for OS, TTR,
50 multivariable analyses, when TPOAb titre by tertiles was considered and in a subgroup of 123
51 patients with plasma collected before adjuvant treatments.

52 **Conclusions:** No evidence for a prognostic role of TPOAb and/or thyroid function in
53 moderate-high risk early breast cancer was found in the largest and longest observational study
54 to date.

55 INTRODUCTION

56 An association between breast cancer (BC) and benign thyroid disorders has been
57 debated for decades, reported in several [1,2], but not all [3] studies; the most recent meta-
58 analyses and reviews reached contrasting conclusions [1,4-6]. Hypothyroidism was found to
59 correlate with both an increased [7,8] or reduced [9-11] risk of developing BC, whilst other
60 authors did not report a significant correlation [12,13]. BC has been particularly associated
61 with thyroid autoimmunity (TA); a higher prevalence of anti-thyroid peroxidase (TPO)
62 autoantibodies (TPOAb) was found among BC patients, compared with healthy controls [8,14].
63 Furthermore, a better BC outcome has been reported in TPOAb positive (TPOAb+) versus
64 TPOAb negative (TPOAb-) patients in some [15-18], but not all [19] studies.

65 Currently no validated major blood prognostic markers for BC are available;
66 carcinoembryonic antigen and cancer antigen 15.3 are the most used, but have low specificity
67 and sensitivity [20]. Circulating tumour DNA and tumour cells seem very promising markers,
68 however further studies are needed to validate them in routine clinical practice [21]. It would
69 therefore be valuable if TPOAb could be confirmed as a blood BC prognostic marker.

70 Two studies evaluated 5-year outcomes in 142 [15] and 47 [16] BC women: Smyth *et al.*
71 *al.* [15] reporting TPOAb- as a poor prognostic factor for disease-free survival (DFS) and
72 overall survival (OS), and Fiore *et al.* [16] reporting 6.7% mortality in patients positive for
73 anti-thyroid autoantibodies (TAb), mainly TPOAb+, compared with 46.9% in TAb negative
74 patients. Farahati *et al.* evaluated 314 newly diagnosed BC patients and found no distant
75 metastases among TPOAb+ patients compared with 6.6% among TPOAb- patients [17]. In
76 contrast, Jiskra *et al.* followed 84 BC patients for 136 months (median), finding no impact of
77 TPOAb on DFS or OS [19].

78 The aim of the present study was to clarify the impact of TPOAb on BC prognosis in a
79 large, well powered patient cohort with long-term follow-up, according to the “REporting
80 recommendations for tumour MARKer prognostic studies (REMARK)” guidelines [22]. The

81 “Taxotere as Adjuvant Chemotherapy Trial (TACT)” recruited 4162 women diagnosed with
82 moderate-high risk early BC, evaluating whether sequential docetaxel (Taxotere) after
83 anthracycline therapy would improve patient outcome compared with standard anthracycline
84 chemotherapy: analyses were conducted at 62 months [23] and 97.5 months [24] follow-up,
85 both showing no evidence of a difference between the two chemotherapy regimens. Of
86 relevance, stored plasma was available in a significant number of these patients.

87 Furthermore, TPO is expressed in BC tissue [25], providing a possible mechanistic link:
88 a thyroid/breast shared autoimmune response might target tumour cells and improve BC
89 outcome. If TPOAb+ was confirmed as associated with a better BC outcome, new BC
90 therapeutic approaches based on antigen-specific immunotherapies targeting TPO could be
91 explored.

92

93 **MATERIALS AND METHODS**

94 **Patients**

95 The TACT study [23] was a multicentre, open-label, phase-III, randomised controlled
96 trial of women aged >18 years diagnosed with operable early BC (pT1-3a, pN0-1, M0), with
97 indication for adjuvant chemotherapy, including both lymph-node positive (node+) patients
98 and lymph-node negative (node-) but high risk (e.g., tumour grade 3, hormonal-receptor
99 expression negative, or lymphovascular invasion) patients.

100 Between February 2001 and June 2003, 4162 women were enrolled across 103 UK
101 and one Belgian centres. All subjects underwent surgery, mastectomy or wide-local-excision
102 (WLE), and were randomized (1:1 ratio) to the experimental regimen FEC-D (n=2073;
103 fluorouracil, epirubicin, cyclophosphamide [FEC] followed by docetaxel) or centre’s choice of
104 control chemotherapy, either FEC (n=1265) or E-CMF (n=824; epirubicin followed by CMF
105 [cyclophosphamide, methotrexate, and fluorouracil]). Adjuvant radiotherapy was mandatory
106 after WLE or used after mastectomy according to local guidelines. Endocrine treatments

107 (tamoxifen or aromatase-inhibitor monotherapy, tamoxifen followed by aromatase-inhibitor)
108 were administered to patients with oestrogen receptor (ER) positive expression (ER+). Patients
109 with human epidermal growth factor receptor-2 (HER2) positive expression (HER2+) were
110 allowed to enter clinical trials assessing trastuzumab. All subjects have given their informed
111 consent and the study protocol has been approved by the institute's committee on human
112 research.

113

114 **Laboratory measurements**

115 Following a protocol amendment (November 2002), blood was taken for future
116 translational research at the time of randomization, or at their next follow-up visit. Plasma
117 samples were stored at -20°C for 6.5-13 years (range) at The Institute of Cancer Research
118 (London, UK), and transferred to the Thyroid Research Group (Cardiff, UK) for TPOAb,
119 thyrotropin (TSH) and free-thyroxine (FT4) analyses (October 2014) using an ADVIA Centaur
120 automated immunoassay analyser (Bayer plc, UK) and Chemiluminescent Microparticle
121 Immunoassay methods by the ARCHITECT® System (ABBOTT Laboratories, USA).
122 According to the assay cut-off, TPOAb values were dichotomized as ≥ 6 kIU/L (positive:
123 TPOAb+) versus < 6 kIU/L (negative: TPOAb-); TPOAb+ were also categorized into tertiles.
124 FT4 and TSH normal ranges were 9.0–19.1 pmol/L and 0.30–4.40 mIU/L, respectively; they
125 were also combined in a thyroid function status variable: euthyroid (FT4 and TSH within the
126 normal ranges), hypothyroid (FT4 < 9.0 pmol/L and/or TSH > 4.40 mIU/L); hyperthyroid (FT4
127 > 19.1 pmol/L and/or TSH < 0.3 mIU/L).

128

129 **Statistical analysis**

130 According to TPOAb prevalence in age-matched females of general population [26,27],
131 20% of BC individuals were expected to be TPOAb+. Power calculations indicated 1158 and
132 1430 samples required to provide respectively 80% and 90% power to detect a 81% 5-year

133 DFS in TPOAb+ versus 73% in TPOAb- subjects (HR, 0.64; two-sided log-rank test with a
134 0.05 probability of a type I error), consistent with a 74.9% 5-year DFS rate in the whole TACT
135 cohort [23].

136 Baseline characteristics, BC treatments and DFS-related characteristics were compared
137 between TACT patients included or not in this study, and presented by dichotomized TPOAb
138 and thyroid function status. Correlations between thyroid biomarkers were assessed using the
139 Spearman rank method.

140 The primary outcome was to assess TPOAb prognostic significance in relation to DFS;
141 secondary outcomes were TPOAb prognostic significance in relation to OS and time-to-
142 recurrence (TTR), and thyroid function in relation to DFS, OS and TTR.

143 For DFS, OS and TTR, Kaplan-Meier curves were plotted and biomarkers compared
144 with the log-rank test, and assessed firstly in a univariate Cox proportional hazards regression
145 model stratified by centre's choice of control chemotherapy regimen and ER status, and
146 subsequently included in a multivariable Cox model along with known BC prognostic factors:
147 age, HER2 status, nodal involvement, tumour size and tumour grade. Additional variables, i.e.
148 trial treatment (experimental versus control), type of surgery, trastuzumab use, radiotherapy
149 and menopausal status, were included if, by stepwise selection ($P < 0.05$), shown to add value.
150 TPOAb, TSH and FT4 were subsequently considered for inclusion if providing independent
151 prognostic information. Interaction tests were used to explore differential effects within
152 subgroups. HR with 95% CI were obtained, with $HR < 1$ indicating a better BC prognosis.

153 All patients with a biomarker value available were included in the analysis, as per an
154 intention-to-treat analysis. All analyses were conducted using Stata version 13.1
155 (STATA CORP, TX) [23,24].

156

157 **RESULTS**

158 All available TACT plasma samples (N=2000) were analysed for thyroid biomarkers,
159 and 1974 samples were considered for the statistical analyses (“analysis population”;
160 **Supplemental Fig. 1**). The median (IQR; range) blood collection time was 15.5 (7.0-24.0; 0.5–
161 57.2) months after surgery.

162 **Supplemental Table 1** reports analysis population’s characteristics; the median (IQR;
163 range) follow-up was 96.7 (87.4-106.3; 3.4-126.4) months. Overall 5-year estimates for DFS,
164 OS and TTR were 79.5% (95% CI, 77.6-81.2), 87.4% (95% CI, 85.9-88.8) and 81.1% (95%
165 CI, 79.3-82.8), respectively.

166

167 **Distribution of TPOAb and thyroid function**

168 TPOAb+ was detected in 406/1974 (20.6%) patients, distributed in the following
169 tertiles: 137 (6.9%) 6-40 kIU/L (T1), 134 (6.7%) 41-238 kIU/L (T2), 135 (6.8%) 240-2000
170 kIU/L (T3). Baseline characteristics were largely comparable between TPOAb+ and TPOAb-
171 patients (**Table 1**), apart from age, with TPOAb+ patients slightly older than TPOAb- patients
172 (mean [SD] age, 50.2 [7.7] years versus 48.8 [8.5] years, respectively; P=0.005).

173 Plasma material was sufficient to determine FT4 and TSH values in 1974/1974 (100%)
174 and 1971/1974 (99.8%) samples respectively. Among the 1974 patients, 1760 (89.2%) were
175 euthyroid, 96 (4.9%) hypothyroid and 118 (6.0%) hyperthyroid; all 3 subgroups had similar
176 baseline characteristics (**Table 1**), apart from age, with hypothyroid and hyperthyroid patients
177 slightly older than euthyroid patients (mean [SD] age, respectively 50.5 [6.6] years and 50.7
178 [7.6] years, versus 48.9 [8.5] years; P=0.03).

179 As shown in **Supplemental Fig. 2**, FT4 and TSH were inversely correlated (Spearman
180 rank, -0.23; P<0.001) and TPOAb was positively associated with TSH (Spearman rank, 0.24;
181 P<0.001). The inverse correlation between TPOAb and FT4 was weak (Spearman rank, -0.04;

182 P=0.09). TPOAb+ cases were more prevalent among hypothyroid and hyperthyroid patients
183 compared with the euthyroid group (73/96 [76.0%] hypothyroid; 45/118 [38.1%] hyperthyroid;
184 288/1760 [16.4%] euthyroid; P<0.001).

185

186 **TPOAb and BC prognosis**

187 The majority of DFS events were related to distant recurrence in both TPOAb+ and
188 TPOAb- groups (**Supplemental Table 2**). There was no evidence of a difference in DFS
189 between TPOAb+ and TPOAb- patients (unadjusted-HR: 0.97, 95% CI: 0.78-1.19, P=0.75,
190 **Fig. 1A**; adjusted-HR: 1.00, 95% CI: 0.81-1.24, P=0.98, **Table 2**). Subgroup analyses showed
191 no evidence of any significant interaction effects (**Fig. 2**). Similarly, there was no evidence of
192 a difference by TPOAb status on OS (unadjusted-HR: 0.86, 95% CI: 0.66-1.11, P=0.24, **Fig.**
193 **1B**; adjusted-HR: 0.89, 95% CI: 0.69-1.14, P=0.35, not shown) and TTR (unadjusted-HR: 0.97,
194 95% CI: 0.78-1.21, P=0.80, **Fig. 1C**; adjusted-HR: 1.02, 95% CI: 0.81-1.27, P=0.89, not
195 shown). TPOAb+ tertiles showed no evidence of a prognostic effect in both univariate (**Fig. 3**)
196 and multivariable (data not shown) analyses for DFS, OS and TTR.

197 Two sensitivity analyses included 126 node+ patients not treated with radiotherapy,
198 similar to Fiore *et al.* cohort [16], and 123 patients with blood taken before any adjuvant
199 therapy. The median (IQR; range) time of blood collection after surgery was 12.4 (4.9-21.6;
200 0.7–47.2) months and 1.1 (0.9-1.4; 0.5-5.9) months, respectively. There was no evidence of a
201 significant impact on DFS by TPOAb status in either of the two analyses, with unadjusted-HRs
202 of 1.48 (95% CI, 0.68-3.25; P=0.32) and 0.83 (95% CI, 0.35-2.03; P=0.69) respectively.

203

204 **Thyroid function and BC prognosis**

205 There was no evidence of a significant difference for DFS, OS and TTR by thyroid
206 function status in either univariate (**Fig. 4**) or multivariable (data not shown) analyses, and

207 when considering FT4 and TSH separately (DFS, **Supplemental Table 3**; OS and TTR, not
208 shown).

209

210 **DISCUSSION**

211 In this large cohort of moderate-high risk early BC patients receiving adjuvant systemic
212 treatments we found that neither the presence nor the titre of plasma TPOAb, assessed after
213 BC diagnosis and measured with standard assays, had a substantial impact on long-term
214 recurrence or mortality; similar findings were observed for thyroid status. These results
215 confirm one previous finding [19], but contrast with two other studies [15,16]. We believe that
216 our study is reliable, considering that our patient cohort is the largest to date, with one of the
217 longest follow-ups, and focused on a well-defined BC population. Previous studies used
218 smaller patient cohorts with shorter follow-ups [15,16,19], mixed different BC stages [19], or
219 provided no information about BC stage [15], histological [15,19] and molecular subtypes
220 [15,16,19], and adjuvant treatments received [15,19]; they may be susceptible to bias and
221 random findings. In addition, the BC population analysed in this study is very similar to that
222 of Fiore *et al.*, who recruited non-metastatic aggressive BC all treated with chemotherapy [16].

223 The long survival of our patient cohort could obscure a minor prognostic effect of
224 TPOAb and/or thyroid function on BC, hypothetically detectable only among patients not
225 suitable for standard treatments (e.g. medical contraindications) and targeted therapies (e.g.
226 triple negative BC). This is possible but unlikely, since our exploratory analysis conducted
227 among different BC subtypes confirmed our negative results. Furthermore, the multivariable
228 analyses confirmed nodal status and tumour size as the two most important BC prognostic
229 factors [28], proving that the cohort used was appropriate for the research question, and the
230 model reasonably sensitive. Similarly, the better BC prognosis characterizing the intermediate
231 age group (50-59 years) is consistent with the results of a recent large cohort study [29].

232 Our study cannot exclude a role of different TA parameters on BC prognosis, i.e. the
233 presence of goitre [15] or incidental TA-related ¹⁸F-FDG PET/CT uptake [18]. Furthermore,
234 differences in the alternative splicing of TPO in the breast as compared to the thyroid have
235 been described [25], therefore this might also result in different TPO epitopes being targeted.

236 TPOAb prevalence in our cohort, similar to our *a priori* predicted value, reflects
237 TPOAb prevalence among women of general population [26,30], increasing with age [26,31].
238 It remains possible that TPOAb+ rates are higher in the BC population, as our study was not
239 designed to compare TPOAb prevalence among BC patients and the general population.

240 The principal limitations of the present study are the lack of clinical history for thyroid
241 diseases or medications and that, similarly to previous studies [15,19], blood was mainly
242 collected during/after adjuvant BC therapy. The first limitation might influence the prognostic
243 role of thyroid function, but marginally of TPOAb, since they should exert an effect when
244 either pre-existing, or appearing at a later time [32]; however, the evidence that thyroid function
245 influences BC outcome is weak [6]. The finding of more cases of hyper- (6.0%) than hypo-
246 thyroidism (4.9%) may reflect over-treatment with levothyroxine in some individuals.

247 Regarding BC adjuvant treatments, an increased risk of hypothyroidism after
248 chemotherapy [33,34] or radiotherapy [35,36] for BC has been suggested in a few small
249 studies, but not confirmed by others [37]. Tamoxifen can exert a modulation of thyroid
250 function, mainly via an anti-thyroid effect [38,39] and the stress related to the surgical
251 procedure itself has been suggested to cause immunomodulation [40]. However no clear large-
252 scale effects of adjuvant treatments for BC, including trastuzumab, on thyroid function and
253 immunity have been described, and our sensitivity analysis in a subgroup of 123 patients in
254 whom blood was collected before BC adjuvant therapy showed no evidence of TPOAb
255 prognostic ability, even if the wide 95% CI suggests a lack of statistical power.

256 To draw definitive conclusions, a prospective study collecting blood before cancer
257 treatments would be ideal, but difficult to realise because of the large patient number required,

258 as shown by our *a priori* power calculation. Furthermore, this study analysed moderate-high
259 risk early BC only. BC is a heterogeneous disease, with many subtypes characterised by
260 different clinical behaviour and prognosis; it could be possible that TPOAb and/or thyroid
261 function affect the prognosis of certain specific BC subtypes and stages only, therefore they
262 should be all investigated separately, with a much higher total patient numbers required to
263 reach significant and definitive results.

264 In conclusion, the present study is to our knowledge the largest currently available
265 investigating the impact of blood TPOAb and thyroid function on BC prognosis, providing a
266 detailed description of the BC population analysed, and therefore representing a key-work to
267 clarify this debate over decades. We found that TPOAb and thyroid function, both measured
268 with standard assays and after BC diagnosis, appear not to influence substantially the long-
269 term recurrence and mortality of moderate-high risk early BC in the modern era. Major
270 confounding in this conclusion due to BC treatments seems unlikely. Future studies might
271 explore different BC stages and/or specific subtypes, also searching for non-conventional or
272 breast-specific immune responses to particular TPO epitopes, to determine whether aspects of
273 TA other than standard TPOAb and thyroid function may be relevant to BC outcome.

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- 414

416 Table 1: Baseline characteristics and treatments for breast cancer by autoantibodies to thyroid peroxidase (TPOAb) and thyroid function status

	TPOAb- N = 1568	TPOAb+ N = 406	P value	Hypothyroid N = 96	Euthyroid N = 1760	Hyperthyroid N = 118	P value
Age (years): mean (SD)	48.8 (8.5)	50.2 (7.7)	0.005 ^a	50.5 (6.6)	48.9 (8.5)	50.7 (7.6)	0.03 ^d
Age group (years): n (%)							
<40	257 (16.4)	49 (12.1)	0.08 ^b	8 (8.3)	287 (16.3)	11 (9.3)	0.62 ^b
40-49	575 (36.7)	151 (37.2)		36 (37.5)	647 (36.8)	43 (36.4)	
50-59	590 (37.6)	167 (41.1)		45 (46.9)	657 (37.3)	55 (46.6)	
≥60	146 (9.3)	39 (9.6)		7 (7.3)	169 (9.6)	9 (7.6)	
Nodal status: n (%)							
Node negative	314 (20.0)	93 (22.9)	0.62 ^b	18 (18.8)	367 (20.9)	22 (18.6)	0.61 ^b
1-3 positive nodes	719 (45.9)	171 (42.1)		33 (34.4)	808 (45.9)	49 (41.5)	
≥4 positive nodes	535 (34.1)	142 (35.0)		45 (46.9)	585 (33.2)	47 (39.8)	
Tumour grade: n (%)							
Grade 1	77 (4.9)	23 (5.7)	0.74 ^b	4 (4.2)	88 (5.0)	8 (6.8)	0.72 ^b
Grade 2	603 (38.5)	155 (38.2)		35 (36.5)	681 (38.7)	42 (35.6)	
Grade 3	883 (56.3)	228 (56.2)		57 (59.4)	986 (56.0)	68 (57.6)	
Unknown	5 (0.3)	0 (0.0)		0 (0.0)	5 (0.3)	0 (0.0)	
Tumour size (cm): n (%)							
≤2	578 (36.9)	147 (36.2)	0.59 ^b	25 (26.0)	659 (37.4)	41 (34.8)	0.38 ^b
>2 and ≤5	857 (54.7)	220 (54.2)		61 (63.5)	952 (54.1)	64 (54.2)	
>5	132 (8.4)	39 (9.6)		10 (10.4)	148 (8.4)	13 (11.0)	
Unknown	1 (0.1)	0 (0.0)		0 (0.0)	1 (0.1)	0 (0.0)	
ER & HER2 status: n (%)							
ER+	1107 (70.6)	289 (71.2)	0.85 ^c (ER) 0.45 ^c (HER2)	69 (71.9)	1248 (70.9)	79 (67.0)	0.62 ^c (ER) 0.84 ^c (HER2)
& HER2+	198 (12.6)	49 (12.1)		13 (13.5)	220 (12.5)	14 (11.9)	
& HER2-	772 (49.2)	201 (49.5)		46 (47.9)	873 (49.6)	54 (45.8)	
& HER2 unknown	137 (8.7)	39 (9.6)		10 (10.4)	155 (8.8)	11 (9.3)	
ER-	461 (29.4)	117 (28.8)		27 (28.1)	512 (29.1)	39 (33.1)	
& HER2+	118 (7.5)	43 (10.6)		8 (8.3)	141 (8.0)	12 (10.2)	
& HER2-	289 (18.4)	61 (15.0)		15 (15.6)	313 (17.8)	22 (18.6)	
& HER2 unknown	54 (3.4)	13 (3.2)		4 (4.2)	58 (3.3)	5 (4.2)	
Molecular subgroup: n (%)							
ER+/HER2 ⁻¹	784 (50.0)	203 (50.0)	0.40 ^c	47 (49.0)	885 (50.3)	55 (46.6)	0.94 ^c
HER2+	316 (20.2)	92 (22.7)		21 (21.9)	361 (20.5)	26 (22.0)	
Triple negative	277 (17.7)	59 (14.5)		14 (14.6)	301 (17.1)	21 (17.8)	

	TPOAb- N = 1568	TPOAb+ N = 406	P value	Hypothyroid N = 96	Euthyroid N = 1760	Hyperthyroid N = 118	P value
Type of surgery and radiotherapy use: n (%)							
Mastectomy	854 (54.5)	225 (55.4)	0.74 ^c (surgery)	53 (55.2)	962 (54.7)	64 (54.2)	0.99 ^c (surgery)
with radiotherapy [^]	688 (80.6)	177 (78.7)		0.61 ^c (radiotherapy)	47 (88.7)	772 (80.2)	
Wide local excision	714 (45.5)	181 (44.6)	0.61 ^c (radiotherapy)	43 (44.8)	798 (45.3)	54 (45.8)	0.33 ^c (radiotherapy)
with radiotherapy [#]	704 (98.6)	176 (97.2)		41 (95.3)	787 (98.6)	52 (96.3)	
Endocrine treatment in ER+ patients: n (%)*							
Tamoxifen monotherapy	696 (62.9)	167 (57.8)	0.13 ^c	43 (62.3)	772 (61.9)	48 (60.8)	0.09 ^c
Tamoxifen followed by AI	354 (32.0)	100 (34.6)		20 (29.0)	409 (32.8)	25 (31.7)	
AI monotherapy	46 (4.2)	15 (5.2)		6 (8.7)	53 (4.3)	2 (2.5)	
No endocrine treatment/unknown	11 (1.0)	7 (2.4)		0 (0.0)	14 (1.1)	4 (5.1)	
Trastuzumab in HER2+ patients: n (%)**							
Yes	40 (12.7)	8 (8.7)	0.36 ^c	1 (4.8)	44 (12.2)	3 (11.5)	0.71 ^c
No/Not known	276 (87.3)	84 (91.3)		20 (95.2)	317 (87.8)	23 (88.5)	
Chemotherapy: n (%)							
Control (FEC)	498 (31.8)	128 (31.5)	0.52 ^c	27 (28.1)	568 (32.3)	31 (26.3)	0.90 ^c
Control (E-CMF)	271 (17.3)	61 (15.0)		16 (16.7)	301 (17.1)	15 (12.7)	
FEC-D	799 (51.0)	217 (53.4)		53 (55.2)	891 (50.6)	52 (44.1)	

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¹ includes ER-, PgR+, HER2-

[^] denominators calculated using patients treated with mastectomy

[#] denominators calculated using patients treated with wide local excision

^{*} denominators calculated using ER+ patients

^{**} denominators calculated using HER2+ patients

^a t-test

^b trend test; note “unknowns” excluded from the test

^c Fisher’s exact test

^d ANOVA

AI, aromatase-inhibitors; ER+, positive estrogen receptor (ER); ER-, negative ER; E-CMF, epirubicin 100 mg/m² for 4 cycles followed by CMF (cyclophosphamide 600 mg/m², methotrexate 40 mg/m² and fluorouracil 600 mg/m²) for 4 cycles; FEC, fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m² for 8 cycles; FEC-D, FEC for 4 cycles followed by docetaxel 100 mg/m² for 4 cycles; HER2+, positive human epidermal growth factor receptor-2 (HER2); HER2-, negative HER2; PgR+, positive progesterone receptor (PgR); SD, standard deviation; TPOAb+, positive TPOAb; TPOAb-, negative TPOAb; triple negative, negative HER2, ER and PgR.

432 **Table 2: Multivariable analysis for disease-free survival by dichotomized autoantibodies to**
 433 **thyroid peroxidase (TPOAb)**

434

		HR	95% CI	P value
TPOAb status	negative (n=1568)	1.00	-	-
	positive (n=406)	1.00	0.81-1.24	0.98
Nodal status	positive (n=1567)	1.00	-	-
	negative (n=407)	0.49	0.37-0.64	< 0.001
HER2 status	negative (n=1323)	1.00	-	-
	positive (n=408)	1.19	0.97-1.46	0.09
	unknown (n=243)	0.93	0.71-1.23	0.63
Age group (years)	<40 (n=306)	1.00	-	-
	40-49 (n=726)	0.78	0.61-1.00	0.05
	50-59 (n=757)	0.75	0.59-0.96	0.02
	≥60 (n=185)	0.95	0.69-1.31	0.76
Tumour grade	Grade 1 (n=100)	1.00	-	-
	Grade 2 (n=758)	1.15	0.74-1.78	0.55
	Grade 3 (n=1111)	1.39	0.89-2.17	0.14
	unknown (n=5)	0.77	0.10-5.75	0.80
Tumour size (cm) *	≤2 (n=725)	1.00	-	-
	>2 and ≤5 (n=1077)	1.37	1.12-1.66	0.002
	>5 (n=171)	1.88	1.41-2.52	< 0.001
Type of surgery	Mastectomy (n=1079)	1.00	-	-
	WLE (n=895)	0.79	0.66-0.95	0.01

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436 HER2, human epidermal growth factor receptor-2; HR, hazard ratio (HR <1 indicates a favorable
 437 breast cancer outcome); WLE, wide local excision; 95% CI, 95% confidence interval.

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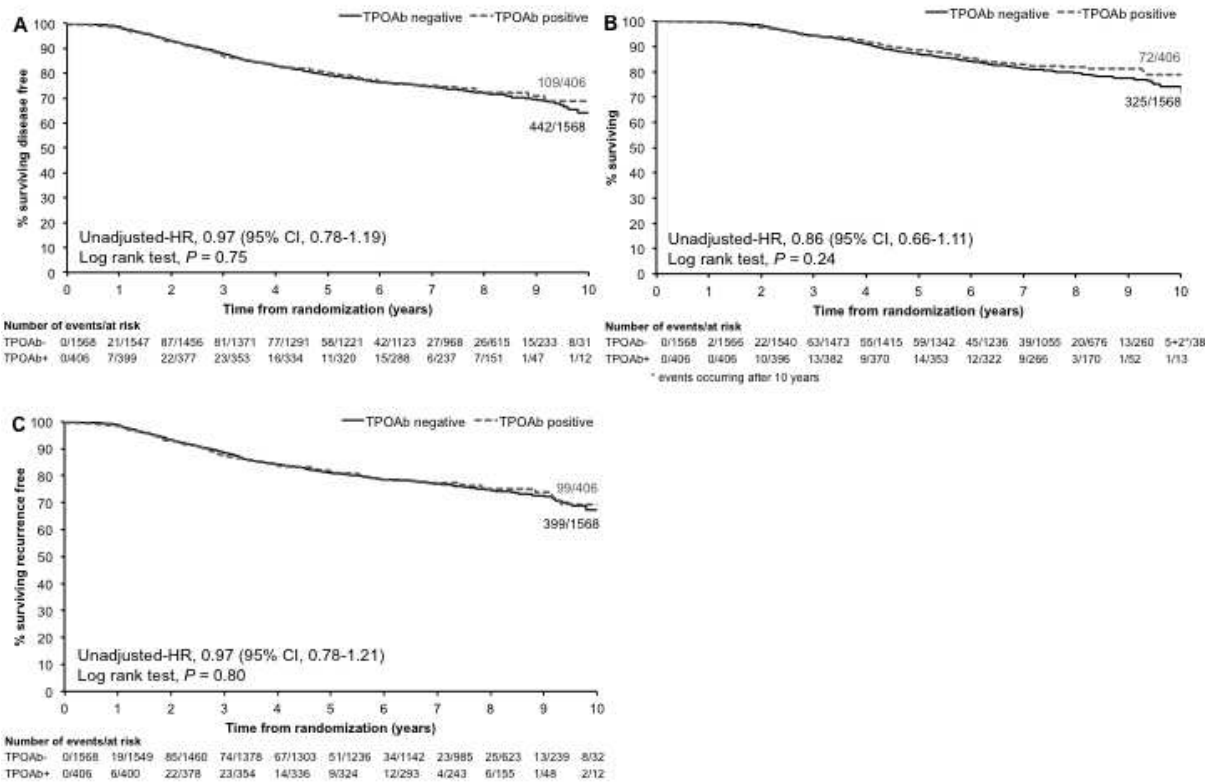
* The patient with unknown tumour size (n=1) has not been considered for this analysis.

439

440 **FIGURES**

441

442 **Fig. 1: Univariate analyses by dichotomized autoantibodies to thyroid peroxidase**
 443 **(TPOAb)**

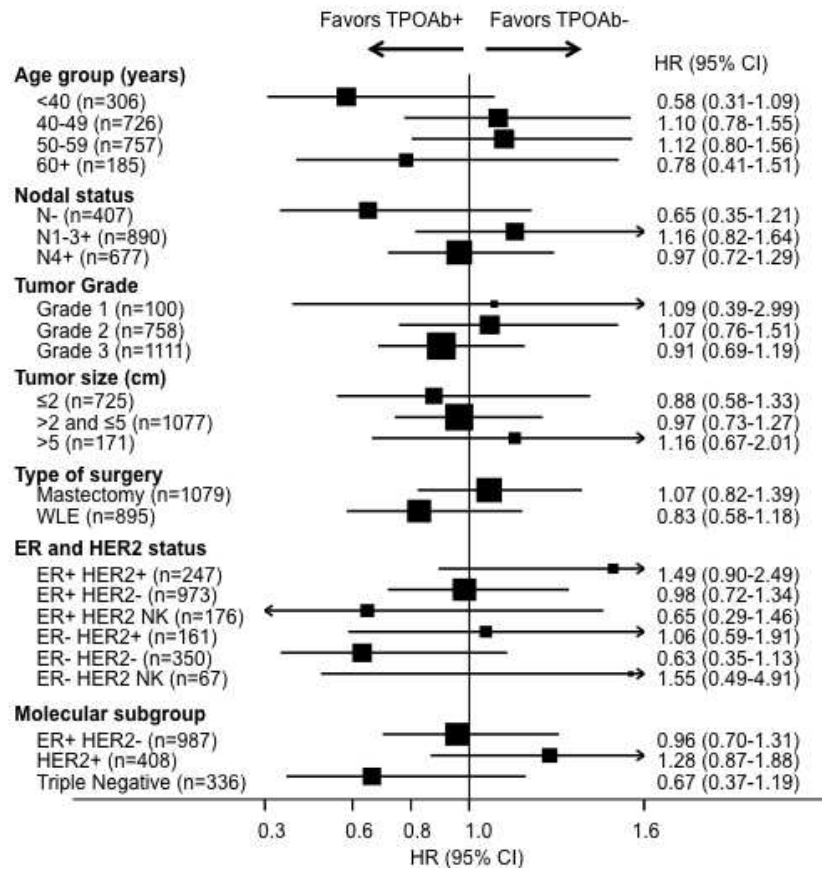


444

445 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)
 446 in patients positive (≥ 6 kIU/L) and negative (< 6 kIU/L) for TPOAb. HR, hazard ratio (HR < 1
 447 indicates a favorable BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free
 448 survival (DFS). Panel B: overall survival (OS). Panel C: time to recurrence (TTR).

449

450 **Fig. 2: Exploratory subgroup analyses for disease-free survival by dichotomized**
 451 **autoantibodies to thyroid peroxidase (TPOAb)**

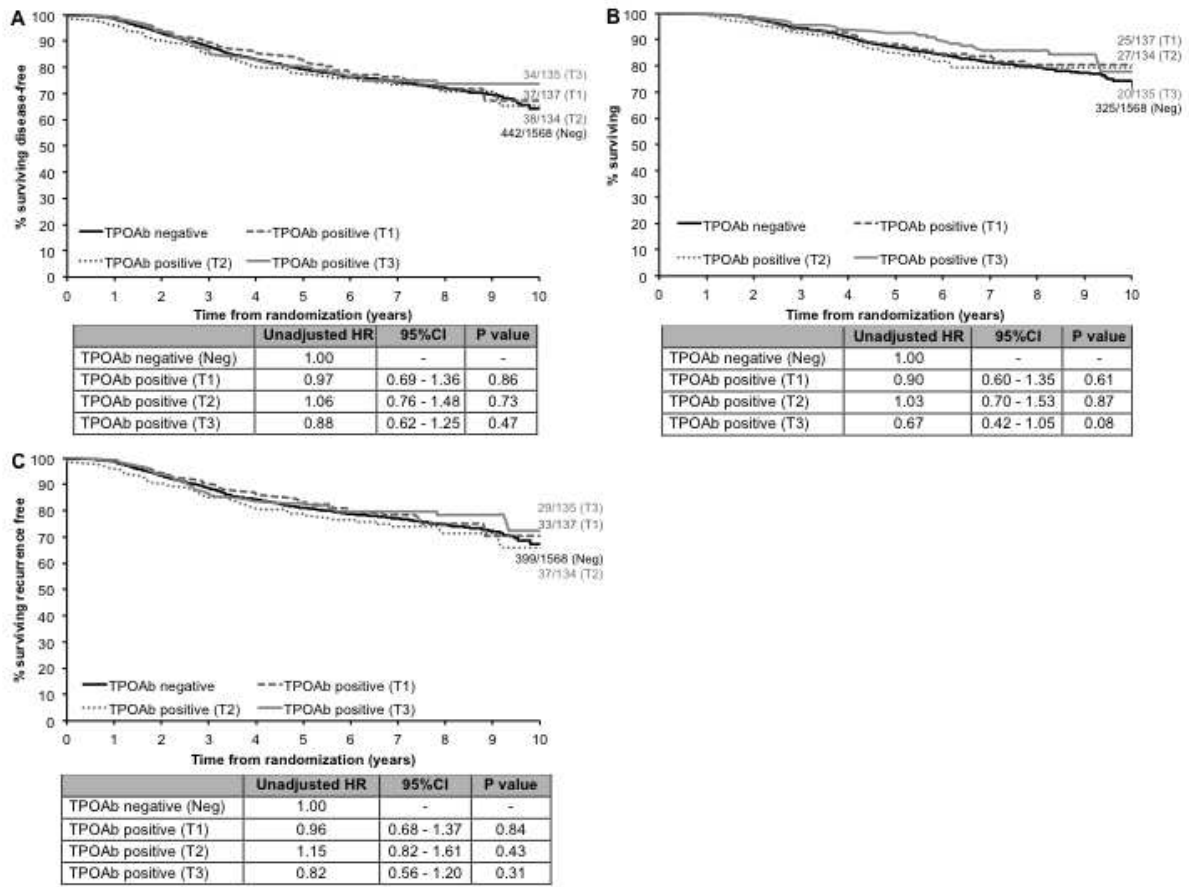


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453 ER+, positive estrogen receptor (ER); ER-, negative ER; HER2+, positive human epidermal
 454 growth factor receptor-2 (HER2); HER2-, negative HER2; NK, not known; N-, lymph-node
 455 negative; N1-3+, 1-3 lymph-nodes positive, N4+, 4 or more lymph-nodes positive; TPOAb+,
 456 positive TPOAb; TPOAb-, negative TPOAb; triple negative, negative HER2, ER and
 457 progesterone receptor; WLE, wide local excision; 95% CI, 95% confidence interval.

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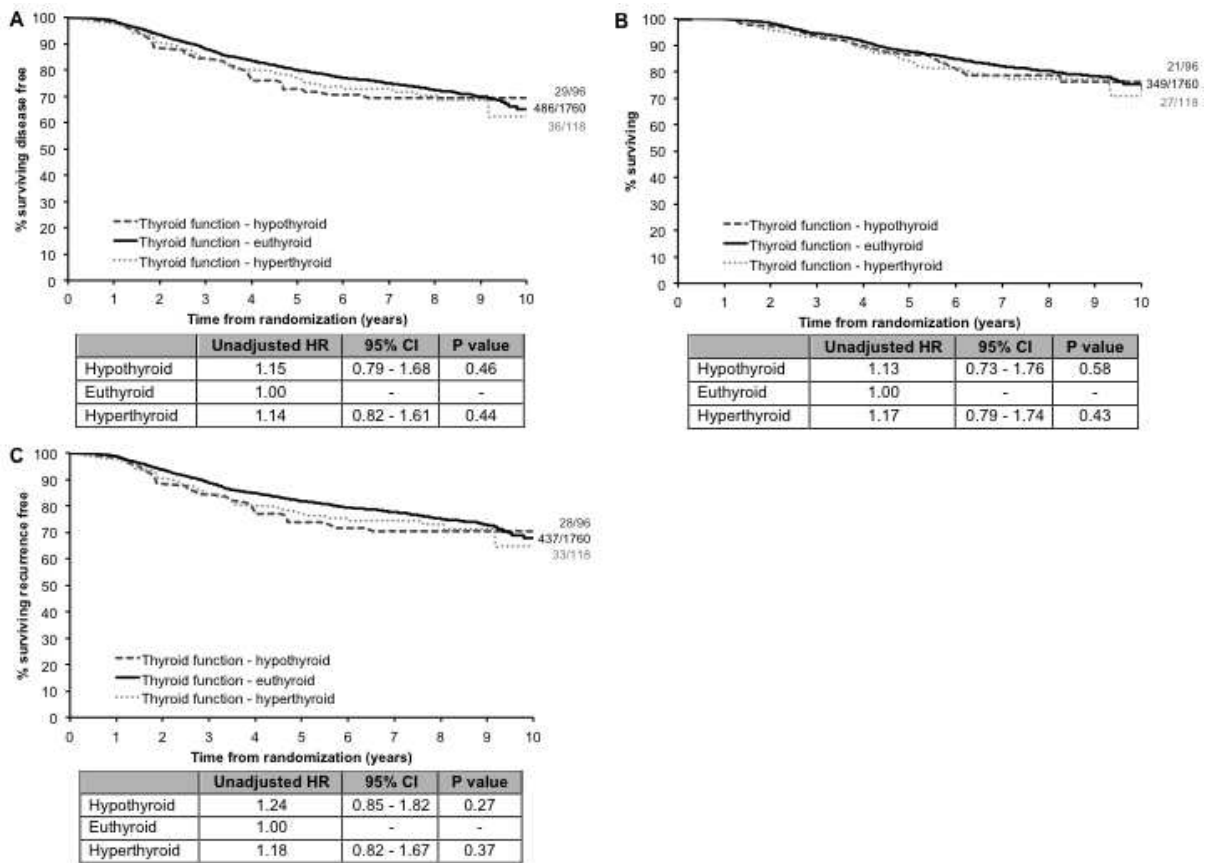
459 **Fig. 3: Univariate analyses by autoantibodies to thyroid peroxidase (TPOAb) categorized**
 460 **into tertiles**



461
 462 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)
 463 in patients negative (<6 kIU/L) and positive for TPOAb categorized into tertiles: 6-40 kIU/L
 464 (T1), 41-238 kIU/L (T2), 240-2000 kIU/L (T3). HR, hazard ratio (HR <1 indicates a favorable
 465 BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free survival (DFS). Panel
 466 B: overall survival (OS). Panel C: time to recurrence (TTR).

467

468 **Fig. 4: Univariate analyses by thyroid function status**



469

470 Kaplan-Meier curves relative to breast cancer (BC) outcome (median follow-up 96.7 months)

471 according to thyroid function status. Euthyroid, free-thyroxine (FT4) 9.0–19.1 pmol/L and

472 thyrotropin (TSH) 0.30–4.40 mIU/L; hyperthyroid, FT4 >19.1 pmol/L and/or TSH <0.3

473 mIU/L; hypothyroid, FT4 <9.0 pmol/L and/or TSH >4.40 mIU/L. HR, hazard ratio (HR <1

474 indicates a favorable BC outcome); 95% CI, 95% confidence interval. Panel A: disease-free

475 survival (DFS). Panel B: overall survival (OS). Panel C: time to recurrence (TTR).

476

477 SUPPLEMENTAL MATERIAL

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479 Supplemental Table 1: Baseline characteristics, treatments for breast cancer and disease-free

480 survival (DFS) related characteristics

481

	Analysis population N = 1974	Not included patients N = 2188	All TACT trial patients N = 4162
Age (years), mean (SD)	49.1 (8.4)	48.2 (8.6)	48.6 (8.5)
Age group (years), n (%)			
<40	306 (15.5)	412 (18.8)	718 (17.3)
40-49	726 (36.8)	841 (38.4)	1567 (37.7)
50-59	757 (38.4)	730 (33.4)	1487 (35.7)
≥60	185 (9.4)	205 (9.4)	390 (9.4)
Nodal status, n (%)			
Node negative	407 (20.6)	428 (19.6)	835 (20.1)
1-3 positive nodes	890 (45.1)	949 (43.4)	1839 (44.2)
≥4 positive nodes	677 (34.3)	811 (37.1)	1488 (35.8)
Tumor grade, n (%)			
Grade 1	100 (5.1)	129 (5.9)	229 (5.5)
Grade 2	758 (38.4)	778 (35.6)	1536 (36.9)
Grade 3	1111 (56.3)	1271 (58.1)	2382 (57.2)
Unknown	5 (0.3)	10 (0.5)	15 (0.4)
Tumor size (cm), n (%)			
≤2	725 (36.7)	711 (32.5)	1436 (34.5)
>2 and ≤5	1077 (54.6)	1253 (57.3)	2330 (56.0)
>5	171 (8.7)	221 (10.1)	392 (9.4)
Unknown	1 (0.1)	3 (0.1)	4 (0.1)
ER & HER2 status, n (%)			
ER+	1396 (70.7)	1479 (67.6)	2875 (69.1)
& HER2+	247 (12.5)	247 (11.3)	494 (11.9)
& HER2-	973 (49.3)	990 (45.2)	1963 (47.2)
& HER2 unknown	176 (8.9)	242 (11.1)	418 (10.0)
ER-	578 (29.3)	709 (32.4)	1287 (30.9)
& HER2+	161 (8.2)	194 (8.9)	355 (8.5)
& HER2-	350 (17.7)	411 (18.8)	761 (18.3)
& HER2 unknown	67 (3.4)	104 (4.8)	171 (4.1)
Molecular subgroup, n (%)			
ER+/HER2-*	987 (50.0)	1014 (46.3)	2001 (48.1)
HER2+	408 (20.7)	441 (20.2)	849 (20.4)
Triple negative	336 (17.0)	387 (17.7)	723 (17.4)
Type of surgery and radiotherapy, n (%)			
Mastectomy	1079 (54.7)	1186 (54.2)	2265 (54.4)
with radiotherapy	865 (43.8)	949 (43.4)	1814 (43.6)
breast	159 (8.1)	254 (11.6)	413 (9.9)
chest wall	709 (35.9)	693 (31.7)	1402 (33.7)
supraclavicular fossa	480 (24.3)	500 (22.9)	980 (23.5)
axilla	85 (4.3)	103 (4.7)	188 (4.5)
Wide local excision	895 (45.3)	1002 (45.8)	1897 (45.6)
with radiotherapy	880 (44.6)	961 (43.9)	1841 (44.2)
breast	856 (43.4)	921 (42.1)	1777 (42.7)
chest wall	31 (1.6)	47 (2.1)	78 (1.9)
supraclavicular fossa	291 (14.7)	283 (12.9)	574 (13.8)
axilla	103 (5.2)	70 (3.2)	173 (4.2)
Endocrine treatment in ER+ patients, n (%)			
Tamoxifen monotherapy	863 (61.8)	927 (62.7)	1790 (62.3)

	Analysis population N = 1974	Not included patients N = 2188	All TACT trial patients N = 4162
Tamoxifen followed by AI	454 (32.5)	439 (29.7)	893 (31.1)
AI monotherapy	61 (4.4)	76 (5.1)	137 (4.8)
No endocrine treatment/unknown	18 (1.3)	37 (2.5)	55 (1.9)
Trastuzumab in HER2+ patients, n (%)			
Yes	48 (11.8)	28 (6.4)	76 (9.0)
No/Not known	360 (88.2)	413 (93.7)	773 (91.0)
Chemotherapy, n (%)			
Control (FEC)	626 (31.7)	639 (29.2)	1265 (30.4)
Control (E-CMF)	332 (16.8)	492 (22.5)	824 (19.8)
FEC-D	1016 (51.5)	1057 (48.3)	2073 (49.5)
Number of patients with event contributing to DFS analysis	551 (27.9)	778 (35.6)	1329 (31.9)
Local recurrence	76 (3.8)	107 (4.9)	183 (4.4)
Distant recurrence	405 (20.5)	572 (26.1)	977 (23.5)
New breast disease	43 (2.2)	44 (2.0)	91 (2.2)
Death from other cause (no recurrence)	27 (1.4)	51 (2.3)	78 (1.9)
Distant relapse ever reported	462 (23.4)	655 (29.9)	1117 (26.8)
New breast disease ever reported	57 (2.9)	67 (3.1)	124 (3.0)
All non-breast cancer second primary	52 (2.6)	54 (2.5)	106 (2.5)
All deaths	397 (20.1)	620 (28.3)	1017 (24.4)
Breast cancer	369 (18.7)	568 (26.0)	937 (22.5)
Death from other causes	28 (1.4)	52 (2.4)	80 (1.9)
Cancer (non-breast)	15 (0.8)	21 (1.0)	36 (0.9)
Treatment toxicity	0	5 (0.2)	5 (0.1)
Other	13 (0.7)	26 (1.2)	39 (0.9)

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* includes ER-, PgR+, HER2-

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AI, aromatase-inhibitors; ER+, positive estrogen receptor (ER); ER-, negative ER; E-CMF, epirubicin 100 mg/m² for 4 cycles followed by CMF (cyclophosphamide 600 mg/m², methotrexate 40 mg/m² and fluorouracil 600 mg/m²) for 4 cycles; FEC, fluorouracil 600 mg/m², epirubicin 60 mg/m² and cyclophosphamide 600 mg/m² for 8 cycles; FEC-D, FEC for 4 cycles followed by docetaxel 100 mg/m² for 4 cycles; HER2+, positive human epidermal growth factor receptor-2 (HER2); HER2-, negative HER2; PgR+, positive progesterone receptor (PgR); SD, standard deviation; TACT, "Taxotere as adjuvant chemotherapy trial"; TPOAb, autoantibodies to thyroid peroxidase.

493 **Supplemental Table 2: Events contributing to disease-free survival (DFS) and numbers of deaths**
 494 **by dichotomized TPOAb status**
 495

	TPOAb- (N = 1568) n (%)	TPOAb+ (N = 406) n (%)
Number of patients with event contributing to DFS analysis	442 (28.2)	109 (26.8)
Local recurrence	59 (3.8)	17 (4.2)
Distant recurrence	327 (20.9)	78 (19.2)
New breast disease	33 (2.1)	10 (2.5)
Death from other cause (no recurrence)	23 (1.5)	4 (1.0)
All deaths	325 (20.7)	72 (17.7)
Breast cancer	301 (19.2)	68 (16.7)
Death from other causes (without distant recurrence)	24 (1.5)	4 (1.0)
Cancer (non-breast)	14 (0.9)	1 (0.2)
Treatment toxicity	0 (0.0)	0 (0.0)
Other	9 (0.6)	3 (0.7)
Vascular (cardiac)	1 (0.1)	1 (0.2)
Vascular (cerebral)	1 (0.1)	0 (0.0)
Vascular (thromboembolic)	0 (0.0)	0 (0.0)
Respiratory	0 (0.0)	0 (0.0)
Accident, suicide, alcoholism	5 (0.3)	0 (0.0)
Infection (not treatment related)	0 (0.0)	1 (0.2)
Gastrointestinal bleed	0 (0.0)	0 (0.0)
Chronic liver disease	1 (0.1)	0 (0.0)
Unknown	2 (0.1)	1 (0.2)

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 497 TPOAb+, positive autoantibodies to thyroid peroxidase (TPOAb); TPOAb-, negative TPOAb.
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499 **Supplemental Table 3: Univariate analyses for disease-free survival by FT4 and TSH**

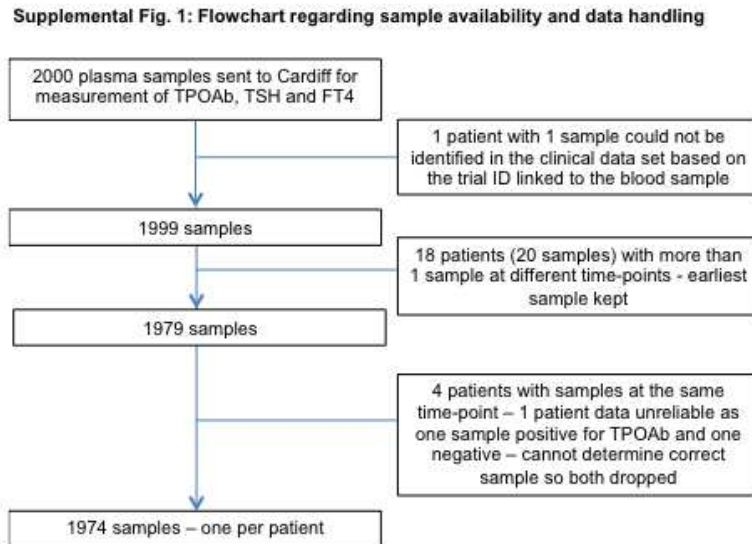
Variable		Unadjusted HR	95% CI	P value
FT4	Continuous	1.00	0.96-1.04	0.91
	<9.0 pmol/L (Hypothyroid; n=13)	1.61	0.67-3.88	0.29
	9.0–19.1 pmol/L (Euthyroid; n=1917)	1.00	-	-
	>19.1 pmol/L (Hyperthyroid; n=44)	1.08	0.62-1.87	0.79
TSH*	Continuous	1.03	0.94-1.13	0.48
	>4.40 mIU/L (Hypothyroid; n=94)	1.08	0.73-1.59	0.71
	0.3–4.40 mIU/L (Euthyroid; n=1781)	1.00	-	-
	<0.3 mIU/L (Hyperthyroid; n=96)	1.19	0.82-1.72	0.36

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FT4, free-thyroxine; HR, hazard ratio (HR <1 indicates a favorable breast cancer outcome); TSH, thyrotropin; 95% CI, 95% confidence interval.

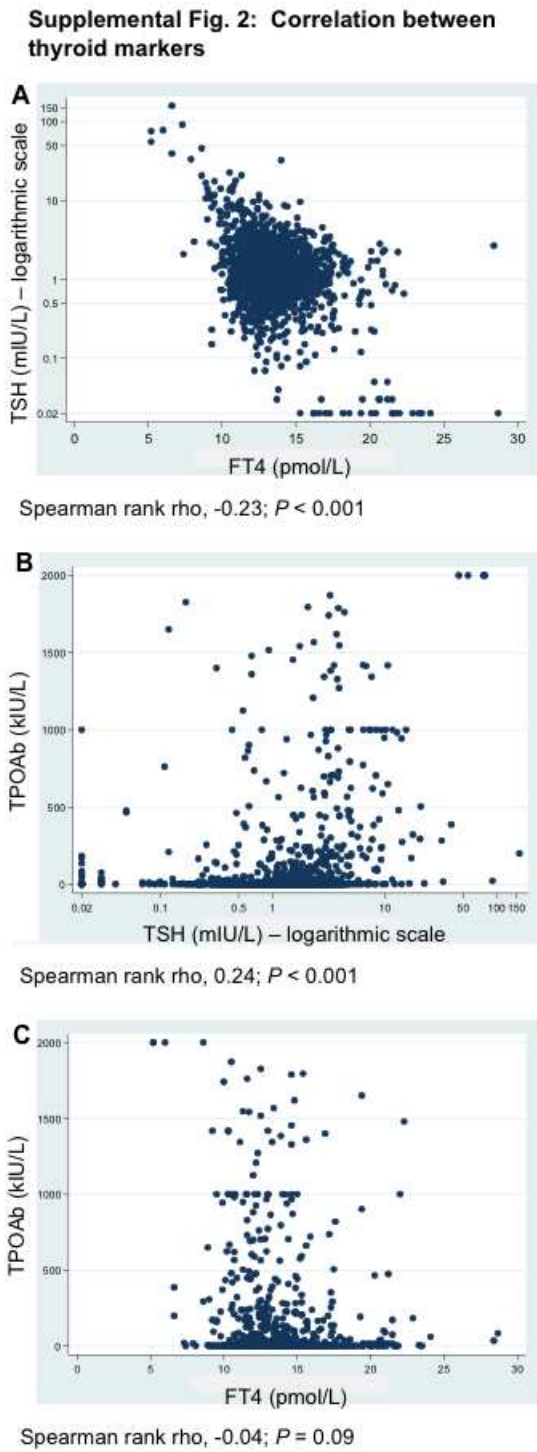
*TSH value was available in 1971/1974 (99.8%) samples

505 **Supplemental Fig. 1: Flowchart regarding sample availability and data handling**



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507 FT4, free-thyroxine; TPOAb, autoantibodies to thyroid peroxidase; TSH, thyrotropin.
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509 **Supplemental Fig. 2: Correlation between thyroid markers**



510

511 FT4, free-thyroxine; TPOAb, autoantibodies to thyroid peroxidase; TSH, thyrotropin. Panel A:

512 inverse correlation between TSH (logarithmic scale) and FT4. Panel B: positive correlation

513 between TPOAb and TSH (logarithmic scale). Panel C: inverse correlation between TPOAb
514 and FT4.