The influence of radioiodine therapy on ocular changes and their relation to urine cotinine level in patients with Graves' Ophthalmopathy

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Abstract

OBJECTIVES: Radioiodine therapy (RIT) is frequently used as the definitive treatment in patients with Graves' hyperthyroidism when remission is not achieved with anti-thyroid drugs (ATDs). In this observational study, we intended to examine whether the use of high doses of radioiodine (RAI) [22 mCi (814 MBq)] with prophylaxis of oral glucocorticoids (oGCS) does not exacerbate Graves ophthalmopathy (GO) in smokers and non-smokers, especially regards to the urine level cotinine and ocular changes before and after RIT.

PATIENTS AND METHODS: The studied group consisted of 26 smokers, aged 28–61 years and 25 non-smoker patients, aged 21–54 years, respectively. The patients were enrolled to RAI after one-year of ineffective ATDs treatment. Criterion for inclusion in the study were patients with mild GO with hyperthyroidism at diagnosis based on the severity (NOSPECTS) and activity (CAS) scale. All the patients were subjected to RIT with oGCS prophylaxis and evaluated prospectively during a one-year follow-up. The ophthalmological examination was performed at various stages of RIT: initial pre-radioiodine administration, at the time of treatment 6, and 12 months after RAI. The present study is unique, because the urine cotinine measurement was employed to detect nicotine exposure, also in regard to smoking intensity.

RESULTS: In smokers, the values of serum TPO-Abs were statistically significant in the second and six month ($p<0.05$) and in the second and after one

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year (p<0.005). The TSHR-Abs concentration was significantly higher in smokers (p<0.05), rising from 22.9±1.2 IU/L before therapy to 29.6±5.3 IU/L – 2 months, 32.6±8.6 IU/L – 6 months, and slightly decreased 28.9±10.6 IU/L – 12 months. These observed changes were statistically different between groups at baseline (p<0.05) and after one-year of follow-up (p<0.005). Mean urine cotinine were considerably higher in smokers comparing to non smokers in each point of observation [903.4±770.0 and 5.2±1.7 ng/mL at baseline (p<0.001), 412.8±277.3 and 3.0±0.6 ng/mL after 2 months (p<0.001), 452.0±245 and 6.6±3.6 after 6 months (p<0.001), 379.4±236.8 and 1.0±1.2 after one year (p<0.001)]. The CAS values in the smoking group before RIT increased statistically from 2.8±0.2 points at baseline to 4.3±0.3 after 6 months, and 4.0±0.5 (12 months), while in the non-smoking patients it was 1.4±0.2, 2.8±0.3 and 2.2±0.2, respectively. The level of urine cotinine correlated positively with CAS and TSHR-Ab in the smoking group (r=0.41; p<0.05) at baseline and during follow-up (2 months: r=0.46; p<0.001, 6 months: r=0.47, p<0.005; 12 months: r=0.46; p<0.005). In the NOSPECS classification, the symptoms changed from mild to moderate, mostly in smoking patients.

CONCLUSIONS: 1) ablative RIT dose with prophylactic oral prednisone is a safe treatment in both smokers and non-smokers with mild GO; 2) The post hoc analysis showed that urinary level of cotinine can be very helpful in the assessment of exacerbation of ophthalmological clinical symptoms before and after RIT particularly in smokers.

Abbrevations:

fT4	- free tetraiodothyroxine
fT3	- free triiodothyronine
GO	- Graves' Ophthalmopathy
RAI	- radioiodine
RAIU	- radioiodine uptake
RIT	- radioiodine therapy
TSH	- thyrotropin
TSHR-Abs	- autoantibodies to the thyrotropin receptor
Tg-Abs	- thyroglobulin autoantibodies
TPO-Abs	- thyroperoxidase autoantibodies

INTRODUCTION

Radioiodine therapy (RIT) is frequently used as the definitive treatment in patients with Graves' hyperthyroidism when remission is not achieved with antithyroid drugs (ATDs) (Singer *et al.* 1995; Cooper 1996). Other currently accepted risk factors for deterioration of ophthalmologic symptoms in patients undergoing RIT comprise a high anti-TSH receptor antibodies (TSHR-Abs) and high serum concentration of free triiodothyronine (fT_3) (Sisson *et al.* 2008; Tallstedt et al 1992; Bartalena & Tanda 2009; Bartalena *et al.* 1998a; Laurberg 2008). Furthermore, only a minority of patients presenting moderate to severe Graves' Ophthalmopathy (GO) after-RIT requires treatment, while majority of them develop only mild and transitory progression (Prummel *et al.* 1990). The causative relation between radioiodine (RAI) and progression of GO has been demonstrated in several studies (Bonnema *et al.* 2002; Tallstedt *et al.* 1992; Bartalena & Tanda 2009,; Kung *et al.* 1994; Vannucchi *et al.* 2009), but some authors reported lack of association (Gorman 1995,; Prummel *et al.* 1990). In addition to RIT, tobacco smoking is presenting a significant influence on the course of orbitopathy as well (Bartalena *et al.* 2011; Marcocci *et al.* 1999; Rasmussen *et al.* 2000; Prummel *et al.* 1997; Tallstedt *et al.* 1992; Bartalena *et al.* 2008; Bartalena 2002; Bartalena et al.1989; Bartalena *et al.* 1998b; Hagg & Asplund 1987; Hegedus *et al.* 2004; Kendler *et al.* 1993; Meberg & Marstein 1986,; O'Hare & Georghegan 1993; Oliveira *et al.* 2009; Tomer & Davies 2003).

Cotinine is a metabolite of nicotine and is used as a biomarker for exposure to tobacco smoke (Triggle 1996). This marker detects smokers, even after several days (up to one week) after cigarette consumption (Florek *et al.* 2006). Furthermore, it cotinine is a valuable marker, in cases when patients do not always tell the doctors truth referring to their smoking. Hitherto, the studies presenting relationship between RIT and smoking in regard to exacerbation of GO including measurement of urine cotinine level were not reported.

In this observational study, we intended to examine whether the use of high doses of radioiodine (RAI) [22 mCi (814 MBq)] with prophylaxis of oral glucocorticoids (oGCS) does not exacerbate Graves ophthalmopathy (GO) in smokers and non-smokers, especially regards to the urine level cotinine and ocular changes before and after RIT.

PATIENTS AND METHODS

Criterion for study inclusion was mild GO in patient with accompanying hyperthyroidism at diagnosis. Hyperthyroidism was elucidated on the basis of severity (NOSPECS) and activity (CAS) scale. The studied group consisted of 26 smokers (23 women and 3 men) aged 28–61 years, and 25 non-smoker patients (21 women and 4 men) aged 21–54 years, respectively (Table 1). The patients in two groups did not reveal significant discrepancy referring to the age at diagnosis, gender, median duration of GO. Patients were enrolled to RAI after one year of ineffective antithyroid drugs (ATDs) treatment. Among the non-smokers, only in one case ATDs could not be used due to granulocytopenia and transaminasemia after combined pegylated IFN-α (Peginterferon alfa-2a, Pegasys) and Ribavirin (Copegus) therapy for chronic hepatitis C (CHC). In this particular case, the administration of IFN-α influenced the development of GO (Czarnywojtek *et al.* 2012).

The diagnosis of GO was established on the basis of following criteria: goiter on palpation and in thyroid ultrasound, proptosis or exophthalmos assessed with an

exophthalmometer (>18 mm), clinical signs of hyperthyroidism (tachycardia, heat intolerance, increased sweating, fine tremor of hands), hormonal profile characterized by suppressed TSH levels and an increased concentration of free thyroxine (fT_4), free triiodothyronine (fT_3) combined with positive autoantibodies in regard to the thyrotropin receptor (TSHR-Abs). Serum free T_4, T_3 and TSH concentrations were measured using fully automated electrochemiluminestent assay (Cobas 6000 system, (Roche Diagnostic). Serum TSH was measured using a third-generation commercial kit assay (sensitivity ≤0.005 μIU/mL. The TSHR-Abs, TgAbs, and TPOAbs (B.R.A.H.M.S AG, Hennigsdorf/ Berlin, Germany) were assayed by commercially available kits (Costagliola *et al.* 1999).

The reference ranges for serum hormone and autoantibody concentrations for our laboratory were as follows: TSH: 0.27–4.2 μIU/ml, fT_4: 11.5–21.5 pmol/L, fT_3: 3.9–6.8 pmol/l, TSHR-Abs: <2 IU/L, thyroglobulin autoantibodies (Tg-Abs): 10–115 IU/mL, and thyroperoxidase autoantibodies (TPO-Abs) <34 IU/mL.

All the patients were subjected to RIT and evaluated prospectively during a one-year follow-up. The following data were recorded: age, gender, time of ATDs therapy and smoking habit. A detailed ophthalmological assessment was performed in all patients at each visit. Ophthalmological examination was conducted at baseline, after 6 and 12 months. Due to delayed action of RAI, an analysis after 2 months was not necessary. Serum TSH, fT4, fT3, Tg-Abs, TPO-Abs, and TSHR-Abs concentrations were measured at baseline, 2, 6, and 12 month.

Thionamides, Methimazole [MMI (in 49 patients)] and propylthiouracil [PTU (in 2 patients)] were the primary therapeutics in the studied group before RIT. ATDs were discontinued at least 24 hours prior to RIT. All the patients received an identical therapeutic dose of 814 mBq [22 mCi] RAI, followed by a complementary therapy with oGCS, i.e. prednisone (0.3–0.5 mg/kg body weight daily) initiating 3 days after RAI and tapering down the dose until withdraw 6 weeks (protective therapy) (Tanta *et al.* 2008).

The study protocol was approved by the ethics committee at the Poznań University of Medical Sciences and all the participants gave their informed consent.

Thyroid ultrasonography

Thyroid ultrasonography was performed using Aloka SSD-500 apparatus (Aloka, Tokyo, Japan) with a 7.5-MHz linear transducer. The thyroid volume was measured by ultrasonography and calculated using the ellipsoid model (width × length × thickness × 0.52 for each lobe) (Knudsen *et al.* 1999).

Ophthalmologic follow-up

A thorough ophthalmological examination was performed at various stages of RIT: initial pre-radioiodine administration, at the time of treatment, and then subsequently after 6 and 12 months after RAI. To make study comparisons possible, it was an important to determine the presence and activity of GO using standardized scales such as the Clinical Activity Scale (CAS) (Mourits *et al.* 1989; Mourits *et al.* 1997) (eye pain, ocular or eyelid erythema, conjunctival or eyelid edema, and worsening of proptosis), and/or NOSPECS (Werner 1969; Werner 1977a,b) (N, no signs or symptoms; O, only signs, no symptoms; S, soft tissue involvement; P, proptosis; E, extraocular muscle involvement; C, corneal involvement; S, sight loss) that reflected the severity of ophthalmopathy. Activity of GO were defined according to the CAS scores of ≤ 3/7. Furthermore, the study has been extended on the basis of consensus of European group on Graves' orbitopathy (EUGOGO) (Bartalena *et al.* 2008; www.eugogo.eu). The criteria used during the ophthalmological examination included: visual acuity, cover test, tonometry, colour test, fundus examination, lid fissure measurement, visual field, Hess-Lancaster screen, and Hertel's exophthalmometer measure. Patients failing to return for follow-up were excluded from the study.

Assessment of smoking history

Urine cotinine level non-smokers: Urine cotinine [(5S)-1-methyl-5-(3-pyridyl)pyrrolidin-2-one)], is a nicotine metabolite and therefore a useful reporter of nicotine traces in the human body. Non-smokers and those not exposed to environmental tobacco smoke (ETS) had urine cotinine levels of 5 ng/mL in urine/mg creatinine; individuals exposed to ETS had 5 to 50 ng cotinine in urine/mg creatinine (passive exposure); and smokers >50 ng cotinine in urine/mg creatinine (from 50 to 500 ng/mL in light smokers; 500–2500 ng/mL in moderate smokers, and above 2500 ng/mL in heavy smokers) (Florek *et al.* 2006).

Chemicals: Cotinine was dissolved in methanol to create a standardized solution in methanol (Wao Pure Chemicals; Osaka, Japan and Sigma Chemical St Louis, MO, USA). The standard solution was added to urine (non-smokers) and distilled water to prepare a range of concentrations: 2.1, 27.6, 55.2, 82.8, 110, 138, 276, 552, 828, 1100 and 1380 ng/ml for cotinine.

Equipment: A gas chromatograph (GC-14B, Kyoto, Japan) equipped with a capillary column and flame thermionic sensor was utilized for quantifying cotinine. The injection port and detector temperature was 260 °C. The column temperature was constant at 150 °C for 2 min, then raised to 260 °C at the rate of 10 °C/min, and held constant for 2 min. as the final temperature. Nitrogen at 15 kPa was the carrier gas.

Measurement: One milliliter of urine was added to 0.1 ml of 12 μg/ml of carbinoxamine maleate in methanol and 1ml of 1M carbonate buffer (pH 9.7). The upper aqueous phase was aspirated and disposed of,

while the remaining organic phase was transferred to a new single use tube. The organic phase was re-extracted with 1ml of 0.1N HCl and vigorously vortexed for 30 s. The resulting aqueous phase was transferred to a new disposable tube and 1ml carbonate buffer, added, and re-extracted using 4 ml dichloromethane ortexed for 30 s. Organic phase was mixed with 20 µl of isoamyl alcohol and dichloromethane, along with succeeding evaporation under a mild flow of nitrogen in a heated block at 35˚C. 1 µl of the remaining alcohol solution was then injected into the gas chromatograph.

Tab. 1. Clinical and biochemical characteristics of patients included in the study.

Clinical parameters	Smoking (n=26)	Non-smoking (n=25)
F/M	3/23	4/21
Median age, range (yr)	38 (28–61)	33 (21–54)
Age at diagnosis (yr)	37±16	36±14
Duration of GO (months)	8±0.5	11±1.7
Recent thyroid status (thyroid treatment)		
ATD before ^{131}I Yes (%) No (%)	 25 (96) 1 (4)	 25 (100) -
Duration ATDs (months)	11.6±15.8	9.8±7.7
^{131}I	3 (11.5)	-
Thyroidectomy	-	2 (8)
Thyroid tests		
TSH (N: 0.27–4.2 µU/mL)	0.07±0.03	0.11±0.05
fT3 (N: 3.9–6.8 pmol/L	11.1±4.3	6.1±2.6
fT4 (N: 11.5–21.5 pmol/L)	22.2±3.7	22.9±3.1
TSHR-Abs (N: <2 IU/L)	**22.9±1.2[1]**	**15.3±0.2[1]**
Tg-Abs (N: 10–115 IU/ml)	197.5±121.6	134.1±123.3
TPO-Abs (N: <34 IU/ml)	284.3±274.8	386.2±343.8
Thyroid volume (ml)	21.3±4.2	16.2±3.1
Smoking status		
Never smoked (%)	-	19 (76)
Ex-smoker (%)	-	2 (8)
Current smoker (%)	26 (100)	-
Passive smoker (%)	-	4 (16)
Urinary cotinine (ng/mL)	**903.4±770.0 [2]**	5.2±1.7 [2]
Radioiodine treatment		
"ablative dose" of ^{131}I (22.0±0.0 mCi) [814.0±0.0 MBq]		

[1,2]- Statistical significant differences are related to dependencies between data labeled with the same Arabic numerals. [1]$p<0.05$, [2]$p<0.001$ (Mann-Whitney test). Numerical variables other than age are given as mean±SD or number (%); M, Male; F, female.

Tab. 2. The clinical and biochemical evaluation of patients at different time after radioiodine therapy (RIT).

Statistical analysis

All alterations in clinical and biochemical parameters between smokers and non-smokers were carefully evaluated, in particular to urine cotinine concentration reference. Statistical analysis was performed using Statistica 7.1, StatSoft Inc. All the data are presented as mean±(±SEM) unless stated otherwise. The Mann–Whitney *U* test was applied in a non-parametric distribution. The statistical correlations were assessed by a calculation of Spearman's coefficient, according to the data distribution. Two-tailed *p*-values <0.05 were considered statistically significant.

RESULTS

The clinical and biochemical characteristics of all the patients studied at baseline and one-year of follow-up after RIT are shown in Table 1 and 2.

Thyroid function and serology

The levels of free T4, and free T3 remained stable in both groups throughout one year observation. Serum values of TSH in both groups were undetectable. In smokers, the mean (±S.E.M) serum fT4 levels before RIT were: 22.2±3.7 pmol/l, 22.5±5.1 pmol/l (for 2 months), 19.5±4.2 U/L pmol/l (6 months) and 18.6±6.1 pmol/l (1 year). In non-smokers, serum fT4 levels at time of RIT were: 22.9±3.1, 21.3±4.9 (for 2 months), 18.8±4.1 (6 months) and 17.2±4.3 pmol/l (1 year). The values of the mean (±S.E.M) serum fT3 levels in non-smokers before RAI were: 6.1±2.6 U/L, 4.7±1.2 after 2 months, 5.1±0.3 (6 months), and 3.7±0.7 pmol/l (1 year). In smokers serum fT3 levels at the time of the RIT administration were: 11.1±4.3, 5.8±0.2 (2 months), 5.3±1.4 (6 months), and 5.0±0.8 pmol/l (1 year), respectively. The observed differences were not statistically significant.

At baseline, after 2, 6 and 12 months after RAI the changes in following groups in levels of Tg-Abs were not significant. In smokers the results were: 197.5±121.6 at baseline, 222.5±159.7 after 2 months, 199.4±124.3 (6 months), and 187.2± 138.4 IU/ml (1 year) and in non-smokers: 134.1±123.3 at baseline, 243.4±161.6 (2 months), 238.4±221.6 (6 months), and 196.4±154.6 IU/ml (1 year), respectively.

In smokers, the values of serum TPO-Abs were: 284.3±274.8 at baseline, 342.4±293.4 after 2 months, 273.4±194.3 (6 months), and 200.7±214.8 IU/ml (1 year). Statistical significant changes were found for values in the second and sixth month ($p<0.05$) and in the second and after one year ($p<0.005$). In non smokers the results were stable and insignificant: 386.2±343.8 at baseline, 410.0±361.6 after 2 months, 343.3±297.4 (6 months), and 214.3±239.7 IU/ml (1 year).

The TSHR-Abs concentration was significantly higher in smokers ($p<0.05$), rising from 22.9±1.2 IU/L before therapy to 29.6±5.3 IU/L – 2 months, 32.6±8.6 IU/L – 6 months, and slightly decreased

28.9±10.6 IU/L – 12 months. In non-smokers the initial TSHR-Abs concentration was lower 15.3±0.2 IU/L), and increased to 18.3±5.3 IU/L (2 months), 17.3±3.3 IU/L (6 months), and decreased to 15.2±6.8 IU/L (12 months, p=NS). These observed changes were statistically different between groups at baseline (p<0.05) and after one-year of follow-up (p<0.005).

The volume of the thyroid gland were also significantly different between two groups after only two months of observation (p<0.05). In the remaining cases, both at baseline and during the one year follow up changes were insignificant [non-smoking group: initially 16.2±3.1 ml; 6 months 15.1±0.1 and one year 13.2±2.1 ml, smoking groups: 21.3±4.2 ml; 6 months 19.3±0.6 ml and one year 17.4±3.2, respectively].

Cotinine and smoking

Mean urine cotinine were considerably higher in smokers comparing to non-smokers in each point of observation [903.4±770.0 and 5.2±1.7 ng/mL at baseline (p<0.001), 412.8±277.3 and 3.0±0.6 ng/mL after 2 months (p<0.001), 452.0±245 and 6.6±3.6 after 6 months (p<0.001), 379.4±236.8 and 1.0±1.2 after one year (p<0.001)]. The level of urine cotinine correlated positively with CAS and TSHR-Ab in the smoking group (r=0.41; p<0.05) at baseline and during follow-up (2 months: r=0.46; p<0.001, 6 months: r=0.47, p<0.005; 12 months: r=0.46; p<0.005).

Ophthalmological symptoms (OS)

In the smoking group most ophthalmological symptoms (OS) were intensified (Table 3). The CAS values in the smoking group before RIT increased statistically from 2.8±0.2 points at baseline to 4.3±0.3 after 6 months, and 4.0±0.5 (12 months), while in the non-smoking patients it was 1.4±0.2, 2.8±0.3 and 2.2±0.2, respectively. A comparison of the CAS values between the two groups showed no statistical differences. In the NOSPECS classification, the symptoms changed from mild to moderate, mostly in smoking patients. The sight loss (Optic Neuropathy) was not taken into consideration because clinical symptoms were mild.

At baseline most frequently observed changes at baseline in smokers were: eyelid redness (100%), pain on attempted up or down gaze (61.5%), redness of the conjunctiva (57.6%) and spontaneous retrobulbar pain (53.8%). Nevertheless assessing the percentage change in one-year of clinical follow-up we noted no significant alteration for levels of swelling of the eyelids (50% vs 53.8%) and conjunctivial edema (50% vs 53.8%). Although, a rising values were reported for redness of the conjunctiva (57.6% vs 96.1%), spontaneous retrobulbar pain (53.8% vs 76.9%), and inflammation of the plica (53.8% vs 69.2%). Only in smokers the corneal involvement and extraocular muscle changes dysfunction, were observed.

In contradiction, the percentages of occurrence before RIT OS in non-smokers ranged from 12% (diplopia intermittent) to 84% (eyelid redness) and after treatment were significantly reduced from 16 % (redness of the conjunctiva) to 48% (pain on attempted up or down gaze). In one year follow up changes were positively observed for symptoms such as: redness of the conjunctiva (48% vs 16%), eyelid redness (84% vs 28%) or pain on attempted up- or down gaze (72% vs 48%). Similarly values remained low for: diplopia intermittent (12% vs 12%), inflammation of the plica (36% vs 32%), swelling of the eyelids (32% vs 28%) and conjunctival oedema (32% vs 28%). Althought proptosis was stable in both groups, there were no significant changes noted between smokers and non-smokers in the observed OS.

DISCUSSION

RIT is the treatment of choice in treating hyperthyroid patients with Graves' disease. The usage of this method in patients with ophthalmopathy, in particular cigarette smokers, is a controversial therapy due to the elevated risk of intensifying exophthalmous.

Parameters	2 months after RIT		1/2 year after RIT		1 year after RIT	
	Smoking	Non-smoking	Smoking	Non-smoking	Smoking	Non-smoking
Serum FT3 levels (pmol/L)	5.8±2.0	4.7±1.2	5.3±1.4	5.1±0.3	5.0±0.8	3.7±0.7
Serum FT4 levels (pmol/L)	22.5±5.1	21.3±4.9	19.5±4.2	18.8±4.1	18.6± 6.1	17.2±4.3
TSHR-Abs (mU/L)	**29.6±5.3**[1]	18.3±5.3	**32.6±8.6**[1,2]	17.3±3.3	**28.9±10.6**[2,3]	**15.2±6.8**[3]
Tg-Abs (IU/L)	222.5±159.7	243.4±161.6	199.4±124.3	238.4±221.6	187.2± 138.4	196.4± 154.6
TPO-Abs (IU/L)	**342.4±293.4**[1,3]	410.0±361.6	**273.4±194.3**[1]	343.3±297.4	**200.7±214.8**[3]	214.3±239.7
Urinary cotinine/creatinine (ng/mL)	**412.8±277.3**[4]	**3.0±0.6**[4]	**452.0±245**[5]	**6.6±3.6**[5]	**379.4±236.8**[6]	**1.0±1.2**[6]
Thyroid volume (ml)	**20.4±0.2**[1]	**15.4±0.3**[1]	19.3±0.6	15.1±0.1	17.4± 3.2	13.2± 2.1

[1,2, 3, 4, 5, 6]- Statistical significant differences are related to dependencies between data labeled with the same Arabic numerals. [1,2]p<0.05, [3]p<0.005, [4,5,6]p<0.001 (Mann-Whitney test). Numerical variables are given as mean±SD.

Tab. 3. The evaluation of severity(former scale of NOSPECS) and activity score (CAS) of GO before radioiodine therapy (RIT) and after 6 and

12 months of follow-up.

Ophthalmological signs	Before RIT		1/2 year after RIT		1 year after RIT	
	Smoking	Non- smoking	Smoking	Non- smoking	Smoking	Non- smoking
Spontaneous retrobulbar pain	14 (53.8)	11 (44)	21(80.7)	13 (52)	20 (76.9)	9 (36)
Pain on attempted up- or down gaze	16 (61.5)	18 (72)	15 (57.6)	15 (60)	14 (53.8)	12 (48)
Swelling of the eyelids	13 (50)	8 (32)	14 (53.8)	10 (40)	14 (53.8)	7 (28)
Eyelid redness	26 (100)	21 (84)	24 (92.3)	18 (72)	23 (88.4)	7 (28)
Redness of the conjunctiva	15 (57.6)	12 (48)	13 (50)	8 (32)	25 (96.1)	4 (16)
Conjunctivial oedema	13 (50)	8 (32)	14 (53.8)	10 (40)	14 (53.8)	7 (28)
Inflammation of the caruncle and/ or plica	14 (53.8)	9 (36)	16 (61.5)	11 (44)	18 (69.2)	8 (32)
Proptosis *						
RE	17.4±2.9	16.2±2.1	18.4±3.4	16.8±2.6	18.8±3.2	16.6±2.2
LE	17.2±2.6	16.1±2.2	18.1±2.9	16.6±2.8	18,9±3.7	16.9±2.8
Diplopia						
- Absent	21 (80.7)	22 (88.0)	13 (50)	21 (84)	15 (58.0)	22 (88)
- Intermittent (morning fatigue)	4 (15.4)	3 (12)	11(42.3)	4 (16)	10 (38.4)	3 (12)
- Constant	1 (3.8)	–	2 (7.6)	–	1 (3.8)	–
Extraocular muscle involvement	2 (7.6)	–	2 (7.6)	1 (4)	4 (15.4)	–
Corneal involvement	–	–	2 (7.6)	–	1 (3.8)	–
Sight loss (Optic Neuropathy)	Not observed					
Evidence of OS in NOSPECS scale						
- mild	26 (100)	25 (100)	19 (73)	24 (96)	17 (65)	23 (92)
- moderate	–	–	7 (27)	1 (4)	9 (35)	2 (8)
- severe	–	–	–	–	–	–
CAS	**2.8±0.2**[1,2]	1.4±0.2	**4.3±0.3**[1]	2.8±0.3	**4.0±0.5**[1,2]	2.2±0.2

[1,2]- Statistical significant differences are related to dependencies between data labeled with the same Arabic numerals. [1]$p<0.05$, [2]$p<0.005$,(Mann-Whitney test). Numerical variables are given as number (%). *Proptosis data are in millimeters. Only CAS and proptosis are shown as mean±SD.

Abbreviations: 1) Absent (NOSPECS 0); 2) Mild (NOSPECS 1: lid lag/retraction); 3) Moderate (NOSPECS 2–3: periorbital edema/proptosis); or 4) Severe (NOSPECS 4–6: eye muscle involvement/corneal involvement/sight loss). We elected to categorize the severity of eye disease according to these criteria because this study was performed throughout a time period of 1 yr and NOSPECS scores were documented in all patients; CAS–Clinical Activity Scale: eye pain, ocular or eyelid erythema, conjunctival or eyelid edema, and worsening of proptosis.

It is estimated that approximately 20% of patients develop clinical GO after RIT (Acharya *et al.* 2008), but those findings are not well documented due to variations in classifying cases as clinical (Marcocci *et al.* 1999; Rasmussen *et al.* 2000). According to Vannucchi (Vannucchi *et al.* 2009), there is no "predictive model" to categorize patients that may be at a higher risk of developing initial or relapsing GO. Currently considered risk factors used to predict GO include: cigarette smoking (associations between GO and ETS) (Bartalena *et al.* 2002; Bartalena *et al.* 1998b, Hagg *et al.* 1987; Hegedus *et al.* 2004; Kendler *et al.* 1993; O'Hare & Georghegan 1993; Tomer & Davies 2003; Utiger 1998; Vestergaard 2002; Wiersinga & Bartalena 2002; Winsa *et al.* 1993; Prummel *et al.* 2004; Prummel & Wiersinga 1993; Bartalena 2011), high serum TSHR-Ab concentration (Rasmussen *et al.* 2000; Laurberg *et al.* 2008), presence of GO at the time of RAI (Laurberg *et al.* 2008), and fT3 concentration at time of treatment initiation (Sisson *et al.* 2008).

Concerning our report a recent study puplished by Vannunchi (Vannunchi *et al.* 2009) revealed other existing risk factors for the development of GO: the duration of Graves' disease (>26 months) and recency of GO development (<18 months), being most significant, and to a lesser extent: age (>52 years) and gender (male).

It is considered that the intensification of GO is initiated by 131I promoted autoantigen interacting with orbital tissues, with a longer course from initial clinical diagnosis of Graves' disease, that is leading to lower autoantigen release after RAI. With duration from diagnosis the infiltration of lymphocytes in both the orbits and thyroid is milder (Armengol *et al.* 2001; Aust *et al.* 2004; Ho *et al.* 2007; Weetman *et al.* 1989). An increased risk of developing GO after RAI was noted in patients with active or reactivated hyperthyroidism (Salvi *et al.* 2009; Prummel *et al.* 1990a; Prummel *et al.* 1990b), while at the same time an increased TSHR-Ab concentration was associated with GO activity, rising

even after RIT (Eckstein *et al.* 2006; Laurberg *et al.* 2008).

Bartalena (Bartalena *et al.* 2002, Bartalena et al.1998; 1998a) was the first who reported that oGCS offered a prophylaxis against GO before and after 131I therapy. In our opinion, the high dose ^{131}I therapy [814 mBq [22 mCi], followed by a complementary therapy with oGCS, is an effective treatment for non-smoking patients (level of urine cotinine was 1.0±1.2 ng/mL) with Graves' hyperthyroidism and does not increase the exophthalmos.

Although the expression "ablative dose" is often used in the literature instead of high dose, in our opinion it should be kept exclusively for thyroid cancer treatment.

The present study concurs with other results in that smoking clearly increases GO, especially after RIT, even with prophylactic of oGCS. Our work is important because nobody until now described the association of cotinine with ophthalmopathy in such a broad sense. The current study is the first to include urinary cotinine in retrospective studies of cigarette smokers and non smokers in Graves' sufferers with mild ophthalmopathy treated with RIT. The urine cotinine concentration is the "gold standard" to measure total nicotine exposure regardless of direct cigarette smoking or environmental exposure (ETS) (Langone *et al.* 1988). The urine cotinine test is very sensitive and manifested by the fact that even non-cigarette smokers showed this marker of ETS. Hence, the presence of urinary cotinine from cigarette exposure, imply even non-smokers at an increased risk of GO. The accepted standard biochemical test for nicotine absorption is urine cotinine concentration which is specific to cigarette smoke and has half life of 20 hours (Florek *et al.* 2006). The measure of cotinine in body fluids, regardless of its usefulness, has its limits due to the costs involved and requirements for specialized laboratory equipment (Mascola *et al.* 1998).

Additionally, the study is presenting the intensity of eye changes associated with RIT-treated patients, in regard of nicotine exposure (Table 3). In particular, the CAS scale is illustrated with the observed intensification of eyelid edema and worsening of proptosis, in smokers even though these changes were not statistically significant, that is proving the benefit of the oGCS prophylaxis. Unfortunately, TSHR-Ab concentration was significantly higher in the smoking group at the beginning and end of our observations. The changes were statistically significant for both groups, which clearly demonstrate that RIT intensifies ophthalmopathy in smokers.

In conclusion: 1) ablative RIT dose with prophylactic oral prednisone is a safe treatment in both smokers and non-smokers with mild GO; 2) The *post hoc* analysis showed that urinary level of cotinine can be very helpful in the assessment of exacerbation of ophthalmological clinical symptoms before and after RIT particularly in smokers.

REFERENCES

1 Acharya SH, Avenell A, Philip S, Burr J, Bevan JS, Abraham P (2008). Radioiodine therapy for Graves' disease and the effect on ophthalmopathy—a systematic review. Clin Endocrinol. **69**(6): 943–950.

2 Armengol MP, Juan M, Lucas-Martín A, Fernández-Figueras MT, Jaraquemada D, Gallart T et al., Pujol-Borrell R (2001). Thyroid autoimmune disease: demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. Am J Pathol. **159**(3): 861–873.

3 Aust G, Sittig D, Becherer L, Anderegg U, Schütz A, Lamesch P, Schmücking E (2004). The role of CXCR5 and its ligand CXCL13 in the compartmentalization of lymphocytes in thyroids affected by autoimmune thyroid diseases. Eur J Endocrinol. **150**(2): 225–234.

4 Bartalena L (2011). The dilemma of how to manage Graves' hyperthyroidism in patients with associated orbitopathy. J Clin Endocrinol Metab. **96**(3): 592–599.

5 Bartalena L, Baldeschi L, Dickinson A, Eckstein A, Kendall-Taylor P, Marcocci C *et al.* (2008). Consensus statement of the European Group on Graves' orbitopathy (EUGOGO) on management of GO. Eur J Endocrinol. **158**(3): 273–285.

6 Bartalena L, Marcocci C, Bogazzi F, Manetti L, Tanda ML, Dell'Unto E *et al.* (1998a). Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. N Engl J Med. **338**(2): 73–78.

7 Bartalena L, Marcocci C, Bogazzi F, Panicucci M, Lepri A, Pinchera A (1989). Use of corticosteroids to prevent progression of Graves' ophthalmopathy radioiodine therapy for hyperthyroidism. N Engl J Med. **321**(20): 1349–1352.

8 Bartalena L, Marcocci C, Pinchera A (2002). Graves' ophthalmopathy: a preventable disease? Eur J Endocrinol. **146**(4): 457–461.

9 Bartalena L, Marcocci C, Tanda ML, Manetti L, Dell'Unto E, Bartolomei MP, Nardi M, Martino E, Pinchera A (1998b). Cigarette smoking and treatment outcomes in Graves ophthalmopathy. Ann Intern Med. **129**(8): 632–635.

10 Bartalena L, Tanda ML (2009). Clinical practice. Graves' ophthalmopathy. N Engl J Med. **360**(10): 994–1001.

11 Bonnema SJ, Bartalena L, Toft AD, Hegedüs L (2002). Controversies in radioiodine therapy: relation to ophthalmopathy, the possible radioprotective effect of antithyroid drugs, and use in large goitres. Eur J Endocrinol. **147**(1): 1–11.

12 Cooper DS (1996). Treatment of thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid. 7th ed. Philadelphia: Lippincott-Raven. 713–733.

13 Costagliola S, Morgenthaler NG, Hoermann R, Badenhoop K, Struck J, Freitag D *et al.* (1999). Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. J Clin Endocrinol Metab. **84**(1): 90–97.

14 Czarnywojtek A, Waligórska-Stachura J, Szczepanek E, Zgorzalewicz-Stachowiak M, Bereszyńska I, Kurdybacha P *et al.* (2012). A rare case of Interferon-alpha-Induced Hyperthyroidism in patients with a chronic hepatitis C with granulocytopenia and transaminasemia treated successfully with radioiodine. Neuro Endocrinol Lett. **33**(3): 268–272.

15 Eckstein AK, Plicht M, Lax H, Neuhäuser M, Mann K, Lederbogen S *et al.* (2006). Thyrotropin receptor autoantibodies are independent risk factors for Graves**'** ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab. **91**(9): 3464–3470.

16 Florek E, Breborowicz GH, Lechowicz W, Wachowiak A, Basior A, Wolna M *et al.* (2006). Cotinine in urine of mother and their newborn and in cord serum and placenta as a biomarker of foetal exposure to tobacco smoke. Przegl Lek. **63**(10): 900–906. In Polish.

17 Gorman CA (1995). Therapeutic controversies. Radioiodine therapy does not aggravate Graves' ophthalmopathy. J Clin Endocrinol Metab. **80**(2): 340–342.

18 Hegedus L, Brix TH, Vestergaard P (2004). Relationship between cigarette smoking and Graves' ophthalmopathy. J Endocrinol Invest. **27**(3): 265–271.

19 Ho VH, Chevez-Barrios P, Jorgensen JL, Silkiss RZ, Esmaeli B (2007). Receptor expression in orbital inflammatory syndromes and implications for targeted therapy. Tissue Antigens. **70**(2): 105–109.
20 Kendler DL, Lippa J, Rootman J (1993). The initial clinical characteristics of Graves' orbitopathy vary with age and sex. Arch. Ophthalmol. **111**(2): 197–201.
21 Knudsen N, Bols B, Bulow I, Jorgensen T, Perrild H *et al.* (1999). Validation of ultrasonography of the thyroid gland for epidemiological purposes. Thyroid. **9**(11): 1069–1074.
22 Langone JJ, Cook G, Bjercke RJ, Lifschitz MH (1988). Monoclonal antibody ELISAfor cotinine in saliva and urine of active and passive smokers. J Immunol Methods. **114**(1–2): 73–78.
23 Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Tørring O (2008). TSH-receptor autoimmunity in Graves' disease therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. Eur J Endocrinol. **158**(1): 69–75.
24 Marcocci C, Bartalena L, Tanda ML, Manetti L, Dell'Unto E, Mazzi B*et al.* (1999). Graves' ophthalmopathy and 131I therapy. Q J Nucl Med. **43**(4): 307–312.
25 Meberg A, Marstein S (1986). Smoking during pregnancy – effects on the fetal thyroid function. Acta Paediatr Scand. **75**(5): 762–6.
26 Mourits MP, Koornneef L, WiersingaWM, Prummel MF, Berghout A, van der Gaag R (1989). Clinical criteria for the assessment of diseaseactivity in Graves' ophthalmopathy: a novel approach. Br J Ophthalmol. **73**(8): 639–644.
27 Mourits MP, Prummel MF, Wiersinga WM, Koornneef L (1997). Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf). **47**(1): 9–14. Erratum in: Clin Endocrinol (Oxf). **47**: 632.
28 O'Hare JA, Georghegan M (1993). Cigarette smoking as a promoter of Graves' disease. European Journal of Internal Medicine. **4**: 289–292.
29 Oliveira E, Moura EG, Santos-Silva AP, Fagundes AT, Rios AS, Abreu-Villaça Y *et al.* (2009). Short- and long-term effects of maternal nicotine exposure during lactation on body adiposity, lipid profile, and thyroid function of rat offspring. J Endocrinol. **202**(3): 397–405.
30 Prummel MF, Wiersinga WM (1993). Smoking and risk of Graves' disease. JAMA. **269**(4): 479–482.
31 Prummel MF, Strieder T, Wiersinga WM (2004). The environment and autoimmune thyroid diseases. Eur J Endocrinol. **150**(5): 605–18.
32 Prummel MF, Wiersinga WM, Koornneef L (1997). Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf) **47**(1): 9–14.
33 Prummel MF, Wiersinga WM, Mourits MP, Koornneef L, Berghout A, van der Gaag R (1990) Effect of abnormal thyroid function on the severity of Graves' ophthalmopathy. Arch Intern Med. **150**: 1098–1101.
34 Rasmussen AK, Nygaard B, Feldt-Rasmussen U (2000). (131)I and thyroid-associated ophthalmopathy. Eur J Endocrinol. **143**(2): 155–160.
35 Salvi M, Vannucchi G, Campi I, Currò N, Simonetta S, Covelli D *et al.* (2009). Rituximab treatment in a patient with severe thyroid-associated ophthalmopathy: effects on orbital lymphocytic infiltrates. Clin Immunol. **131**(2): 360–365.
36 Singer PA, Cooper DS, Levy EG, Ladenson PW, Braverman LE, Daniels G *et al.* (1995). Treatment guidelines for patients with hyperthyroidism and hypothyroidism. JAMA. **273**(10): 808–812.
37 Sisson JC, Schipper MJ, Nelson CC, Freitas JE, Frueh BR (2008). Radioiodine therapy and Graves' ophthalmopathy. J Nucl Med. **49**(6): 923–930.
38 Tallstedt L, Lundell G, Tørring O, Wallin G, Ljunggren JG, Blomgren H et al., Taube A (1992). Occurrence of ophthalmopathy treatment for Graves' hyperthyroidism. The Thyroid Study Group. N Engl J Med. **326**(26): 1733–1738.
39 Tomer Y, Davies TF (2003). Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. Endocr Rev. **24**(5): 694–717.
40 Utiger RD (1998). Effects of smoking on thyroid function. Eur J Endocrinol. **138**(4): 368–369.
41 Vannucchi G, Campi I, Covelli D, Dazzi D, Currò N, Simonetta S *et al.* (2009). Graves' orbitopathy activation radioactive iodine therapy with and without steroid prophylaxis. J Clin Endocrinol Metab. **94**(9): 3381–3386.
42 Vestergaard P (2002). Smoking and thyroid disorders – a meta-analysis. Eur J Endocrinol. **146**(2): 153–161.
43 Weetman AP, Cohen S, Gatter KC, Fells P, Shine B (1989). Immunohistochemical analysis of the retrobulbar tissues in Graves' ophthalmopathy. Clin Exp Immunol. **75**(2): 222–227.
44 Werner SC (1977a). Modification of the classification of the eye changes in Graves'disease. Am J Ophthalmol. **83**(5): 725–727.
45 Werner SC (1977b). Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. J Clin Endocrinol Metab. **44**(1): 203–204.
46 Werner SC (1969). Classification of the eye changes of Graves' disease. Am J Ophthalmol. **68**(4): 646–648.
47 Wiersinga WM, Bartalena L (2002). Epidemiology and prevention of Graves' ophthalmopathy. Thyroid. **12**(10): 855–860.
48 Winsa B, Mandahl A, Karlsson FA (1993). Graves' disease, endocrine ophthalmopathy and smoking. Acta Endocrinol (Copenh). **128**(2): 156–160.