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## Supplemental Methods

*Design and study population*

In addition to the study visits with saliva collections, data on patient characteristics were obtained from the medical record. These included age, sex, tumor characteristics (TNM stage, histology), prior radioiodine treatments if applicable, co-morbidities, and medications used. Xerostomia-related drugs were defined as oral beta blockers, diuretics or neuropsychiatric drugs (antidepressants, antipsychotics, anxiolytics)*(1)*.

*Treatment and follow-up*

A pre-therapy diagnostic whole body scan (WBS) was performed after administration of 40 or 74 MBq of radioiodine ($^{131}$I) before ablation or repeat therapy, respectively. One week after radioiodine administration, a planar post-therapy WBS was routinely performed, as well as a SPECT/CT scan of the neck. After ablation therapy, patients started with thyroid hormone suppression therapy using liothyronine (T3) or levothyroxine (T4). When patients were disease-free, follow-up started, and levothyroxine treatment was universally applied. In case of high-risk, persistent or recurrent disease, repeat radioiodine treatments were considered.

*Saliva collection*

During the two study visits, (un)stimulated whole and glandular saliva were collected using standardized methods*(2,3)*. Patients were asked not to not eat, drink, chew chewing gum, brush teeth or smoke for at least 60 minutes prior to the saliva collection. Unstimulated whole saliva was collected during five minutes, by regularly

spitting in a container. Thereafter, paraffin-stimulated whole saliva was collected in a similar fashion during five minutes. During the glandular saliva collection, saliva of the left and right parotid, and the submandibular/sublingual glands were separately collected for ten minutes. The salivary glands were stimulated by applying a cottonwool swab with 2% citric acid solution on both the lateral surfaces of the tongue every 30 seconds. For the parotid saliva collection, Lashley cups were positioned in the cheek on both orifices of the parotid gland (Stenson's duct). A negative pressure was applied on the outer chamber for attachment of the cup to the cheek; saliva that accumulated in the inner chamber drained into a tube. Simultaneously, saliva from the submandibular and sublingual glands that pooled in the anterior floor of the mouth (where Wharton's ducts drain), was aspirated using a syringe. The saliva flowrate in milliliter/minute (ml/min) was calculated as the weight of the saliva divided by the collection time in minutes, assuming a specific gravity of saliva of 1.0 g/ml. After collection, saliva was centrifuged, and the supernatant stored at -80 °C.

*Sialochemistry*

Sialochemistry analyses were performed at the end of the study period. Samples were thawed at room temperature, and briefly vortexed and centrifuged. Subsequently, sodium and potassium were quantified using atomic emission spectrometry (Thermo Fisher Scientific, Inc) using 589.0 nm and 766.5 nm wavelengths, respectively. Dilutions of 1:100 and 1:1000 were applied for sodium, and 1:1000 for potassium measurement. Chloride, amylase, and total protein were measured using a Roche Modular analyzer (Roche, Mannheim, Germany). Sialochemistry analyses were dependent upon saliva quantity, as at least 0.1 and 0.2

ml were needed for sodium/ potassium and chloride/ total protein/ amylase quantification, respectively.

*Imaging protocol*

Planar imaging for the pre- and post-therapy WBS was performed at a speed of 8 cm/min (vertex of the skull to the knees, matrix 256x1024, with a 364 keV photopeak and 20% energy window). Planar imaging was performed 24 hours after administration of 40 MBq for ablation planning or 72 hours after administration of 74 MBq radiodiodine for planning of repeat therapy, and 7 days after high-activity radioiodine administration using a dual headed SPECT/(CT) Siemens gammacamera system (Symbia S or T2, Siemens, Knoxville, TN) equipped with high energy collimators. SPECT/CT imaging of the head and neck was performed immediately after the post-therapy WBS using the Siemens Symbia T2. SPECT/CT images were acquired using 64 steps at 25 s per step with a 128 × 128 matrix. The CT scan was acquired using CARE DOSE4D, with parameters 110 kV and 30mAs and 3 mm slices. SPECT data were processed using Siemens Esoft MI Apps with incorporated iterative reconstruction, attenuation correction, scatter correction and resolution recovery software.

On the GE Infinia gammacamera or GE Discovery NMCT670 (GE, Haifa, Israel) gammacameras used in the Isala Clinics, acquisition protocols were largely similar with a few modifications: planar imaging for the pre- and post-therapy WBS was performed using separate spotviews of 10 min each. The CT scan was acquired with parameters 100 kV and 50-250 modulated mAs and reconstructed into 3mm slices.

*Iodine uptake measurement on diagnostic scans*

Iodine uptake in both parotid and submandibular glands was scored semi-quantitatively on the planar pre- and post-therapy WBS. This was performed in consensus reading by two clinicians (AHB and ENKH) using three categories for radioiodine uptake: 1) none or very limited, 2) intermediate or 3) high. Furthermore, radioiodine uptake in the salivary glands was quantified on the post-therapy SPECT/CT scan. An aliquot of (radioiodine) activity was scanned along with the patient for dosimetry measurements. Regions of interest (ROI), representing the anatomic borders of the salivary glands, were drawn using the low-dose CT images, and the number of counts in the ROIs was calculated using Siemens syngo MultiModality Workplace software, VE36A (Siemens, Knoxville, TN). Subsequently, we calculated the radioiodine activity (in Bq and Bq/ml) concentrated in each gland as follows:

The radioiodine activity (in Bq) concentrated in each gland (left/right parotid and submandibular glands) was calculated as:

**A:** Counts per salivary gland (ROI) as measured on SPECT/CT

---------- $*10^6$

**B:** Mean calibration factor of all eligible analyzed patients

With:

**A:** Regions of interest (ROI) representing the anatomic borders of the salivary glands. ROIs were drawn, and the number of counts in the particular ROI was calculated using Siemens syngo MultiModality Workplace software, VE36A (Siemens, Knoxville, TN).

**B:** Mean calibration factor was calculated as the mean of the calibration factors per patient. Calibration factor per patient was calculated as:

$$\frac{\text{Counts of aliquot of radioiodine measured on SPECT/CT}}{\text{Activity of aliquot of radioiodine (in MBq)}}$$

With,

Counts of aliquot of radioiodine activity measured in a standardized volume of 100 $cm^3$ using the same software as mentioned above (see explanation A). And, activity of aliquot of radioiodine calculated as: EXP (- days between SPECT/CT scan and calibration of aliquot * 0,693/8,3) * calibration activity of source (in MBq).

Furthermore, we assessed the concentration of radioiodine in each parotid and submandibular salivary gland (in Bq/ml), by dividing the concentration of radioiodine in each gland (in Bq), by the volume of the gland, that is, the ROI (in ml).

*Statistical analyses*

In case of gland dysfunction at baseline, or when oral anatomy did not allow glandular saliva collection, the patient was excluded for analysis with regard to the particular flow-rate. Baseline gland function should be >0.1 and >0.2 ml/min for unstimulated or stimulated whole saliva, respectively (otherwise, the baseline flow rate was already too low to reliably show any effect of radioiodine treatment). With regard to stimulated parotid and submandibular glands, flow rates should be >0.05 and >0.1 ml/min to be eligible for analysis, respectively.

Differences between patients who did, or did not have a decrease of at least 50% in stimulated whole saliva flow rate were tested using chi-squared test, t-test

and non-parametric tests, as appropriate. The 50% decrease was chosen since this decrease indicates salivary gland dysfunction*(4)*, and is generally accompanied by xerostomia*(5)*. The patient characteristics age, sex, TNM stage, tumor histology, and cumulative radioiodine activity were evaluated.

## Supplemental Results

**Supplemental Table 1**. Normal reference metrics of saliva flow rate*(5-9)*.

| Flow rate (ml/min) / Parameter | Mean | SD | Lower limit reference range | Upper limit reference range | Range |
|---|---|---|---|---|---|
| Unstimulated whole saliva | 0.30 | 0.24 | 0.10 | 0.50 | 0 - 1.71 |
| Stimulated whole saliva | 2.26 | 1.00 | 0.20 | 3.0 | 0.9 - 6.9 |
| Stimulated parotid saliva§ | 0.33 | 0.11 | 0.11 | 0.55 | 0.10 – 1.30 |
| Stimulated submandibular saliva*§ | 0.31 | 0.12 | 0.07 | 0.55 | 0.07 – 1.22 |

* Since we collected mixed submandibular saliva from both glands, the lower limit reference range should be 0.14 ml/min for both glands § reference range is defined as mean±2*SD. ref. range = reference range.

**Supplemental Table 2**. Outcome xerostomia inventory

| **Ablation therapy** (n=56) | **Ablation therapy** (n=56) | | | **Repeat therapy** (n=11) | | |
|---|---|---|---|---|---|---|
| | **Before ablation** | **After ablation** | p-value | **Before therapy** | **After therapy** | p-value |
| XI total score | 17 [15–24] | 20 [16–27] | .064 | 25 [19–31] | 26 [16–30] | .878 |
| Score per item, *median [IQR]* | | | | | | |
| 1. I sip liquids to aid in swallowing food | 1 [1–2] | 1 [1–2] | .750 | 2 [1–3] | 3 [1–3] | .748 |
| 2. My mouth feels dry when eating a meal | 1 [1–2] | 1 [1–2] | .263 | 2 [2–3] | 3 [1–3] | .739 |
| 3. I get up at night to drink | 2 [1–3] | 2 [1–3] | .386 | 1 [1–2] | 1 [1–2] | .480 |
| 4. My mouth feels dry | 2 [1–3] | 2 [2–3] | **.001*** | 3 [1–4] | 3 [1–4] | .180 |
| 5. I have difficulty in eating dry foods | 1 [1–2] | 2 [1–2] | .142 | 2 [1–3] | 2 [1–4] | .564 |
| 6. I suck sweets or lollies to relieve dry mouth | 1 [1–2] | 1 [1–2] | .412 | 2 [1–3] | 2 [1–3] | .408 |
| 7. I have difficulties swallowing certain foods | 1 [1–2] | 1 [1–2] | .514 | 2 [1–3] | 2 [1–3] | .317 |
| 8. The skin of my face feels dry | 2 [1–3] | 2 [1–3] | .181 | 2 [1–3] | 2 [1–3] | .722 |
| 9. My eyes feel dry | 2 [1–3] | 2 [1–3] | .857 | 2 [1–3] | 2 [1–3] | .891 |
| 10. My lips feel dry | 3 [1–3] | 3 [2–3] | .583 | 3 [1–4] | 2 [2–3] | .739 |
| 11. The inside of my nose feels dry | 1 [1–3] | 1 [1–3] | .698 | 2 [1–3] | 2 [1–3] | .317 |

Scores of the xerostomia inventory per item: 1=never, 2=hardly ever, 3=occasionally, 4=frequently, 5=always.

* remains significant after Bonferroni correction for multiple testing (Bonferroni-corrected alpha for significance = .005)

**Supplemental Table 3**. Correlation between semi-quantitatively assessed radioiodine uptake in salivary glands on the pre- and post-therapy WBS, and percentage change in saliva flow rate of the particular gland(s) post-radioiodine treatment, as compared to pre-treatment.

| ***Semi-quantitative uptake*** | | **Nr of patients evaluated*** | **Correlation coefficient** | **p-value** |
|---|---|---|---|---|
| **Pre-therapy WBS correlations** | | | | |
| **$^{131}$I uptake in glands** | **Saliva flow-rate** | | | |
| All 4 glands | Unstimulated whole saliva | 49 | .169 | .246 |
| All 4 glands | Paraffin-stim. whole saliva | 55 | -.102 | .459 |
| Parotid glands | Acid-stim. parotids combined | 33 | -.092 | .609 |
| Submandibular glands | Acid-stim. SM combined | 40 | .111 | .494 |
| **Post-therapy WBS correlations** | | | | |
| **$^{131}$I uptake in glands** | **Saliva flow-rate** | | | |
| All 4 glands | Unstimulated whole saliva | 50 | -.075 | .605 |
| All 4 glands | Paraffin-stim. whole saliva | 55 | -.102 | .457 |
| Parotid glands | Acid-stim. parotids combined | 32 | .177 | .332 |
| Submandibular glands | Acid-stim. SM combined | 39 | .101 | .541 |

In case a salivary gland (for example left parotid) was not evaluable due to high radiation of surrounding tissue, the uptake of the other gland (in this example right parotid) was used to calculate the uptake of parotid glands combined, or uptake in all 4 glands. SM = submandibular glands. All 4 glands indicate the left and right parotid and submandibular glands.

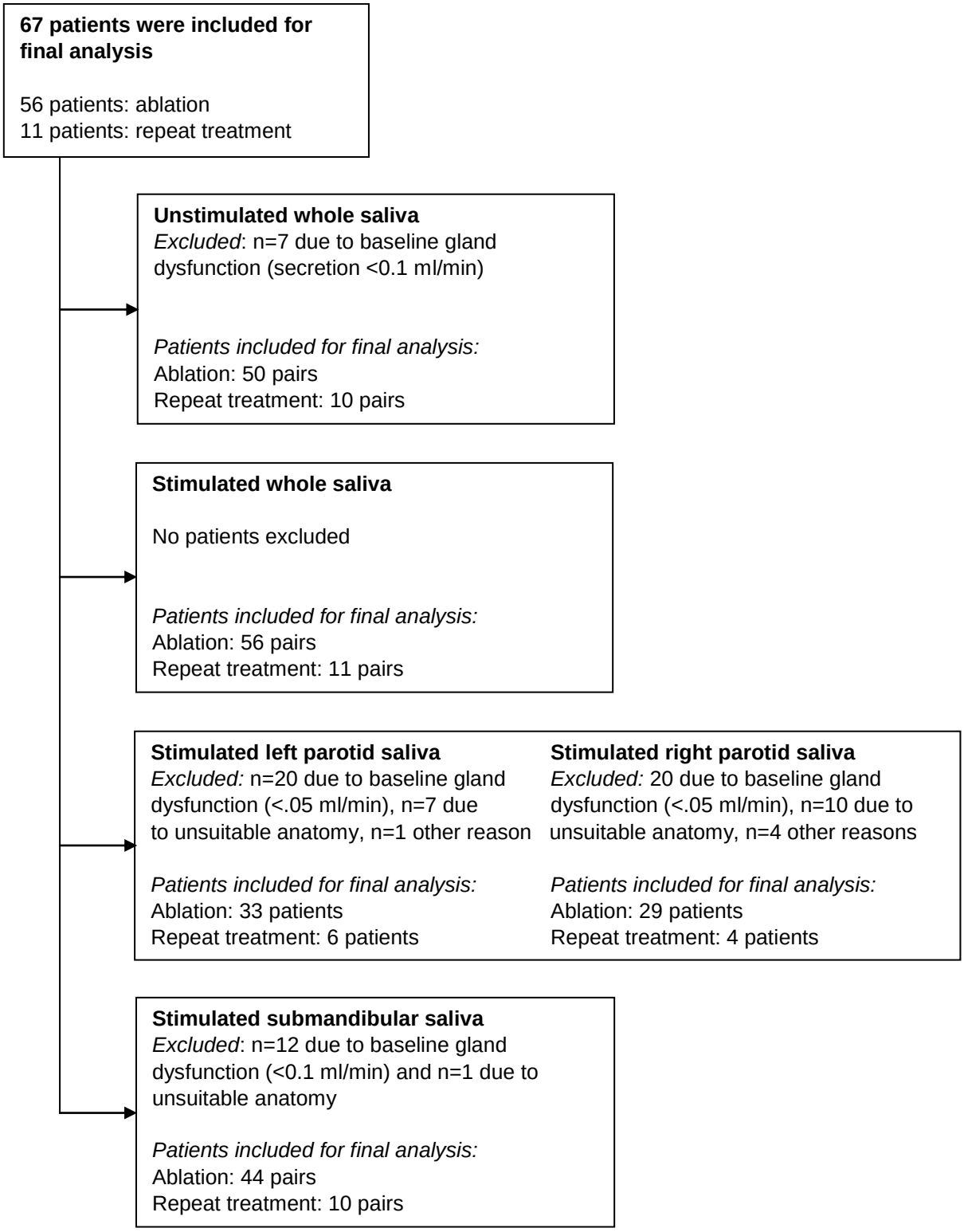
* Patients could not be evaluated when excluded for a particular flow rate analysis (Supplemental Figure 1), in case of non-evaluable uptake of radioiodine in salivary glands due to high radiation levels of surrounding tissue, or when scan has not been performed (in case of pre-therapy WBS). These patients underwent ablation therapy. Data of patients that underwent repeat treatment are not shown.

**Supplemental Table 4.** Correlation between quantitatively assessed radioiodine uptake in salivary glands on the post therapy SPECT/CT scan (represented as Bq and Bq/ml), and percentage change in saliva flow rate of the particular gland(s) post-radioiodine treatment compared to pre-treatment.

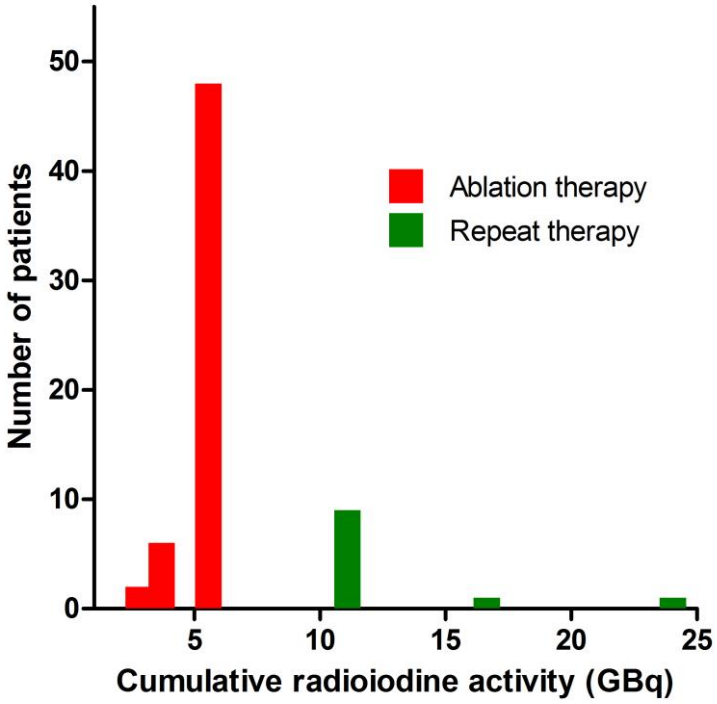
| Salivary glands | Saliva flow rate | Nr of patients evaluated* | Correlation coefficient | p-value |
|---|---|---|---|---|
| All 4 glands | Unstimulated whole saliva | | | |
| Activity $^{131}$I (Bq) | | 41 | -.134 | .404 |
| Concentration $^{131}$I (Bq/ml) | | 41 | -.201 | .209 |
| All 4 glands | Paraffin-stim. whole saliva | | | |
| Activity $^{131}$I (Bq) | | 45 | -.105 | .492 |
| Concentration $^{131}$I (Bq/ml) | | 45 | -.097 | .528 |
| Parotid glands | Acid-stim. parotid saliva | | | |
| Activity $^{131}$I (Bq) | | 25 | -.103 | .624 |
| Concentration $^{131}$I (Bq/ml) | | 25 | -.207 | .321 |
| Submandibular glands | Acid-stim. submandibular saliva | | | |
| Activity $^{131}$I (Bq) | | 33 | -.113 | .531 |
| Concentration $^{131}$I (Bq/ml) | | 33 | -.071 | .695 |

All 4 glands indicate the left and right parotid and submandibular glands.

* Patients could not be evaluated when excluded for a particular flow rate analysis (Supplemental Figure 1), in case of non-evaluable uptake of radioiodine in salivary glands due to high radiation levels of surrounding tissue, or in case of missing scan data. These patients underwent ablation therapy. Data of patients that underwent repeat treatment are not shown.

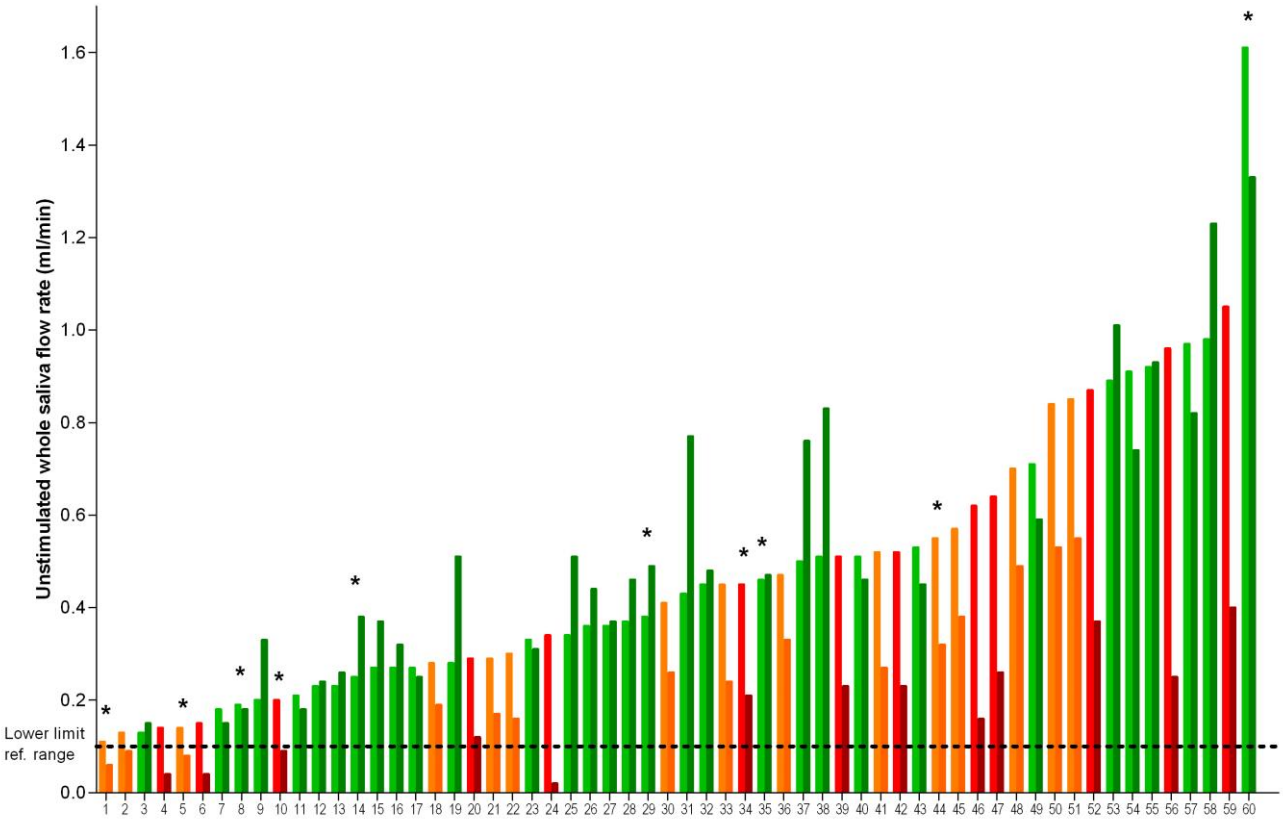



**Supplemental Figure 1.** We only included patients for analyses when a paired measurement was available (i.e., when both pre-and post-treatment measurements were available of a particular gland in an individual). In this figure an overview is presented of the numbers of patients of whom a paired flow rate measurement was available. Furthermore, reasons for exclusion are given (baseline gland dysfunction, an unsuitable intra-oral anatomy for Lashley cup placement, or other reasons).

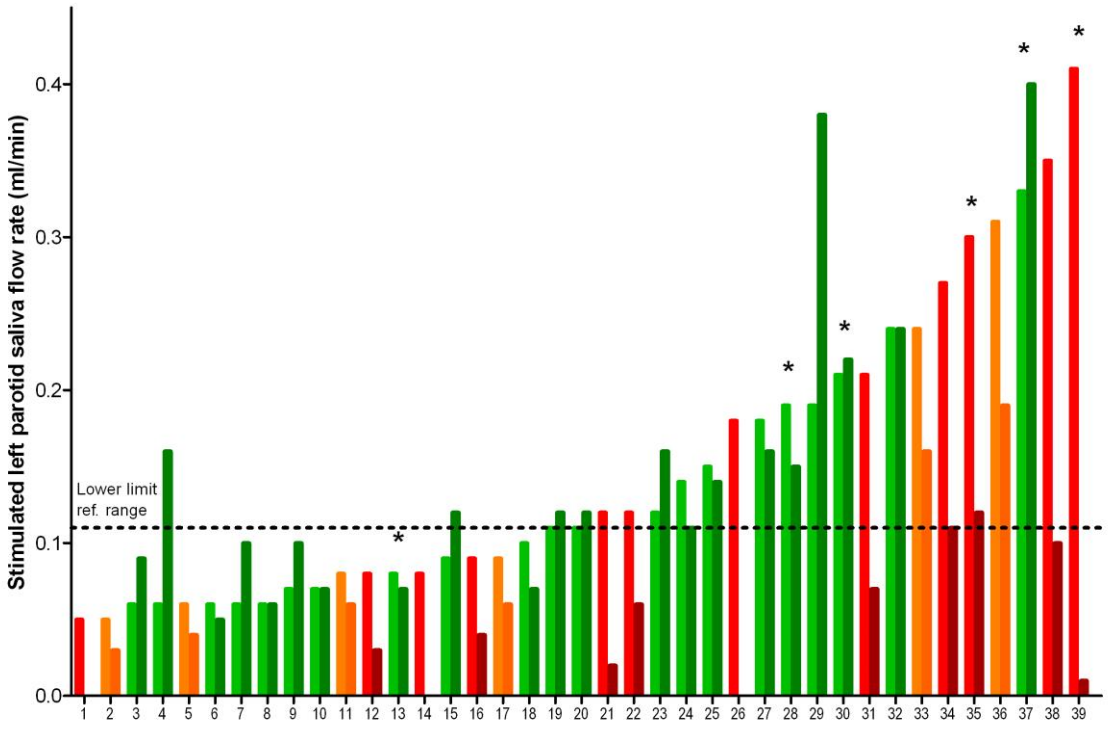



**Supplemental Figure 2.** Overview of administered cumulative radioiodine activities.

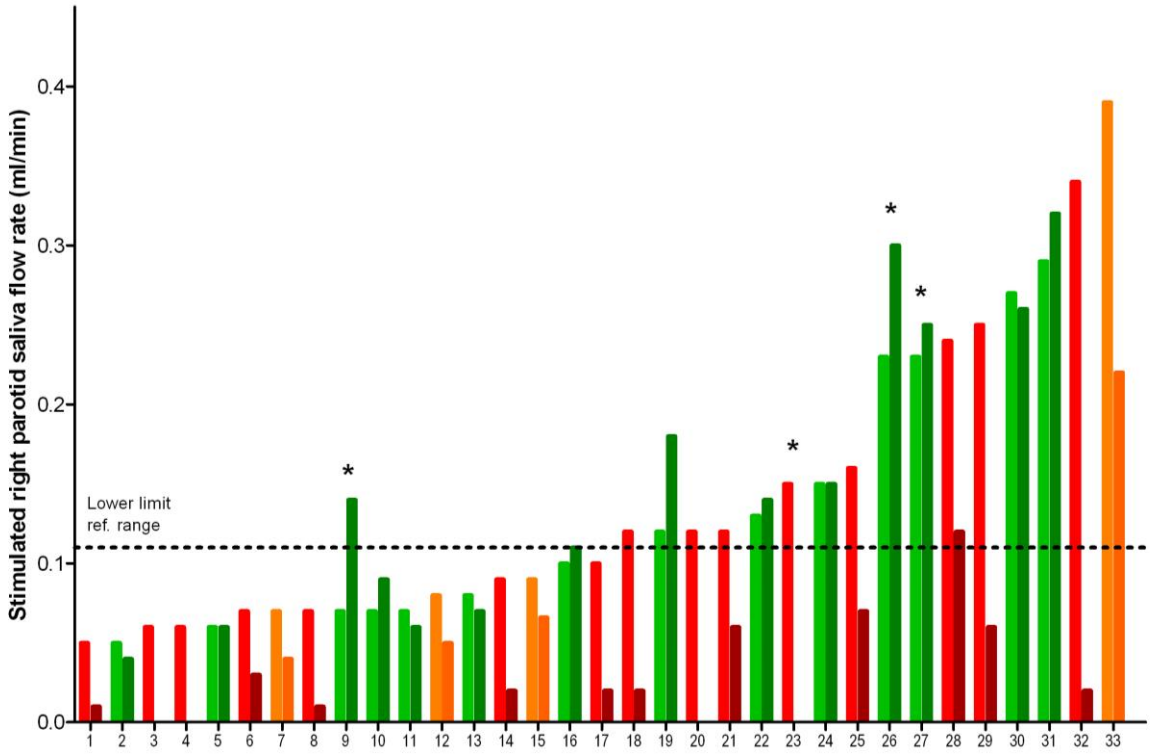
**A.**


Unstimulated whole saliva flow rate (ml/min)
Lower limit ref. range

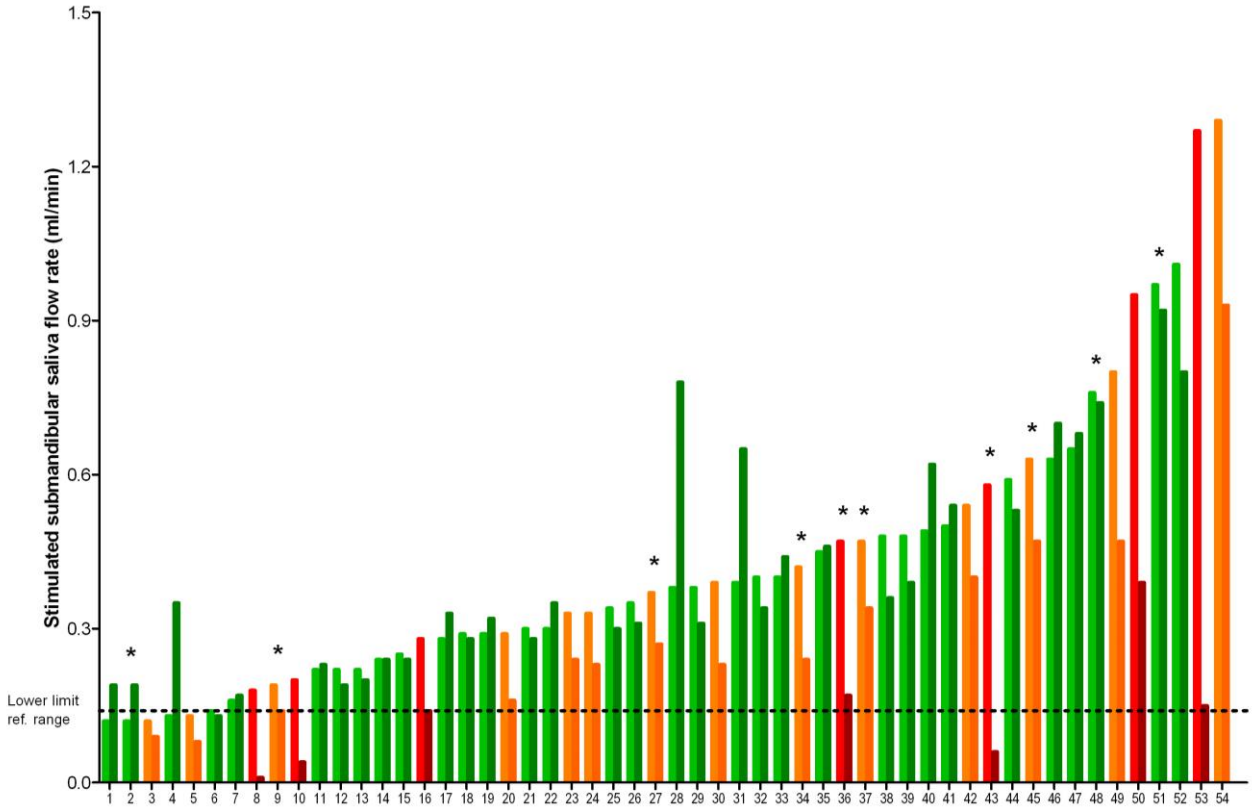
**B.**


Stimulated left parotid saliva flow rate (ml/min)
Lower limit ref. range

**C.**

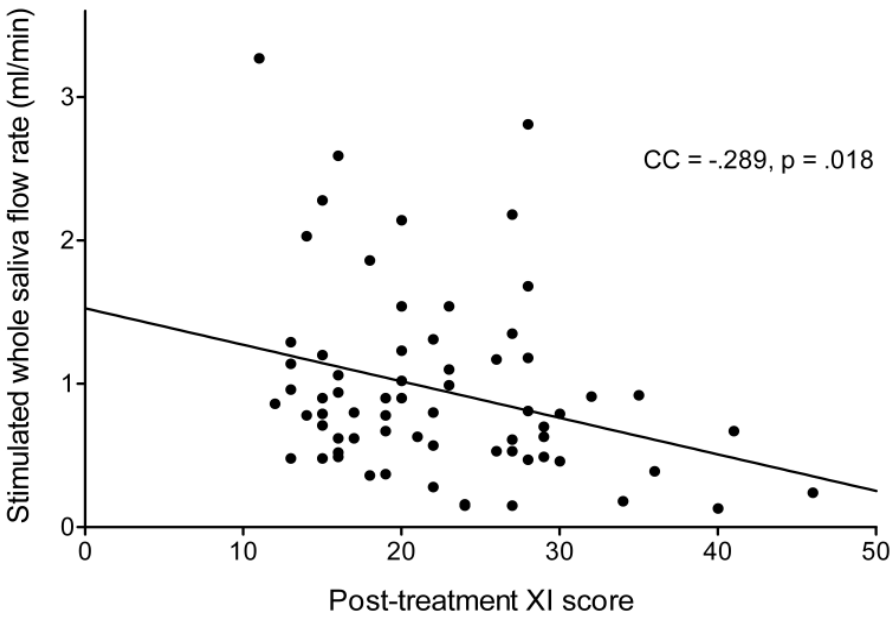



**D.**




**Supplemental Figure 3.** Displayed are changes in unstimulated whole saliva (A), and stimulated left parotid (B), right parotid (C), and submandibular (D) flow rates for each study subject with available

paired flow rate measurements, ordered by baseline flow rate. The lighter and paired darker bars represent flow rates before and after radioiodine treatment, respectively. The flow rate changes are color coded – green bars for subjects with less than 25% decrease, orange for 25-50% decrease, and red for > 50% decrease in the saliva flow rates. * Over the bars identifies patients with repeat treatment.




**Supplemental Figure 4.** Correlation between post-therapy stimulated whole saliva flow rate and xerostomia inventory score.

**Supplemental references**

1. Villa A, Wolff A, Aframian D, et al. World workshop on oral medicine VI: A systematic review of medication-induced salivary gland dysfunction: Prevalence, diagnosis, and treatment. Clin Oral Investig. 2015; 19:1563-1580.

2. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci. 1993; 694:72-77.

3. Kalk WW, Vissink A, Spijkervet FK, Bootsma H, Kallenberg CG, Nieuw Amerongen AV. Sialometry and sialochemistry: Diagnostic tools for sjogren's syndrome. Ann Rheum Dis. 2001; 60:1110-1116.

4. Ghezzi EM, Lange LA, Ship JA. Determination of variation of stimulated salivary flow rates. J Dent Res. 2000; 79:1874-1878.

5. Ship JA, Fox PC, Baum BJ. How much saliva is enough? 'normal' function defined. J Am Dent Assoc. 1991; 122:63-69.

6. Ghezzi EM, Lange LA, Ship JA. Determination of variation of stimulated salivary flow rates. J. Dent. Res. 2000; 79:1874-1878

7. Bergdahl M. Salivary flow and oral complaints in adult dental patient. Community Dent. Oral Epidemiol. 2000; 28:59-66.

8. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J. Prosthet. Dent. 2001; 85:162-169

9. Veerman ECI, Vissink A. *Speeksel en speekselklieren. Betekenis voor de mondgezondheid*. Houten, The Netherlands: Bohn Stafleu van Loghum; 2014:23-34. (Dutch)