European Journal of Endocrinology, 2024, **190**, 12–22
https://doi.org/10.1093/ejendo/lvad172
Advance access publication 20 December 2023
Original Research

Randomized double-blind placebo-controlled trial on levothyroxine and liothyronine combination therapy in totally thyroidectomized subjects: the LEVOLIO study

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Abstract

Objective: Despite having normal thyroid-stimulating hormone levels, many hypothyroid patients are dissatisfied with the treatment. The primary aim of this study was to evaluate the effect of twice-daily, combination therapy with levothyroxine (LT4) and liothyronine (LT3), at doses adapted according to TSH-level, on peripheral tissues as reflected by sex hormone binding globulin (SHBG) levels in totally thyroidectomized patients. Changes in other tissue markers and quality of life considering *DIO2*-rs225014 and *MCT10*-rs17606253 genetic variants were also assessed.

Design: Double-blind, randomized, placebo-controlled.

Methods: One hundred and forty-one subjects were randomized to LT4 + LT3 group (LT4 + LT3 in the morning and LT3 in the evening; $n = 70$) or placebo group (LT4 in the morning and placebo in the evening; $n = 71$). Pituitary-thyroid axis compensation was assessed after 6, 12, and 24 weeks. Clinical parameters, quality of life, and tissue markers (sex hormone binding globulin, serum lipids, bone markers) were evaluated at 12 and 24 weeks. *DIO2* and *MCT10* single nucleotide polymorphisms were genotyped.

Results: The LT4 + LT3 group was treated with mean daily LT3 doses of 5.00 µg, with a mean daily LT4 reduction of 15 µg. After 6 months of treatment, neither SHBG and other tissue markers nor quality of life differed significantly between groups. Combination treatment required greater dose adjustments than placebo (25% vs 54%, $P < .001$), due to thyroid-stimulating hormone reduction, without hyperthyroidism signs or symptoms. At the end of treatment, the LT4 + placebo group had significantly lower fT3/fT4 compared to the LT4 + LT3 group (0.26 ± 0.05 vs 0.32 ± 0.08, $P < .001$). No preference for combination therapy was found. Genetic variants did not influence any outcomes.

Conclusions: Six months of combination therapy with twice-daily LT3 dose adapted according to TSH-level do not significantly change peripheral tissue response or quality of life, despite an increase in the fT3/fT4 ratio.

Keywords: hypothyroidism, combination treatment, levothyroxine, liothyronine, thyroidectomy

Significance

This represents one of the largest studies to date to evaluate the efficacy of combined therapy with levothyroxine and liothyronine. By administering 6-month dose adapted according to TSH-level and respecting the physiological relationships between the 2 hormones, the treated subjects did not have significant changes in tissue markers of thyroid hormone action nor in quality of life, despite an improvement in the ratio between the 2 circulating thyroid hormones.

Introduction

The gold standard treatment for hypothyroidism is synthetic levothyroxine (LT4), a pro-hormone further converted into the active form, triiodothyronine (T3). Under physiological conditions, 20% of T3 is produced by the thyroid, while the remaining percentage derives from the activation of the pro-hormone in the peripheral tissues by deiodinases.[1] This activation is considered sufficient to ensure adequate peripheral levels of thyroid hormones in subjects treated with LT4. However, the persistence of hypothyroidism-related symptoms under LT4 has been announced since the 1990s[2] and it is still an unresolved issue.[3] The main concerns

Received: December 22, 2022. **Revised:** November 28, 2023. **Editorial Decision:** December 4, 2023. **Accepted:** December 4, 2023
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of symptomatic patients treated with LT4, despite normal thyroid-stimulating hormone (TSH), are impaired cognition and fatigue,[4,5] with significant impairment of quality of life (QoL).[3]

LT4 does not normalize thyroid hormone levels in adipose tissue, lung, and liver of thyroidectomized rats.[6] In humans, serum levels of free triiodothyronine (fT3) and thyroxine (fT4) during LT4 are altered compared to euthyroid controls. After thyroidectomy, LT4 treatment leads to an increase in fT4 serum levels, with TSH normalization, but fT3 serum levels are significantly lower than before surgery.[7] Thus, LT4 guarantees euthyroidism at the pituitary level, with consequent TSH normalization, but probably not at the periphery. These findings have rekindled interest in combination therapy with LT4 and liothyronine (LT3). However, the current evidence on the efficacy of combination therapy (LT4 + LT3) is conflicting, deriving from studies penalized by methodological limitations,[8,9] hindering a clear position in guidelines on this issue.[10,11] Limitations of previous studies can be summarized in: (1) Administration of LT3 once daily in the morning,[12-21] not respecting the known half-life and circadian rhythm of T3,[22,23] (2) lack of adequate washout period in cross-over studies,[12-14,16,21,24] (3) fixed LT3 doses, leading to various LT4/LT3 ratios with the risk of over- or undertreatment,[14,21,24,25] and (4) hypothyroidism of any nature, with varying degrees of residual function.[12,14,19,21,24,26] Moreover, these studies generally evaluated the QoL, using questionnaires not specific to hypothyroidism.

Finally, LT4 + LT3 may be advisable only for patients with characteristics yet to be defined. Carriers of the rs225014 single nucleotide polymorphism (SNP) of the type 2 deiodinase (DIO2) coding gene have lower T3, despite normal TSH[27] and seem to prefer LT4 + LT3.[28] Carriers of this variant exhibit lower DIO2 activity and therefore less ability to generate T3 from exogenous LT4.[27] Also, the influence of thyroid hormone transporters at the cellular level has been advocated, since they regulate the passage of T3 in the central nervous system and the hypothalamus-pituitary unit:[29] Subjects with monocarboxylate transporter (MCT)-10 rs17606253, alone or together with the derived *DIO2* allele, seem to prefer LT4 + LT3.[28]

Thus, the present study was designed to evaluate the effect of combination therapy with LT4 + LT3 on peripheral tissues, as reflected by sex hormone binding globulin (SHBG) levels, in totally thyroidectomized patients without residual thyroid function, partially replacing LT4 with customized twice-daily doses of LT3, respecting circadian rhythm and the physiological T3/T4 ratio. Changes in other tissue markers and quality of life considering *DIO2*-rs225014 and *MCT10*-rs17606253 genetic variants were also assessed.

Methods

Study design

The longitudinal, prospective, double-blinded, randomized, placebo-controlled study was registered on ClinicalTrials.gov (NCT03053115) (EudraCT number 2016-000687-41). The research was conducted in accordance with the World Medical Association Declaration of Helsinki. The institutional review board of Modena approved the study (protocol n. 98/16). The Italian Medicines Agency (AIFA) approved the study (protocol n. 59166/16).

From March 2017 to March 2020, 160 hypothyroid patients in LT4 replacement therapy were enrolled at the Unit of Endocrinology of the Azienda Ospedaliero-Universitaria Policlinico of Modena, Italy. Patients were selected according to the following criteria: 18 years old or older, capable of understanding and willing, able to understand and fill in a questionnaire in Italian, without residual thyroid function (totally thyroidectomized for benign or malignant disease, with serum thyroglobulin < 0.2 ng/mL and negative anti-thyroglobulin antibodies, to avoid interference in the thyroglobulin dosage method), and in good compensation during replacement therapy with LT4 in tablets at stable dosage for at least 3 months. Patients were excluded if pregnant or with a history of cardiac arrhythmias, severe liver, kidney, or bone metabolism disorders, or if they were taking LT4 at TSH-suppressive dosage, corticosteroids, antiresorptive therapies for osteoporosis (current or in the previous 12 months), amiodarone, cholestyramine, or iron supplementation.

Patients were randomized into groups treated with LT4 + LT3 (LT4 + LT3 group) or LT4 and placebo (LT4 + placebo group). The study protocol included screening visits, baseline visits (V0), and visits at 6 (V1), 12 (V2), and 24 (V3) weeks from V0 (Figure 1).

During the screening visit, inclusion and exclusion criteria were considered, and consent has been obtained from each patient after a full explanation of the purpose and nature of all procedures used. At V0, any symptom or sign of hypothyroidism was collected. At V0, V1, V2, and V3, TSH serum levels (with reflex fT4 testing in case of altered TSH) were measured at 8 AM, fasting and before taking therapy. Dose adjustments were made to keep the patient on replacement therapy with good compensation (see "Experimental treatment"). At V0, V2, and V3, clinical data such as weight, height, body mass index (BMI), heart rate (HR), and systolic and diastolic blood pressure (SBP, DBP) were collected. At V0, V2, and V3, serum was collected by sampling at 8 AM, fasting and before taking the therapy and they were stored at −20 °C. Whole blood samples in ethylenediaminetetraacetic acid were collected at V0 and stored at −80 °C. At the end of the study, samples were thawed and analyzed on the same day under the same conditions. The following serum parameters were measured: TSH, fT4, fT3, antibodies to thyroperoxidase (TPOAb), total cholesterol (total-CH), high-density lipoprotein cholesterol (HDL-CH), triglycerides, SHBG, osteocalcin, carboxy-terminal collagen crosslinks (CTX), and bone isoenzyme of alkaline phosphatase (Table S1).

The fT3/fT4 ratio was calculated and it was considered physiological between 0.27 and 0.37.[7,9]

DIO2-rs225014 and *MCT10*-rs17606253 variants were genotyped at baseline. See Supplementary methods for details.

The Italian version of the ThyPRO questionnaire[30] was administered at V0, V2, and V3 to assess QoL. At each visit, compliance to thyroid replacement therapy was verified by checking the daily compilation of the therapeutic scheme delivered to the patient and administering the validated questionnaire Morisky Medication Adherence Scale.[31] See Supplementary methods for questionnaire details.

Experimental treatment

LT3, LT4, and placebo drops were provided by IBSA Institut Biochimique SA (Switzerland). See Supplementary methods for details on the custom calculation of doses.

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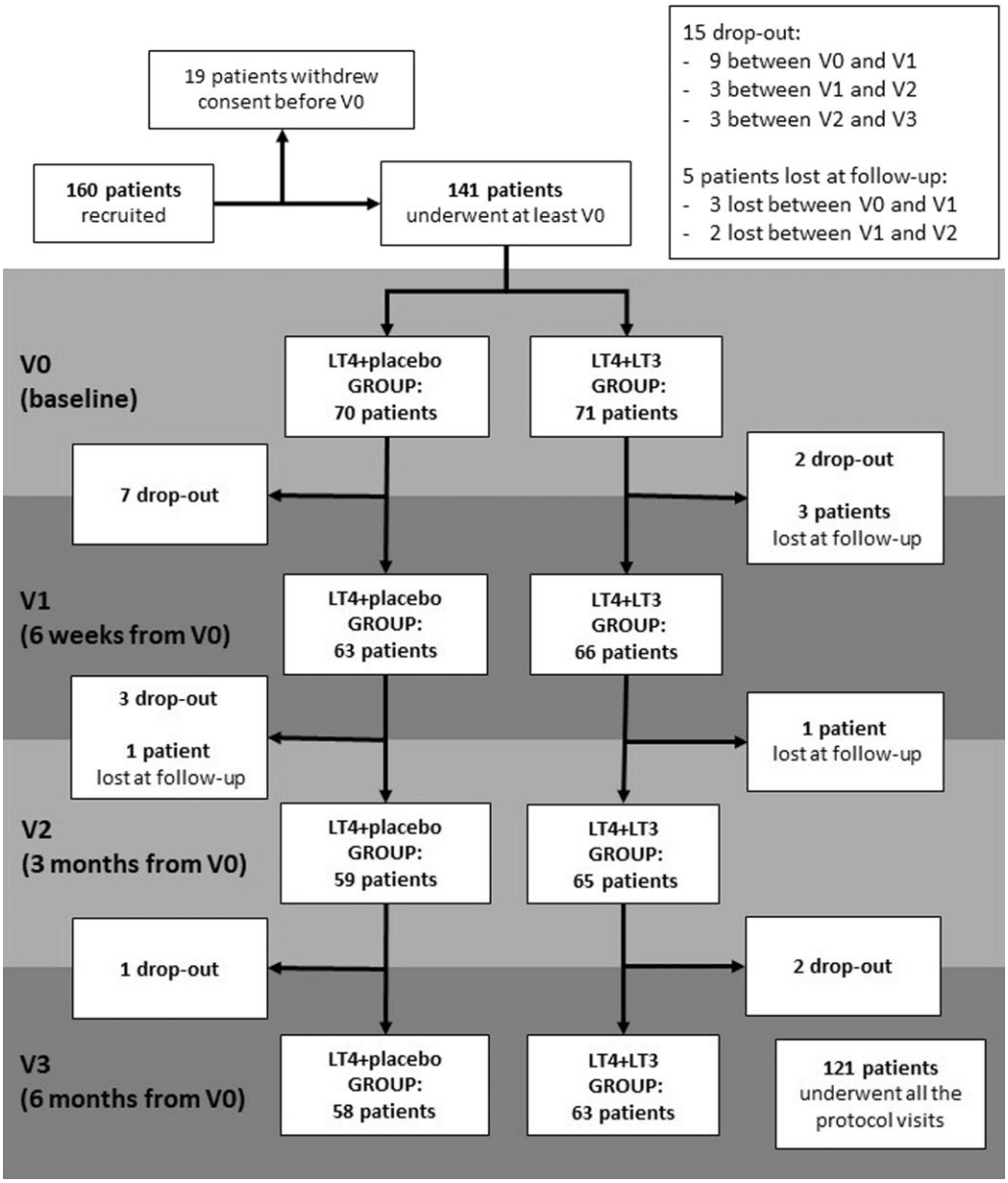

Figure 1. Flow chart of the study.

Placebo was clear and colorless. Both LT3, LT4, and placebo drops were identical in color and taste. It was checked for LT4 sodium identification (negative) and LT3 sodium identification (negative).

The treatment lasted 6 months. At 6, 12, and 24 weeks, TSH was checked to guide dosage adjustments. If TSH was out of the reference range, the randomizing clinician made the change. In the LT4 + LT3 group, the reduction/increase was first performed on the daily total LT4 dose in μg corresponding to the sum of the thyroid hormones prescribed in the previous visit. The dose modification was made case by case, according to clinical practice and international guidelines (at V1, changes of at least 12.5-25 μg/daily were made, but subsequently even lower changes were considered to achieve goal TSH levels).[32] Then, the new reduced/increased dosage was converted into doses of LT4 and LT3 following the formula described in Supplementary methods, in order to maintain T4/T3 ratio between 13:1 and 20:1. In the LT4 + placebo group, the dosage of LT4 in drops taken in the morning was changed. The extent of the reduction/increase was indicated by the investigators in both groups (eg, ±10 μg daily).

At each visit, patients were asked about side effects, including clinically relevant hypo/hyperthyroid symptoms, posology changes of existing therapies, or initiation of new therapies.

After the end of the study, a telephone questionnaire was conducted with predefined multiple-choice questions ("Did you prefer the experimental or the previous therapy? Why?", "Did you find the experimental therapy uncomfortable? Why?") to investigate the preference for experimental therapy and any difficulties with the new therapeutic scheme. See Supplementary methods for the complete version of the telephone questionnaire.

Blinding

The randomization was performed using a permuted block randomization scheme which allowed to randomize participants within blocks such that an equal number are assigned

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to each treatment, using the SPSS 29.0 for Windows (SPSS Inc., Chicago, IL, USA) software. The randomization was performed with a 1:1 ratio, considering: the LT4 + LT3 group and the LT4 + placebo group. Participants, nurses, and investigators performing the visits were blinded throughout the entire study period, ignoring which treatment participants were receiving.

The customized doses of LT3 and LT4 were calculated by a clinician of the Endocrinology Unit, called randomizing clinician, who had access to the randomization list but never met patients during the study. The figure of the randomizing clinician was essential to calculate the precise dosages, while maintaining the double blindness of the investigators.

Both groups took the same formulations, in order not to understand which treatment they were taking: Drops (containing LT4 in the placebo or LT3 in the LT4 + LT3 group) plus tablets (containing LT4) in the morning and drops in the evening (containing placebo or LT3). Each subject at each visit received 2 bottles, 1 for the morning (marked "morning") and 1 for the evening (marked "evening"), accompanied by a therapeutic scheme, which indicated the precise number of drops to be taken in the morning and the evening and the dose of LT4 in tablets to be taken in the morning. The patient was asked to check the doses taken on the therapeutic scheme daily. The randomizing clinician packed the drug bottles in boxes identical and indistinguishable from those of the LT4 + placebo group, with the sole indication of the patient number. Then the randomizing clinician delivered the box to the investigators who in turn delivered it to the patient during the visit, together with the new therapeutic scheme, showing any dosage changes, when necessary (see "Treatment duration and dosage adjustments" paragraph for details).

Sample size calculation

The sample size was a priori determined by power analysis, assuming serum SHBG variations as the most relevant indicator of thyroid hormones tissue effect. Walsh et al.[33] demonstrated an SHBG increase by approximately 9 nmol/L, increasing the LT4 dose of 50 µg for 2 months. Setting the sample size calculation on the size effect reported by Walsh et al. (8.14′ ± 9.94′ nmol/L) and considering a 2-sided repeated measurement after baseline, with a power of 90% and an alpha-error = .05, we estimated 113 patients for each group. Considering a drop-out rate of 15%, the total sample size was 260 patients.

Study outcomes

The primary outcome of the study was: Serum SHBG variations after 6 months of treatment.

The secondary outcomes of the study were: BMI, quality of life changes evaluated by the ThyPRO questionnaire scores, TSH, fT3, fT4, total-CH, HDL-CH, triglycerides, CTX, osteocalcin, and bone alkaline phosphatase.

The following exploratory outcomes (not predefined on clinicaltrials.gov) were added during the clinical trial: fT3/fT4, HR, SBP, and DBP.

Statistics

The randomization list and dosage choices, depending on the study arm assigned to each patient, were managed solely by the randomizing clinician. The primary endpoint was the serum SHBG variations after 6 months of treatment.

The "live" TSH reflex measurement at each visit served only to guide any therapeutic adjustments. The analysis was performed according to the intention-to-treat approach, to reduce the potential influence of dropouts on the final result.

Statistical analyses on TSH, fT3, and fT4 were performed using the measurements on all sera obtained at the end of the study. The distribution of the collected variables was studied using the Kolmogorov-Smirnov test. Thyroid hormones, as well as the tissue markers and the questionnaire scores, were not normally distributed. However, all the primary and secondary outcomes, comparing LT4 + LT3 and LT4 + placebo groups at visit 3, were evaluated with generalized linear model analysis, as previously suggested.[34] Categorical variables were compared by Fisher's exact test. Correlation analyses were performed by Spearman Rho's test. To test the predictive value of genotypes on the studied outcomes, we compared continuous variables according to genotype applying Kruskal-Wallis test. Deviation from Hardy-Weinberg equilibrium was analyzed using the chi-square test. Statistical analyses were performed using SPSS 29.0 for Windows (SPSS Inc., Chicago, IL, USA). Values of $P < .05$ were considered significant.

Results

One hundred and sixty patients have been enrolled into the study. Nineteen subjects withdrew their consent before randomization, mainly persuaded by relatives once they returned home or because, during the pandemic, they did not want to access the hospital for the visits required by the protocol. Finally, 141 subjects were randomized in LT4 + LT3 or LT4 + placebo group and underwent at least V0, 71 in the study (74.6% females) and 70 in the LT4 + placebo group (67.1% females). Fifteen patients dropped out (10.6%), and 5 were lost at follow-up (Figure 1). All dropouts were due to the patient's choice, and none to adverse events. Six of the 8 patients lost after V1 left the study during the lockdown due to the coronavirus disease 2019 (COVID-19) pandemic. Finally, 121 patients, 63 in the LT4 + LT3 and 58 in the LT4 + placebo group, underwent all visits (Figure 1).

Baseline characteristics of the LT4 + LT3 and LT4 + placebo groups are shown in Table 1. Only 9 patients had positive TPOAb. At baseline, TPOAb positive subjects had worse QoL, considering emotional susceptibility ($P = .04$), impaired daily life ($P = .02$), cosmetic complaints ($P = .05$), and item12 ($P = .05$) compared to TPOAb negative subjects. In subsequent analyses, no comparisons were made based on TPOAb, given the small size of the positive group.

Subjects randomized in the LT4 + LT3 group were treated with mean daily LT4 and LT3 doses of 100.13 ± 26.25 and 5.00 ± 1.31 µg, respectively, thus with an LT4/LT3 ratio of 20 (Table S2).

Primary and secondary outcome values at baseline and the end of treatment are shown in Table 2.

Changes in tissue markers of peripheral thyroid hormone action

No significant differences in tissue markers were observed between the LT4 + LT3 and LT4 + placebo groups at the end of treatment (Table 2). SHBG was slightly higher in the LT4 + LT3 group (52.9 ± 25.8 nmol/L) compared to the placebo (48.9 ± 33.1 nmol/L) ($P = .47$, power 59.5%). On performing

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Table 1. Baseline characteristics of levothyroxine + liothyronine (LT4 + LT3) and levothyroxine + placebo (LT4 + placebo) groups.

	LT4 + LT3 group ($n = 71$)	LT4 + placebo group ($n = 70$)
Age (years)	55.5 ± 10.8	56.4 ± 13.0
Females *n* (%)	53 (74.6)	47 (67.1)
BMI (kg/m^2)	28.5 ± 5.5	28.7 ± 5.8
HR (bpm)	73.2 ± 8.6	73.3 ± 10.5
SBP (mm Hg)	128.4 ± 17.9	128.7 ± 17.3
DBP (mm Hg)	78.2 ± 10.3	77.6 ± 11.0
Months post-thyroidectomy	113.5 ± 97.6	107.1 ± 78.8
Papillary carcinoma *n* (%)	57 (82.6)	57 (85.1)
	Stage I: 52 (91.2)	Stage I: 53 (93.0)
	Stage II: 5 (8.8)	Stage II: 4 (7.0)
Follicular carcinoma *n* (%)	8 (11.6)	2 (3.0)
	Stage I: 8 (100)	Stage I: 2 (100)
Papillary and follicular carcinoma *n* (%)	2 (2.9)	2 (3.0)
	Stage I: 2 (100)	Stage I: 2 (100)
Medullary carcinoma *n* (%)	1 (1.4)	3 (4.5)
Benign nodular goiter *n* (%)	1 (1.4)	3 (4.5)
RAI remnant ablation	52 (73)	42 (60)
Daily LT4 dosage (μg/daily)	112.1 ± 28.7	114.9 ± 34.6
LT4 dosage per kg (μg/kg)	1.5 ± 0.3	1.5 ± 0.4
Number of hypothyroidism-related symptoms	2.1 ± 1.6	2.0 ± 1.6
Asthenia *n* (%)	36 (50.7)	36 (51.4)
Duration of asthenia (months)	84.5 ± 111.0	70.5 ± 65.3
Weight gain *n* (%)	34 (47.9)	29 (41.4)
Duration of weight gain (months)	71.2 ± 60.1	56.8 ± 52.8
Reduced memory *n* (%)	26 (36.6)	23 (32.9)
Duration of reduced memory (months)	69.3 ± 63.0	66.5 ± 73.1
Dry scalp *n* (%)	18 (25.4)	17 (24.3)
Duration of dry scalp (months)	87.5 ± 79.4	38.1 ± 67.5
Cold intolerance *n* (%)	3 (4.2)	2 (2.9)
Duration of cold intolerance (months)	185.3 ± 297.0	150.0 ± 110.3
Number of comorbidities	0.8 ± 1.0	0.8 ± 1.2
Hypertension *n* (%)	26 (36.6)	29 (41.4)
Diabetes mellitus *n* (%)	3 (4.2)	5 (7.1)
Dyslipidemia *n* (%)	9 (12.7)	10 (14.3)
Gastroesophageal reflux disease *n* (%)	8 (11.3)	4 (5.7)
Psychiatric disorders *n* (%)	8 (11.3)	2 (2.9)
Hypoparathyroidism *n* (%)	9 (12.7)	9 (12.9)
Number of drugs	1.3 ± 1.8	1.3 ± 1.7
Calcium supplements *n* (%)	12 (16.9)	10 (14.3)
Vitamin D supplements *n* (%)	20 (28.1)	20 (28.6)
Antihypertensive drugs *n* (%)	25 (35.2)	29 (41.4)
Antiplatelet drugs *n* (%)	6 (8.5)	8 (11.4)
Anticoagulants drugs *n* (%)	0 (0.0)	1 (1.4)
Lipid-lowering drugs *n* (%)	8 (11.3)	11 (15.7)
Proton pump inhibitors *n* (%)	8 (11.3)	4 (5.7)
Psycho-active drugs *n* (%)	8 (11.3)	3 (4.3)
Antidiabetic drugs *n* (%)	3 (4.2)	5 (7.1)

Data are presented as mean ± SD or number of patients (percentages).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HR, hearth rate; RAI, radioiodine; SBP, systolic blood pressure.

the same analysis separately for males and females, no difference within and between the placebo group and the LT4 + LT3 group was found.

Effects of experimental therapy on thyroid function compensation

Patients treated with LT4 + LT3 showed a TSH decrease at V1 and until V3 ($P < .001$). The proportion of patients with TSH below the reference range was higher in the LT4 + LT3 group compared to the LT4 + placebo group, both in V1 (56.1% vs 24.2%, $P < .001$) and in V2 (46.2% vs 25.4%, $P = .01$) (Table S3, Figure 2). In V3, the number of patients with TSH below the reference range was higher in the LT4 + LT3 group than in the placebo group (33.3% vs 19.0%, $P = .06$). However, if overt iatrogenic thyrotoxicosis is considered (TSH < 0.35 μIU/mL and fT4 > 12 pg/mL), only 1 event was recorded at V2 in the LT4 + placebo group.

The incidence of reduced thyroid compensation was very low during the study. Only at V3, the incidence of elevated TSH was statistically significantly higher in the placebo (15.5%) than in the LT4 + LT3 group (1.6%) ($P < .05$) (Table S3, Figure 2). No cases of overt hypothyroidism (defined as TSH > 4.94 μIU/mL and fT4 < 6 pg/mL) occurred in any group.

In the LT4 + LT3 group, there was a statistically significant fT4 decrease in agreement with the progressive reduction of the dose of LT4 provided ($P = .04$). Conversely, there was a significant fT3 increase ($P = .01$), despite the progressive reduction in LT3 dose. In the LT4 + placebo group, there were no changes in fT4 and fT3 through visits ($P = .42$ and $P = .29$).

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Table 2. Primary and secondary outcomes, comparing study and placebo groups at visit 3.

	Visit 0 (baseline)		Visit 3 (6 months)		Comparison between groups at visit 3		
	LT4 + LT3 group ($n = 71$)	LT4 + placebo group ($n = 70$)	LT4 + LT3 ($n = 63$)	LT4 + placebo ($n = 58$)	Mean difference	*P*	95% CI
Primary outcome							
SHBG (nmol/L)	51.6 ± 29.1	49.7 ± 30.4	52.9 ± 25.8	48.9 ± 33.1	4.0	.47	−6.9, 14.9
Secondary outcomes							
Body mass index (kg/m^2)	28.5 ± 5.5	28.7 ± 5.8	28.1 ± 5.3	28.7 ± 5.6	−0.6	.51	−2.6, 1.3
ThyPRO questionnaire scores							
Goiter symptoms	9.5 ± 8.6	9.5 ± 11.3	8.3 ± 10.0	8.9 ± 9.8	−0.5	.78	−4.1, 3.1
Hyperthyroid symptoms	20.2 ± 17.0	20.7 ± 15.2	16.1 ± 12.2	18.5 ± 13.2	−2.4	.31	−7.1, 2.2
Hypothyroid symptoms	21.1 ± 18.1	20.6 ± 17.3	17.3 ± 17.5	17.8 ± 15.0	−0.4	.88	−6.4, 5.5
Eye symptoms	15.7 ± 15.1	11.9 ± 11.8	13.0 ± 13.6	12.2 ± 12.9	0.7	.76	−4.0, 5.6
Tiredness	17.9 ± 17.3	13.6 ± 13.4	14.8 ± 15.5	14.0 ± 14.7	0.8	.76	−4.7, 6.3
Cognitive problems	25.9 ± 22.2	21.7 ± 17.6	19.1 ± 15.2	18.6 ± 16.1	0.5	.85	−5.2, 6.2
Anxiety	26.4 ± 20.4	26.9 ± 19.4	17.3 ± 14.8	21.2 ± 19.9	−3.8	.23	−9.9, 2.2
Depressivity	30.8 ± 18.1	30.7 ± 17.0	24.7 ± 13.9	29.0 ± 20.1	−4.3	.18	−10.6, 1.9
Emotional susceptibility	31.8 ± 18.8	31.4 ± 16.3	25.1 ± 14.8	28.4 ± 17.9	−3.3	.29	−9.2, 2.9
Impaired social life	13.2 ± 16.8	11.8 ± 14.7	7.6 ± 11.5	9.2 ± 14.6	−1.6	.53	−6.8, 3.4
Impaired daily life	13.8 ± 17.6	10.2 ± 12.9	9.2 ± 10.6	11.8 ± 15.2	−2.6	.29	−7.9, 2.2
Impaired sex life	21.7 ± 27.7	21.1 ± 23.2	21.4 ± 25.2	21.1 ± 24.6	0.3	.93	−9.0, 9.7
Cosmetic complaints	18.2 ± 22.7	13.7 ± 16.3	11.6 ± 16.4	9.1 ± 12.2	2.5	.36	−2.8, 7.8
Overall impact	25.0 ± 24.6	17.2 ± 23.9	13.8 ± 20.5	12.7 ± 19.0	1.0	.77	−6.2, 8.4
TSH (μIU/mL)	1.4 ± 1.5	1.1 ± 1.2	1.3 ± 2.7	2.6 ± 5.3	−1.3	.08	−2.8, 0.2
fT3 (pg/mL)	3.0 ± 0.3	3.0 ± 0.3	3.2 ± 0.5	2.9 ± 0.4	0.3	.002	0.1, 0.5
fT4 (pg/mL)	11.9 ± 2.2	11.7 ± 1.8	10.4 ± 2.2	11.3 ± 2.0	−0.9	.02	−1.7, −0.2
Total-CH (mg/dL)	206.5 ± 33.3	203.4 ± 48.0	210.0 ± 36.6	219.6 ± 44.7	−9.5	.21	−24.4, 5.3
HDL cholesterol (mg/dL)	52.1 ± 13.2	50.2 ± 13.4	54.9 ± 14.4	54.8 ± 14.0	0.1	.98	−4.7, 5.0
Triglycerides (mg/dL)	108.0 ± 67.2	105.2 ± 79.6	100.9 ± 70.8	110.0 ± 71.2	−9.1	.49	−35, 16.8
CTX (ng/dL)	9.6 ± 7.1	11.4 ± 8.7	12.3 ± 10.0	11.7 ± 10.1	0.5	.79	−3.2, 4.2
Osteocalcin (ng/mL)	17.3 ± 4.6	17.4 ± 4.7	18.3 ± 4.5	17.0 ± 4.9	1.3	.14	−0.4, 3.0
Bone alkaline phosphatase (μg/L)	11.3 ± 3.9	11.8 ± 3.6	11.7 ± 4.1	11.5 ± 3.6	0.1	.86	−1.3, 1.6

Data are presented as mean ± SD. The *P*-value reported is obtained by the generalized linear model analysis.
Abbreviations: CTX, C-terminal telopeptide of type 1 collagen; fT3, serum free triiodothyronine; fT4, serum free thyroxine; HDL-CH, high-density lipoprotein cholesterol; SHBG, sex hormone binding globulin; TSH, thyroid-stimulating hormone; Total-CH, total cholesterol.

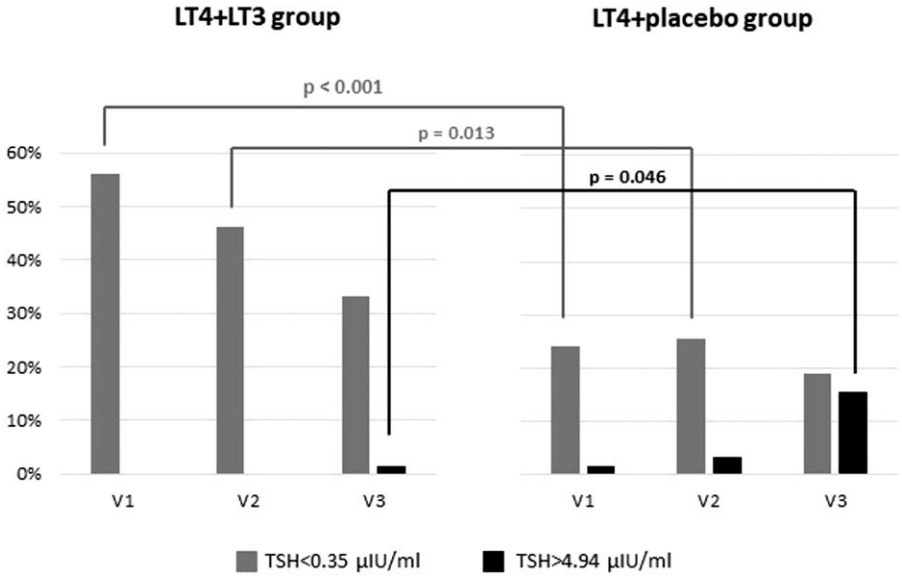

Figure 2. Occurrence of alteration of TSH serum levels, above or below the reference range in the groups, at visit 1 (V1, 6 weeks after baseline), visit 2 (V2, 3 months after baseline), and visit 3 (V3, 6 months after baseline).

Then, we considered the fT3/fT4 ratio. At baseline, fT3/fT4 was below the physiological range (0.27-0.37[7,9]) in both groups (0.26 ± 0.04 in the LT4 + LT3 group, 0.25 ± 0.04 in the LT4 + placebo group), suggesting a suboptimal compensation under LT4 alone, despite mean normal TSH. At the end of treatment, fT3/fT4 remained low in the LT4 + placebo group, while the ratio significantly increased in the LT4 + LT3 group normalizing (0.32 ± 0.08 in the LT4 + LT3 group vs 0.26 ± 0.05 in the LT4 + placebo group, $P < .001$) (Figure 3).

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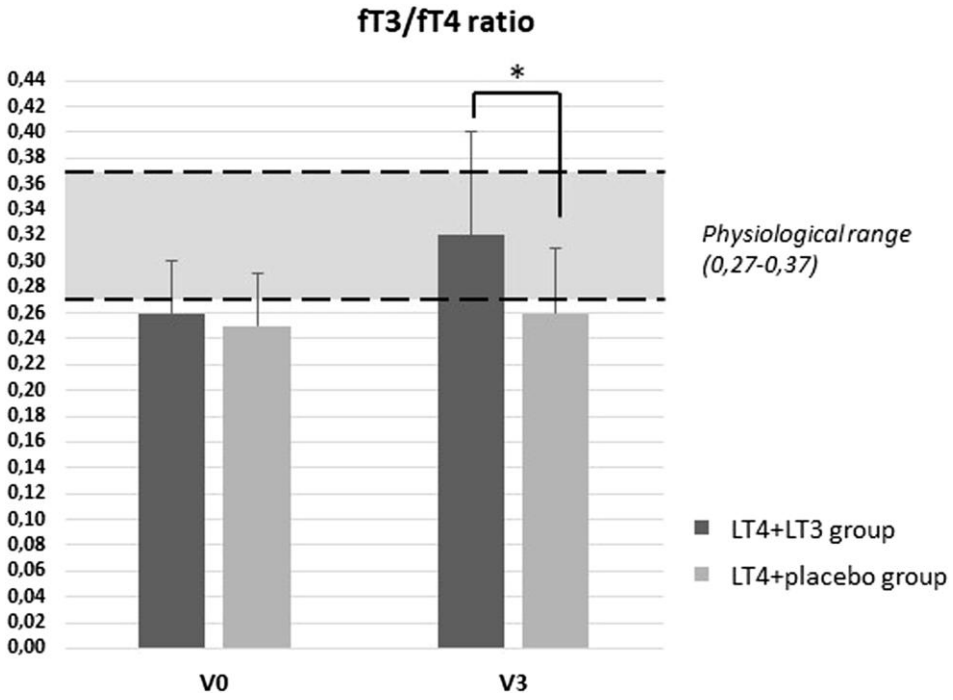

Figure 3. Comparison of fT3/fT4 ratio between groups at baseline (V0) and after 6 months (V3) of LT4 + LT3 group and LT4 + placebo group (*$P < .001$). Data are reported as mean and SD. Mean TSH serum levels were within the reference range both in the LT4 + LT3 group and the LT4 + placebo group at V0 and V3. The physiological range of the fT3/fT4 ratio (mean = 0.32, interquartile range 0.27-0.37) in the presence of physiological TSH levels has been proposed in the consensus document on the evidence-based use of levothyroxine/liothyronine combinations in treating hypothyroidism approved by the American Thyroid Association (ATA), British Thyroid Association (BTA), and European Thyroid Association (ETA).[9]

Changes in clinical parameters

Body mass index did not differ between groups at the end of treatment (Table 2). Even HR, SBP, and DPB did not differ at V3: HR was 73.6 ± 9.0 and 70.4 ± 9.7 bpm in the LT4 + LT3 group and the placebo group, respectively ($P = .08$); SBP was 127.3 ± 19.4 and 128.0 ± 20.6 mm Hg in the LT4 + LT3 group and the placebo group, respectively ($P = .87$), and DBP was 79.3 ± 10.9 and 78.6 ± 10.7 mm Hg in the LT4 + LT3 group and the placebo group, respectively ($P = .87$).

Changes in quality of life

At the end of treatment, no significant differences were found in the totalized score in ThyPRO items between groups (Table 2). The same results were confirmed, performing separate analysis for males and females. No significant correlations were found between the scores in any of these items and TSH, fT4, fT3, and fT3/fT4 ($P > .05$).

The study was not powered on a QoL outcome, and it is underpowered for a ThyPRO outcome.

Compliance, safety, and dose adjustments

Only 10.6% of subjects had complete adherence to ongoing LT4 monotherapy at baseline. Despite the greater complexity of the experimental therapeutic scheme, compliance remained unchanged during the study, with a rate of moderate or complete adherence of 67% and 8% in the LT4 + LT3 group, and 72% and 9% in the LT4 + placebo group, respectively. No significant differences in compliance rate were found between the LT4 + LT3 group and the LT4 + placebo group ($P > .05$).

The safety profile of the combination therapy was good. Only 1 serious adverse event was recorded: One subject from the LT4 + LT3 group was hospitalized for acute hepatitis, probably from gallstones, between V2 and V3. The liver damage spontaneously resolved, and the experimental therapy was not considered etiologically responsible and was continued.

The remaining adverse events were not serious. The most frequent was TSH which was out of the reference range (Figure 2), without signs or symptoms of altered thyroid compensation. Accordingly, changes in LT4 and LT3 dosage were made. At V1, dose changes were more frequent in the LT4 + LT3 group than in the LT4 + placebo group ($P < .001$). This difference was lost at V2, even if dose adjustments were required again in 30% (LT4 + placebo) and 45% (LT4 + LT3) of subjects. Considering therapeutic changes, there was a greater incidence of dose reduction in the LT4 + LT3 compared to the LT4 + placebo group at V1. On the contrary, dose increase occurred rarely and only in LT4 + placebo group. Beyond TSH alterations, the remaining adverse events were insomnia, palpitations, anxiety, and headache (Table S3), without differences between groups when considered globally ($P = .08$).

Finally, no dose changes of drugs taken for comorbidities were registered during the study. Few new drugs were started during the experimental treatment, with a significant increase between V1 and V2 in the LT4 + LT3 group ($P = .01$): One subject started integrative therapy with cholecalciferol, 1 lipid-lowering supplement, 1 acyclovir, and 1 antihypertensive treatment.

Preference

Only 11% of subjects in the LT4 + LT3 group preferred the experimental treatment, without difference from the

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LT4 + placebo group ($P = .86$). The 56% and 60% of patients in the LT4 + LT3 group and the LT4 + placebo group ($P = .58$), respectively, found the new scheme more complicated than LT4 tablet monotherapy. The 2 major difficulties were drop counting and the twice-daily administration.

Influence of DIO2 and MCT10 SNPs on experimental treatment outcomes

There were no significant genotype-related differences between the LT4 + LT3 group and the LT4 + placebo group for the studied outcomes. See Supplementary results.

Discussion

This study shows that patients treated with customized twice-daily doses of LT3 in combination with LT4 have a significant reduction of TSH together with an increase of fT3 and fT3/fT4 compared to placebo. However, no significant differences occur between groups in peripheral tissue markers of thyroid function, QoL, and BMI.

To date, there is no consistent strong evidence of the superiority of combination therapy over LT4 monotherapy. Guidelines recommend against the routine use of combination treatment as a form of thyroid replacement therapy in patients with primary hypothyroidism.[35] Combination treatment might be considered as an experimental approach in LT4-treated patients who have persistent complaints despite serum TSH values within the reference range, and it must be discontinued if no improvement is experienced after 3 months.[36]

Our data confirm that LT4 monotherapy, although capable of normalizing TSH serum levels, has some limits, at least in thyroidectomized subjects. First, conventional replacement monotherapy does not normalize the fT3/fT4 ratio in up to 70% of treated subjects. Second, despite being a simple and safe therapy, compliance to the treatment is moderate but not complete in most patients. Third, a high percentage of patients complain of residual symptoms of hypothyroidism or that they correlate with thyroidectomy. We could speculate that although TSH serum levels during LT4 monotherapy resulted within the reference range, this conventional approach is not able to recreate circulating free thyroid hormone levels comparable to the physiological state.[7,37,38] Accordingly, it has been shown that TSH-suppressive LT4 doses are required to achieve preoperative serum fT3 levels[39] and to improve QoL after thyroidectomy.[40]

Only subjects treated with combination therapy normalize fT3/fT4, while those treated with LT4 and placebo still have reduced values despite normal TSH. This limitation of LT4 monotherapy is well known since Gullo et al.[7] showed that fT3/fT4 was significantly lower in athyreotic individuals on LT4 replacement compared to individuals with intact pituitary-thyroid axis despite similar and normal TSH serum levels. The normalization of fT3/fT4 is a sign of a good LT4/LT3 ratio, therefore that the dosages used have made it possible to recreate values of free thyroid fractions close to physiological ones. However, it is not clear if restoring a physiological fT3/fT4 ratio has benefits for patients in terms of symptoms and/or biochemical parameters.[41] In our study, the fT3/fT4 increase after combined therapy seems not to be related to either QoL or metabolic homeostasis. Moreover, our data do not show a beneficial effect of combined therapy on SHBG hepatic secretion and bone turnover markers, which are known as a proxy of the effect of thyroid hormones on these 2 target organs. Although these analytes are influenced by many factors, we would have expected a greater variation, in conjunction with the effect on thyroid compensation and QoL, as seen in other studies.[15,19,25] Perhaps, 6 months were not sufficient to see an improvement in these endpoints. However, it remains true that other more specific tissue markers are needed to measure the effect of thyroid hormones outside the hypothalamus-pituitary unit.[42]

LT4 + LT3 treated subjects show an improvement in some aspects of QoL related to the emotional sphere and the overall impact of thyroid disease, but without reaching significant differences at the end of treatment in comparison to the LT4 + placebo group. The observation that many patients on LT4 monotherapy complain of fatigue, mood, or memory alterations despite normal TSH[3] suggests that LT4 alone could fail to restore T3 levels in the brain. However, the normalization of the fT3/fT4 ratio in LT4 + LT3 treated subjects did not lead to QoL improvement. Previous data on this topic are conflicting: Some authors did not find changes in mood or psychological health,[15,17-19] but they all prescribed LT3 only once daily; others found an improvement in psychological parameters.[12,13,16,25,43] It must also be considered that the TRUST trial clearly demonstrated that not even LT4 improves thyroid-related symptoms, at least in older adults with subclinical hypothyroidism.[44]

Previous studies also recorded evident patient preference for combination therapy,[12,13,16] even in the absence of clear clinical benefits.[14,25] The main limitation of these data is linked to its evaluation, which is extremely variable from study to study and not performed with ad hoc validated tools. In our study, subjects treated with combination therapy did not prefer the experimental treatment. Going deeper, the therapeutic scheme was perceived as too complex, due to drop counting and twice-daily administration. These difficulties could have smoothened the beneficial effect of combined therapy on QoL. Another interesting aspect is compliance. Most of the subjects declared moderate compliance with LT4 therapy at baseline and only 10% of the total had complete compliance, based on the stripping criteria of the Morisky Medication Adherence Scale. These data did not change despite the greater complexity of the therapeutic scheme during the study, regardless of the treatment received. This may have influenced the results, concealing a potentially greater effect of combined treatment.

Previous studies suggested that patients carrying the derived allele of genes encoding deiodinases or thyroid hormones transporters may prefer combination therapy rather than LT4 alone.[28,45] In the present study, we did not find any consistent influence of *DIO2*-rs225014 and *MCT10*-rs17606253, alone or in combination, on preference for combination therapy. Moreover, we found that these derived genetic variants do not influence pituitary-thyroid axis compensation, peripheral tissue markers of thyroid function, or QoL in patients treated with LT4 + LT3. However, we used a dominant model in this analysis as we did not have sufficient power to use the method more generally applied in this area, which is a recessive model.

In the context of studies on the efficacy of combination therapy, the other side of the coin is its safety. In our study, the safety profile of LT3 is good with few adverse effects in the short term. The most frequent adverse effect was TSH reduction below the normal range, despite the dose of combination

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therapy being meticulously calculated considering the physiological T4/T3 ratio as suggested by the ETA guidelines.[36] Thus, the LT4/LT3 suggested ratio should be more precisely defined by future studies. However, there was no increase in free thyroid hormones beyond reference ranges and no subjects had signs/symptoms of hyperthyroidism.

The main strength of our study is that it respects most recommendations of the 2021 Consensus on studies on combination therapy:[9] Perspective, randomized placebo-controlled, double-blind design; duration of treatment greater than 4 months; recruitment of patients with low thyroid residual function and on LT4 replacement therapy with substitutive TSH target; administration of LT4 + LT3 respecting the physiological ratio, even in case of dosage changes during the study and with customized dosage for each patient, with LT3 twice daily; assessments of peripheral tissue markers of TH function; assessment of clinical benefits through specific questionnaires for QoL in thyroid diseases; assessment at the end of the protocol on patient preference; and genotyping for the main *DIO2* and *MCT10* variants.

However, we must admit the following limitations of the study: The development of part of the study during the COVID-19 pandemic severely compromised the chances of enrolling due to the drastic reduction of clinical activity and subsequent dropouts; the measurement of serum fT3, instead of total T3; and the recruitment of both asymptomatic and symptomatic subjects. Thus, the study was underrecruited due to the pandemic and hence was underpowered for the primary endpoint, SHBG and we did not have sufficient power to analyze the effect of genotype by a recessive model. Despite the failure to reach the expected sample size, to date, our study remains the one with the highest number of participants and with the longest duration of treatment among those who administered LT3 twice daily.

In conclusion, LT4 + LT3 therapy at a dose adapted according to TSH-level, with twice-daily LT3, is capable to restore the physiologic fT3/fT4 ratio, but the impact of such an effect is still to be defined. No effects emerged either on peripheral tissue markers of thyroid hormone action, on QoL or on clinical parameters. Patients did not prefer LT4 + LT3 and found the scheme too complex. Neither preference nor therapeutic compensation is influenced by *DIO2*-rs225014 and *MCT10*-rs17606253. However, these results may not apply to the general hypothyroid population, since all the enrolled subjects had post-thyroidectomy hypothyroidism. Future studies with an easier therapeutic scheme, for example, with sustained release of T3 preparation, should be conducted, hopefully with new and more specific markers of the effect of thyroid hormones at the periphery.

Acknowledgments

The study was supported by IBSA Institut Biochimique SA, without involvement in study design, collection, analysis, and interpretation of data, writing of the report, nor any restrictions regarding the submission of the report for publication.

G.B. is supported by a grant from the Italian Ministry of University under the "Departments of Excellence" program.

The authors are grateful to Torquill Watt from the Department of Endocrinology, Copenhagen University Hospital Rigshospitalet (Copenhagen, Denmark) for providing the validated Italian version of ThyPRO questionnaires.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Conflict of interest: None declared.

Authors' contributions

Giulia Brigante (Conceptualization [lead], Data curation [equal], Formal analysis [equal], Funding acquisition [supporting], Investigation [lead], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Daniele Santi (Conceptualization [supporting], Data curation [supporting], Formal analysis [lead], Investigation [equal], Methodology [equal], Writing—review & editing [supporting]), Gisella Boselli (Data curation [equal], Investigation [equal]), Gianluca Margiotta (Data curation [equal], Investigation [equal]), Rossella Corleto (Data curation [equal], Investigation [equal]), Maria Laura Monzani (Data curation [equal], Investigation [equal]), Andrea Craparo (Data curation [equal], Investigation [equal]), Michela Locaso (Investigation [equal]), Samantha Sperduti (Investigation [equal], Writing—original draft [supporting]), Neena Roy (Investigation [equal], Writing—original draft [supporting]), Livio Casarini (Conceptualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Tommaso Trenti (Conceptualization [equal], Investigation [equal]), Simonetta Tagliavini (Conceptualization [equal], Investigation [equal]), Maria Cristina De Santis (Investigation [equal]), Laura Roli (Investigation [equal]), Vincenzo Rochira (Conceptualization [equal], Investigation [equal], Writing—original draft [equal], Writing—review & editing [equal]), and Manuela Simoni (Conceptualization [lead], Funding acquisition [lead], Methodology [equal], Project administration [lead], Supervision [lead], Writing—original draft [equal], Writing—review & editing [equal])

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