Early detection of biochemically occult autonomous thyroid nodules

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Abstract

Objective: Autonomously functioning thyroid areas may be associated with subclinical or overt hyperthyroidism, but may exist even in the presence of normal TSH. This study was aimed at comparing the rate of autonomously functioning areas and their cardiac sequelae in patients with nodular goitre studied with the usual and a novel approach.

Design and methods: In total 490 adult outpatients with thyroid nodular goitre, living in a mild iodine-deficient area, were selected in our referral centre for thyroid diseases from 2009 to 2014 on the basis of a suspicion of thyroid functional autonomy. They were divided in three groups according to a non-conventional approach (excessive response to thyroxine treatment: group 1) or conventional approach (low/normal TSH with clinical suspicion or low TSH: groups 2 and 3). All patients of the study with the suspicion of thyroid functional autonomy underwent thyroid scan with radioactive iodine (I^{131}) uptake (RAIU).

Results: The percentage of confirmed thyroid functional autonomy was 319/490, being significantly higher in group 3 than in groups 1 and 2 (81.5 vs 64.7 vs 52.6%; chi-square $P < 0.0001$). However, the diagnosis with non-conventional approach was made at a significant earlier age ($P < 0.0001$). Cardiac arrhythmias as well as atrial fibrillation were similarly detected by conventional and non-conventional approaches (chi-square test: $P = 0.2537$; $P = 0.8425$).

Conclusions: The hyper-responsiveness to thyroxine treatment should induce the suspicion of thyroid functional autonomy at an early stage, allowing to detect autonomous functioning areas in apparently euthyroid patients.

Introduction

The natural history of nontoxic goitre, in areas of iodine deficiency, encompasses a progressive increase in size (diffuse goitre) and/or development of multiple nodules (nodular goitre, NG) (1, 2, 3). Despite its long-lasting euthyroid phase, thyroid functional autonomy may occur in both diffuse and NG, with greater frequency in the latter (6.4% vs 21.2%) (4). The progression towards thyroid autonomy depends on genetic predisposition, somatic mutations of TSH receptor, iodine intake and older age (1, 2).

Autonomously functioning thyroid nodule (AFTN) may not only be associated with subclinical or overt hyperthyroidism (2, 5), but also with a normal TSH level

(6). Increased cardiovascular morbidity and mortality have been reported in patients with overt hyperthyroidism, as well as in those with subclinical hyperthyroidism (7). In addition, atrial arrhythmias (sinus tachycardia, atrial premature beats and atrial fibrillation) are frequent sequelae of overt hyperthyroidism (5). Similar effects on cardiac function have been described even in subclinical hyperthyroid patients (8, 9, 10), mostly in the elderly (11). Indeed, atrial fibrillation (12) is diagnosed in 2% of euthyroid people, 14% of overt hyperthyroid patients and 13% of patients with subclinical hyperthyroidism (5, 13).

Radioiodine thyroid scan (TS) represents so far the gold standard to detect autonomously functioning

areas, and ATA guidelines suggest performing it in patients with low TSH (14). However, a recent meta-analysis underlines the importance of TS also in goitrous patients with TSH within the normal references to detect autonomous areas (6). In fact, low serum TSH is present only in half of patients with autonomous thyroid functional areas, as ascertained with TS (6). This raises the question whether occult autonomous areas may be not diagnosed in multinodular goitres showing normal serum TSH. So, there is a need for an early recognition of these occult autonomous areas in the thyroid even to prevent the cardiac sequelae (15).

Serum TSH is a recognized growth factor for thyroid nodules (16). In iodine-deficient areas (17), thyroxine treatment is used as yet to lower TSH to prevent growth of nodules and the appearance of new ones (16, 18). However, the efficacy and appropriateness of this treatment has been challenged, mainly in iodine sufficient areas (2, 3, 19), also due to side effects related to the use of high doses of thyroxine (5). To date, increasing evidence showed the importance of using an individually tailored thyroxine dose to reach more safely target TSH (20, 21, 22, 23).

This study aims to evaluate the potential reliability of the use of tailored LT4 dose in detecting AFTN. This non-conventional approach was compared, in terms of potential clinical sequelae, to the conventional one adopting TS in patients with low TSH.

Subjects and methods

Subjects

A total of 2940 Caucasian outpatients with thyroid NG, living in a mild iodine-deficient area, were examined in our referral centre for thyroid diseases from 2009 to 2014. Of these, a total of 568 (19.3%) patients with NG were selected based on the following suspicious features of thyroid functional autonomy: (1) patients treated with levothyroxine (L-T$_4$) that showed TSH less than the expected level (TSH target: 0.1–0.4 mU/L) or a TSH target with a L-T$_4$ dose lower than the one observed in a carefully checked reference group ('non-conventional' suspicion); (2) untreated patients with TSH value in the lower part of the normal range (0.4–2.0 mU/L) in the presence of cardiovascular (i.e. increased heart rate, arrhythmia) and extra-cardiovascular symptoms (anxiety, irritability, etc.) ('fairly conventional' suspicion); (3) untreated patients with subnormal TSH value (<0.4 mU/L) and in the presence of clinical symptoms of hyperthyroidism ('conventional' suspicion).

Exclusion criteria were (a) overt hyperthyroidism; (b) autoimmune, factitious, post-inflammatory or iatrogenic hyperthyroidism; (c) recent use of iodine products and drugs able to affect thyroid function (24, 25); (d) lack of patient's compliance; and (e) pregnancy and breastfeeding.

Based on these criteria, 78 patients were excluded and the remaining 490 patients represented the study group (384 female and 106 male, median age: 59 years): 156 patients belonging to group 1 (136F/20M; median age: 52 years), 188 belonging to group 2 (145F/43M; median age: 62 years) and 146 to group 3 (103F/43M; median age: 66 years) (Table 1). The diagnosis of NG was based on clinical and ultrasonographic features, as described previously (26). In adult patients belonging to group 1, thyroxine treatment has been used to lower TSH, as still accepted in iodine-deficient areas in patients with evidence of non-neoplastic nodular growth (16, 17, 18). Elderly patients were in long-lasting treatment, either started previously in our institution or, more often, prescribed in other hospitals. In all patients, any side effect possibly related to thyroxine treatment has led to an immediate drug withdrawal.

The internal reference group, needed as a benchmark for thyroxine treatment, consisted of 124 patients (110F/14M; median age: 54 years; 88 patients with less than 60 years and 36 patients with more than 60 years) with nontoxic NG (NTNG) in semi-suppressive L-T$_4$ treatment (serum TSH target: 0.1–0.4 mU/L). The individually daily thyroxine dose was titrated on the basis of patients' body weight (kg) and age, as described previously (21, 26, 27). In this group, the presence of thyroid functional autonomy, gastrointestinal disorders and other major interferences (see Liwanpo for rev (25)) as well as cardiac disorders has been positively excluded. Patients belonging to the reference group and those with 'non-conventional' suspicion were educated to take thyroxine fasting since at least 3h and to wait at least 1h before ingest any food or drink, except water. Moreover, we informed patients to wait at least 3h before to take other drugs, in order to avoid interferences with the absorption kinetic of thyroxine, according to more recent literature (28, 29, 30, 31).

Table 1 Patients' characteristics.

	Group 1	Group 2	Group 3
Patients (n)	156	188	146
Sex (female/male)	136F/20M	145F/43M	103F/43M
Median age (years)	52	62	66
Median TSH (mU/L)	1.21	0.80	0.12

A single levothyroxine brand was used in all treated patients (Eutirox – Bracco, Milan, Italy; since July 2013 Merck Serono S.p.A.), to prevent possible differences in bioequivalence (32).

Design of the study

The 156 patients with NG in semi-suppressive thyroxine therapy ('non-conventional' suspicion, group 1) were subdivided into two age-related groups (adult <60 years and elderly >60 years) and each group has been compared with the corresponding age-related reference group (Table 2). This was done to avoid bias due to the lower T_4 requirement in elderly (1, 5). So far, in the adult reference group the desired serum TSH (median = 0.20 mU/L) was reached at a median L-T_4 dose of 1.56 µg/kg/day. In the study group, despite the lower L-T_4 dose ($P < 0.0001$), the adult patients exhibited a lower TSH level ($P < 0.0001$) (Table 2). Similar results were observed in the elderly patients: a median L-T_4 dose of 1.33 µg/kg/day and a median serum TSH of 0.22 mU/L were observed in the reference group, while, in the study group, a significantly lower L-T_4 dose ($P < 0.0001$) led to a median serum TSH of 0.07 mU/L ($P = 0.0727$) (Table 2). Hence, these patients were considered hyper-responsive to thyroid treatment regardless of age. Data were, thus, merged together and compared with those of goitrous patients with conventional suspicion of thyroid autonomy.

All patients of the study with the suspicion of thyroid functional autonomy underwent TS with radioactive iodine (I^{131}) uptake (RAIU). Obviously, in the patients with 'non-conventional' suspicion, the L-T_4 treatment was previously discontinued. When the results of TS were inconclusive, the patients underwent to a second TS and radioiodine uptake after triiodothyronine (T_3) administration (20–25 µg for three times in the day, for 8–10 days) (Werner's test) (33).

Study has been conducted upon written informed consent and as part of the diagnostic and therapeutic workup of the patients involved, according to the local ethical rules and to the guidelines in the Declaration of Helsinki.

Methods

Serum TSH levels were measured by commercial kit (Thermo Scientific, BRAHMS TSH RIA) (normal range: 0.4–4.0 mU/L; sensitivity: 0.04 mU/L; intra-assay and inter-assay variation were 2.5% and 4.1%, respectively). Levels of serum free T_4 were detected by commercial kit (Thermo Scientific, BRAHMS FT$_4$ RIA) (normal range: 10–25 pmol/L, which is the equivalent of 0.78–1.94 ng/dL), as the levels of serum free T_3 (normal range: 3.5–8.1 pmol/L or 2.3–5.3 pg/mL). Serum anti-thyroid peroxidase antibodies (TPO-Ab) levels were detected by commercial kit (Thermo Scientific, BRAHMS anti-TPO) (normal range: <60 U/mL). The anti-TSHreceptor antibodies (TRAb) levels were measured by commercial kit (Thermo Scientific, BRAHMS TRAK Human RIA).

Statistical analysis

Data are expressed as median value (Interquartile range: IQ1 and IQ3) and were analysed by nonparametric Mann–Whitney U test. The differences among the groups were calculated using the Fisher's exact test or chi-square test. INSTAT GraphPad Prism 5.0 software for Windows was used in the statistical analysis.

Results

The 156 treated patients with NG, despite a low dose of thyroxine (median $T_4 = 1.17$ µg/kg/day), had a median TSH of 0.079 mU/L (IQ1–IQ3 = 0.02–0.30 mU/L) ('non-conventional' suspicion, group 1). After withdrawal of thyroxine treatment, TSH level was restored into normal range (median TSH = 1.21 mU/L). All these patients underwent scants, which confirmed the diagnosis of thyroid functional autonomy in 101/156 patients (64.7%), while in the remaining 55 patients (35.3%) the suspicion was not confirmed (Fig. 1). Interestingly, the median TSH, without thyroxine treatment, was not different in the patients with or without confirmed areas of autonomy (median TSH: 1.28 vs 1.12 mU/L; $P =$ ns). In contrast, both the early iodine uptake at 4th hour (16.27 vs 13%; $P = 0.0378$) and the total iodine uptake at 24th hour (31.91 vs 25.28%; $P = 0.0006$) were significantly different in confirmed and non-confirmed patients (Fig. 2A).

Table 2 Age-related characteristics of thyroxine treated patients.

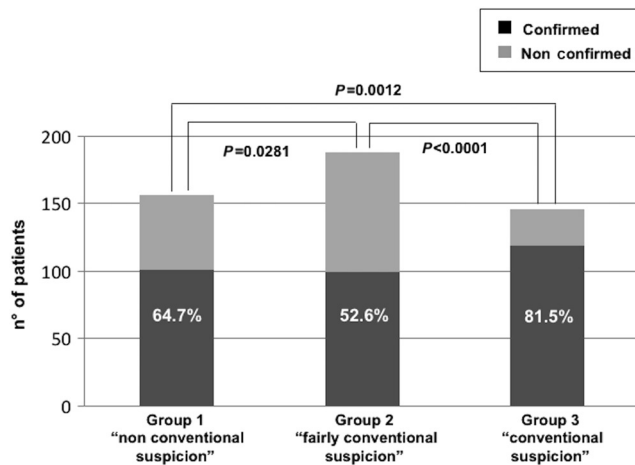|	Group 1	Reference group	P
<60 years (n)	114	88	
Median TSH with T_4 (mU/L)	0.08	0.20	<0.0001
T_4 dose/weight (µg/kg/day)	1.20	1.56	<0.0001
>60 years (n)	42	36	
Median TSH with T_4 (mU/L)	0.07	0.22	0.0727
T_4 dose/weight (µg/kg/day)	0.93	1.33	<0.0001

Figure 1

Distribution of percentages of patients with confirmed areas of autonomy in the study groups. Data were analysed by nonparametric Mann–Whitney U test.

These data were compared with those obtained in the other groups of patients investigated on the basis of 'fairly conventional' and 'conventional' suspicion. The 188 patients with NG and 'fairly conventional' suspicion of thyroid functional autonomy (group 2) showed a median TSH=0.80 mU/L (IQ1–IQ3: 0.60–0.95 mU/L). In 99/188 patients (52.6%) the suspicion of an autonomously functioning area was confirmed and excluded in the remaining 89. Unlike group 1, the median iodine uptake at 4th hour and 24th hour was similar, 12.34 vs 12.83% and 26% vs 26%, respectively, in the confirmed and in non-confirmed patients (P=ns) (Fig. 2B). In the 146 patients with NG and 'conventional' suspicion of thyroid functional autonomy (group 3) a median TSH level of 0.12 mU/L (IQ1–IQ3: 0.03–0.26 mU/L) was observed. In 119/146 patients (81.5%) the presence of an autonomously functioning area was confirmed by the TS, while in 27 was excluded. The median radioiodine uptake at 4th hour was significantly higher (P=0.013) in those with proven areas of autonomy than in those non-confirmed (median uptake: 14% vs 11.9%). A similar trend was observed even at 24th hour (28.16% vs 25.09%; P=0.0094) (Fig. 2C).

So far, the percentage of patients with confirmed thyroid autonomy was higher in group 3 (low serum TSH) than in patients with normal TSH (groups 1 and 2) (chi-square P<0.0001) (Fig. 1), whereas the percentage of thyroid autonomy in group 1 ('non-conventional' approach) (64.7%) is equal to the one in group 2 and 3 pooled together ('fairly' and 'conventional' approach) (65%) (Fisher's exact test: P=0.9192).
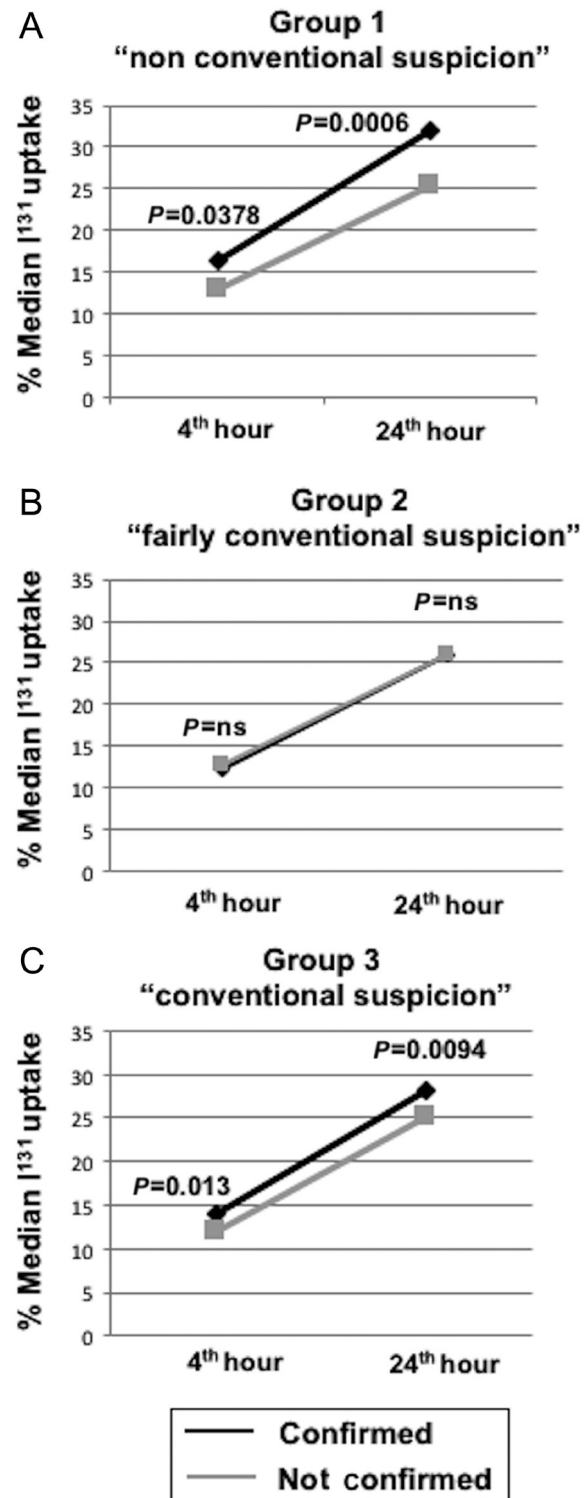

Figure 2

The median iodine uptake at 4th and 24th hour in patients with confirmed areas of autonomy and in those without diagnostic confirmation in the study groups. Data were analysed by nonparametric Mann–Whitney U test.

Table 3 Cardiac arrhythmias in the study groups.

	n (F/M)	Median age, years	Group 1	Group 2	Group 3	P
All patients with cardiac arrhythmias (CA)	85 (69/16)	64	22/156	39/188	24/146	0.2537
Patients with CA and confirmed areas of autonomy	62 (50/12)	65	16/101	25/99	21/119	0.2003
Patients with atrial fibrillation and confirmed areas of autonomy	25 (20/5)	69	7/101	9/99	9/119	0.8425

Owing to the fact that autonomously functioning areas is highly associated with cardiac arrhythmias (tachycardia, extrasystoles, atrial fibrillation) (9), the presence of these disorders were investigated in the whole study group. A total of 85 (69F/16M; median age: 64 years) out of 490 patients with NG (17.3%) showed heart rhythm disorders. Notably, a cardiac arrhythmia has been found in 22/156 patients (14.1%) in group 1, in 39/188 patients (20.7%) in group 2 and in 24/146 patients (16.4%) in group 3, being these differences not statistically significant (chi-square test: $P=0.2537$) (Table 3). Also no difference ($P=0.2755$) was observed by merging data from 'non-conventional' and 'fairly conventional' suspicion, showing similar TSH values, and comparing them with data in patients with low serum TSH.

Among the 319 patients with proven areas of thyroid autonomy, 62 patients (50F/12M; median age: 65 years) had a cardiac arrhythmias (19.4%), representing 72.9% of all patients (62/85) with heart rhythm disorders (Fig. 3). Analysis of the subgroups revealed that there were no differences in rate of cardiac arrhythmias among the groups (chi-square test: $P=0.2003$) (Table 3). Noticeably, the median age was significantly higher in patients with low serum TSH (group 3; median age: 70 years) than in patients with normal TSH, both untreated (group 2; median age: 64 years; $P=0.0103$) and treated with T_4 (group 1; median age: 55 years; $P=0.0019$) (Table 3).

Overall, among 85 patients with heart rhythm disorders, some 30 patients (35%) had one or more events of atrial fibrillation (paroxysmal or chronic). In 5 of these, atrial fibrillation was not related with thyroid disorder and therefore it represented the 40% (25/62) of all arrhythmias diagnosed in patients with thyroid autonomy. Again, the rate of atrial fibrillation was similarly detected by conventional and non-conventional approaches (group 1: 7/101 (6.93%); group 2: 9/99 (9.1%); group 3: 9/119 (7.56%); chi-square test: $P=0.8425$] (Table 3).

Discussion

The findings of this study have shown that (a) the low serum TSH level is a reliable but belated index associated with hyperfunctioning nodules; (b) normal serum TSH may be also associated with autonomous thyroid areas; (c) by the described 'non-conventional' approach, we have identified a substantial percentage of occult autonomous areas as well; (d) heart arrhythmias were not related to serum TSH but rather to the presence of autonomous areas, even occult.

In iodine-deficient areas, the natural history of nontoxic goitre encompasses its functional transition to thyroid autonomy (2, 34), which increases with age and may be recognizable in some 15% of elderly patients (35). This study agreed with the other reports stating that low serum TSH is a good predictor for the existence of thyroid areas with functional autonomy (14). Nevertheless, this study demonstrated that the TS, performed in carefully selected patients with normal TSH, detects early a significant number of patients with autonomous thyroid function areas and cardiac arrhythmias. This is not fully in keeping with Recommendation 2 (moderate quality evidence) in
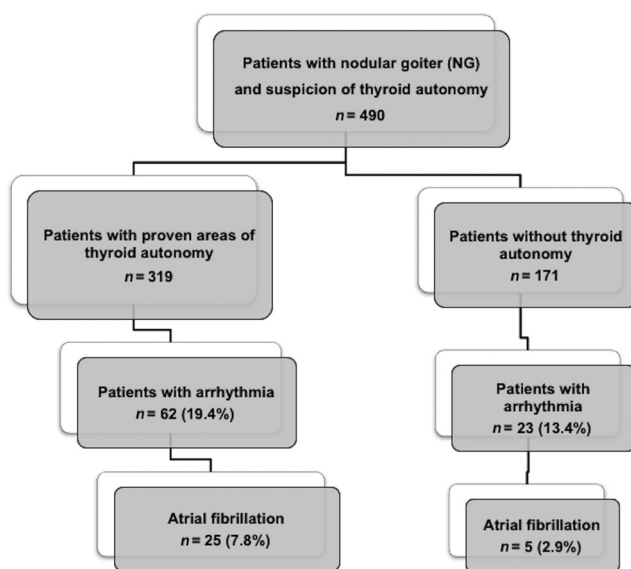
Figure 3

Overview of arrhythmias and atrial fibrillation in patients with or without proven areas of autonomy.

the ATA thyroid nodules guidelines which support the notion that scintiscan should be performed only when serum TSH is low (14). However, the recognition of these hyperfunctioning areas through that approach may not be early enough; recent studies, in fact, demonstrated that thyroid functional autonomy may exist even in patients with normal serum TSH levels and our results showed that this occurs several years before the appearance of low serum TSH. Coexistence of normal serum TSH levels and AFTN may occur in patients with apparently euthyroid goitre and in those with goitre associated with Hashimoto's thyroiditis (6, 15, 36, 37). Indeed, in euthyroid goitres, small/subcentimetric areas of 'hot' thyroid tissue partially inhibit the remaining follicles, but the little amount of autonomous tissue and/or the low synthesis rate of iodothyronines prevent TSH suppression (6, 15). Similarly, in patients with untreated Hashimoto's thyroiditis, the hypothyroidism, due to the progressive autoimmune destruction of thyroid tissue, is counteracted by the presence of one or more autonomous areas (36). The final balance among these opposite functional activities may sometime lead to normal TSH level (36). These areas of thyroid autonomy may remain occult for years (34), but the risk of developing hyperthyroidism may occur in both cases (6, 36) and so novel and easy diagnostic tools are needed to identify them at earlier stages.

On this ground, our data suggest that hyper-responsiveness to thyroxine treatment may be useful to suspect the presence of an occult autonomous thyroid areas at an early stage. We used a similar approach when we studied patients with T_4 malabsorption (22, 23, 38, 39): in that case, TSH levels higher than expected led us to look for occult gastrointestinal disorders while in this study, a TSH lower than expected helped us to detect functional thyroid autonomy. This 'non-conventional' approach is as efficient as the conventional methods; in fact, the diagnosis of thyroid functional autonomy has been confirmed in about two-third of patients who hyper-responded to thyroxine treatment, a fraction similar to the one recorded with the conventional approach. Noticeably, after T_4 treatment withdrawal, these patients had a normal TSH level, showing that this approach identified thyroid autonomy when still in an occult phase.

Arrhythmias are a major concern in patients with subclinical or overt hyperthyroidism, since their prevalence is greatly increased in these patients as compared with general population (7, 13). The linkage between autonomous functioning areas and cardiac arrhythmias, independently from thyroid function, is as yet scarcely described. Our study shows that one-fifth of patients with thyroid autonomously functioning areas have cardiac arrhythmias and some one-tenth has atrial fibrillation. It should be emphasized that the number of arrhythmias and that of atrial fibrillation were similar in patients with low and normal serum TSH, despite patients with low TSH were older, in agreement with the literature (40). Based on these data, thyroid functional autonomy may represent in itself a risk factor for atrial fibrillation, even when serum TSH is normal. Interestingly, there was no whatsoever difference in the frequency of arrhythmias and atrial fibrillation depending on the approach used. To note, the non-conventional approach (hyper-responsiveness to thyroxine treatment) to thyroid autonomy has been proven equivalent to conventional approach (low TSH or normal TSH with symptoms), even in detecting cardiac arrhythmias.

In conclusion, this study shows that the hyper-responsiveness to thyroxine treatment should induce the suspicion of a thyroid functional autonomy at an early stage, allowing to detect autonomous functioning areas in apparently euthyroid patients. Cardiac arrhythmias, and chiefly atrial fibrillation, can be also associated to occult thyroid functional autonomy, though TSH would be normal. These data indicated that a low serum TSH may be not an early and exhaustive marker to diagnose thyroid functional autonomy and related arrhythmias.

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