**2.2. Treatment protocol**

The CT images were exported to TPS for contouring. The Clinical Target Volume (CTV) was defined following the limits of the ring radiopaque marker placed clinically by the radiation oncologist in CT simulation. The CTV definition is clinical, based on clinical recommendations, and follows our clinical institutional protocol. Breast CTV was divided into 3 sub-volumes: GTV-SIB (Gross Target Volume - Simultaneous Integrated Boost), proximal CTV and distal CTV, according to Zunino et al. [16]. The OARs contoured by a dosimetrist were the spinal cord, ipsilateral lung, contralateral lung, contralateral breast, esophagus, and left cardiac region, as shown in Figure 1-d. The left cardiac region (LCR) is considered as the left anterior descending artery (LAD) and the heart left ventricle. Our institution focused on the protection of this region instead of the heart because LCR has a direct clinical effect on cardiovascular diseases due to relevant cardiac toxicities [18-20]. The radiation-induced cardiovascular disease encompasses direct damage to the coronary arteries, fibrosis of the pericardium and myocardium, microvascular damage, and valvular stenosis [21-23].

The CTVs were uniformly expanded 5 mm in all directions to create PTVs corresponding to different dose levels to account for the uncertainty of the patient's daily setup and the patient movement. The PTVs were identified according to the nomenclature described by AAPM report TG-263 [24]. The union of the different PTVs was created and named as zPTV\_Total! The PTVs were cropped 4 mm inside to the body for target evaluation in FIF. The PTVs were described by Nicolini et al. [17] for target evaluation in VMAT.

The clinical institutional breast treatment planning protocol included breast irradiation in 20 fractions with 3 dose levels. The dose prescription for GTV-SIB was 5600 cGy, proximal CTV 4600 cGy and distal CTV 4300 cGy.

For FIF and VMAT plans, the same dose-volume constraints were followed, as described in Table 1. In the case of planning treatment with FIF, first the dose differentiation was taken into account by the use of subfields (maintaining homogeneous dose) and subsequently the restriction to the homolateral lung dose was considered. The treatment plans are comparable due to both plans (FIF and VMAT) follow the dose normalization to zPTV\_High\_5600!: D95% to 5320 cGy (95% of 5600 cGy). The D95% must be similar up to 3% for the zPTV\_Mid\_4600! and zPTV\_Low\_4300! The V20 Gy is lower than 10% for the homolateral lung.

**2.4. FIF treatment plan**

FIF is a forward plan based on achieving the homogeneous dose to the PTV. The plan was based on an adaptation of the FIF technique proposed by Kestin et al. [10]. An isotropic margin of 4 mm was used between the PTVs and the MLC to define the field shape to account for the beam penumbra. The isocenter was placed on the PTV as shown in Figure 2-a. This was selected to provide more anatomical information for image-guided verification. This isocenter selection is related to a higher angular difference between the tangential fields.

For the zPTV\_Low\_4300! the field angle was optimized manually to minimize the beam divergence along the posterior beam edge to reduce irradiation of the LCR and the homolateral lung. 2 cm of air “flash” was used. The tangential beams were aligned such that the medial and lateral markers placed at the time of CT scan are superposed. Minor adjustments of gantry angle were allowed to avoid contralateral breast. The dose was calculated with these open tangent fields and the weight of each one was set to 50% (segment 1).

To improve dose homogeneity (between 95% and 107% of the prescribed dose) each field was duplicated twice. Dose objects volumes corresponding to 108% and 112% of the dose were created and used for additional segments. Using beam eye view, the MLC was accommodated to block the regions with a dose superior to 112% (segment 2) and superior to 108% (segment 3). Segment 1 (internal and external fields) was weighted to deliver approximately 86% of the tangential dose. Segment 2 (internal and external fields) was weighted 8% and segment 3 (internal and external fields) was weighted 6%.

To achieve the dose in the zPTV\_Mid\_4600! a fourth segment (segment 4) angle-equivalent to segment 1. The MLC was used to block the 4600 cGy region. The zPTV\_High\_5600! was irradiated using the same isocenter with two oblique fields (usually with two segments) and a direct beam. Special care was taken while determining the oblique field angles to protect the contralateral breast. The direct beam was typically weighted at 50% and each tangent field at 25%. The use of oblique and direct beams is used only for zPTV\_High\_5600! The contribution of the beams to zPTV\_Total! is less and it does not affect the lung dose, thus it reduces the probability of breast fibrosis.

All beam (and segment) weights were optimized manually to increase the homogeneity across the PTVs and their dose differentiation. The dose distribution obtained through the fields used in the FIF technique of breast treatment plan is shown in Figure 2-a.

**2.5. VMAT treatment plan**

VMAT is an inverse plan based on achieving the homogeneous dose to the PTV. It was generated by the use of RapidArc™ (Varian Medical Systems, Palo Alto, CA). The plans consisted of two semi-arcs (clockwise and counterclockwise) of 240º (from 300º to 180º) with complementary 20º collimator angles.

The isocenter was placed at the zPTV\_Total! center of mass. The plan was based on a reported planning strategy by Nicolini et al. [17]. The strategy consisted of the use of duplicated CT image series (modified\_CT and original\_CT) for inverse planning and dose calculation, respectively. Both image sets shared the planning structures. The modified\_CT included two planning structures: The first is a “ring” to reduce the contralateral breast and lung dose. The second is a “surface” to have fluence expansion. As institutional protocol, it was considered a safety margin for target volume coverage and the breast motion (5 mm PTV-margin plus 7 mm to avoid build-up region), the surface structure was created with 12 mm expansion of the body and the PTVs towards the body external direction along the breast whole extension. A density of 1 was assigned to this region.

The CTVs and PTVs of the original\_CT were trimmed 5 mm within the body. The used dose-volume constraints are shown in Table 1. Once the inverse planning achieved the planning objective, the optimized plan was pasted into the original\_CT, dose distribution was calculated and normalized as shown in Figure 2-c. Both plans (FIF and VMAT) were normalized to the zPTV\_High\_5600! dose (Figure 2-b). The plan was considered acceptable if the PTV dose objectives and the constraints for the homolateral lung (Table 1) were achieved.

**2.6. TLD Dosimetry**

A batch of 96 thermoluminescent dosimeters TLD-700 (3.2×3.2×0.9 mm³) manufactured by Bicron- NE Harshaw (USA) was used. TLDs measured dose at an equivalent tissue depth of approximately 1 mm due to their thickness and electron density [25]. Dosimeters were characterized and calibrated in the same treatment beam (6 MV) and read 24 hours after irradiation in the range from 10 to 280 cGy as shown in Figure A.

TLDs were read on the Harshaw 4000™ reader (Thermo Fisher, MA, USA). The protocol used was: preheating at 90°C for 5 s, a heating rate of 5°C/s for 40 s, temperature raising from 90 to 290°C. The GCA-New v3.0 Ciemat software (Ciemat AMS Group, Madrid, Spain) was used for glow curve analysis. The TL signal relation with dose was obtained by peaks 4 and 5 associated respectively with temperatures (193 ± 2) °C and (216 ± 2) °C [25].

**2.7. OSLD nanoDot Dosimetry**

A batch of 10 OSLD nanoDot® (10×10×2 mm³) manufactured by Landauer Inc (Glenwood, USA) was used. The dosimeters were calibrated following AAPM TG-191 recommendations [26]. Dosimeters were read on the MicroStar® reader (Landauer Inc, Glenwood, USA) 72 h after exposure. Each dosimeter was read 5 times and the average measurement () was obtained. The dosimeters were bleached for 24 h using a 40 W halogen bulb. Following the TG-191, the dose at point P is calculated using equation 1.

(1)

Where is the background measurement, is the individual sensitivity factor associated with each dosimeter in the batch. The factor is associated with the depletion process. The factor is associated with the fading process. The factor corresponds to the energy correction. The factor corresponds to the angular correction. The factor allows the conversion of the OSLD measurement to dose. This factor was obtained using a calibration curve. These factors have been studied in greater detail by Viamonte et al. [27].

The measurements for a single calibration curve were obtained in a water phantom, for doses from 30 cGy to 300 cGy. The irradiation conditions were source-surface distance (SSD) 90 cm, depth 10 cm, and 10x10 cm² field size. The curve was generated by the correlation of the OSLD corrected by the corresponding correction factors and the dose measured by the ionization chamber (IC) Farmer NE 2571 following the TRS 398 [28]. The IC effective point corresponded with the OSLD center of the sensitive volume as shown in Figure 3.

The factors , , and were determined before the clinical implementation of dosimetry with OSLD. The irradiation conditions were 6 MV beam, dose 50 cGy, SSD 95 cm, depth 5 cm, and 10x10 cm² field size. The linear dose calibration curve was obtained as shown in Figure A.

**2.8. Treatment and in vivo dosimetry**

The phantom was placed on the treatment couch. Fiducials were located along the breast volume. ExacTrac® version 6.0 system (Brainlab AG, Munchen, Germany) was used to acquire stereotactic X-ray images to define the treatment position by fiducials. Anterior and lateral portal images were additionally used to verify the position. The dosimeters were placed at positions indicated in Figure 2, directly on the surface of the phantom, without bolus, in a similar way to the work of O'Grady et al. [29]. For each detector (TLD and OSLD), two different irradiations using both techniques (VMAT and FIF) were realized. The readings were averaged for each technique and detector. The doses were compared to the prescribed dose at zPTV\_High\_5600! In order to get a good confidence level, it was acquired on three different days, for the phantom, two measurements for each technique at all the locations shown in Figure 2 using TLD and OSLD.

The breast volume for the anthropomorphic phantom calculated by TPS Eclipse version 15.5 (Varian Medical Systems, Palo Alto, CA) was 306.5 cc. It corresponds to a small breast. To verify the skin doses reported in this work to other breast volumes, *in vivo* measurements were performed. The size and volume of breast values ​​were taken by the work of Zunino et al. [16]. The classification considered was: small (160-400 cc), medium (400 - 700 cc), and large (700 - 1100 cc).

In order to have patient data of skin dose measurements for different breast sizes, nine patients were included in this study to have a preliminary approximation of values. The inclusion criterion was to select three patients for each breast size classification. The patients gave their informed consent. Future research on *in vivo* dosimetry will be done. This work focuses on the experimental determination of skin dose in an anthropomorphic phantom.

The *in vivo* dosimetry was done under the supervision of the radiation oncologist responsible of quality and protocols. The clinical control was performed by the radiation oncologist responsible once a week during the 4 weeks of the treatment. In this work, only acute (early) reactions are evaluated, given the time elapsed between the treatment irradiation and the presentation of this study.

The patients were placed on the treatment couch and fiducials were located along breast volume for positioning. ExacTrac® version 6.0 system (Brainlab AG, Munchen, Germany) was used to acquire stereotactic X-ray images to define the treatment position by fiducials and to visualize the bone structures. In addition, anterior and lateral portal images were acquired to verify the treatment position.

TLDs were placed at three different points in the homolateral breast area closest to the zPTV\_High\_5600! The VMAT technique was executed. The TLD readings were normalized to the mean zPTV\_High\_5600! dose for each patient.

**2.9. Patient Specific Quality Assurance**

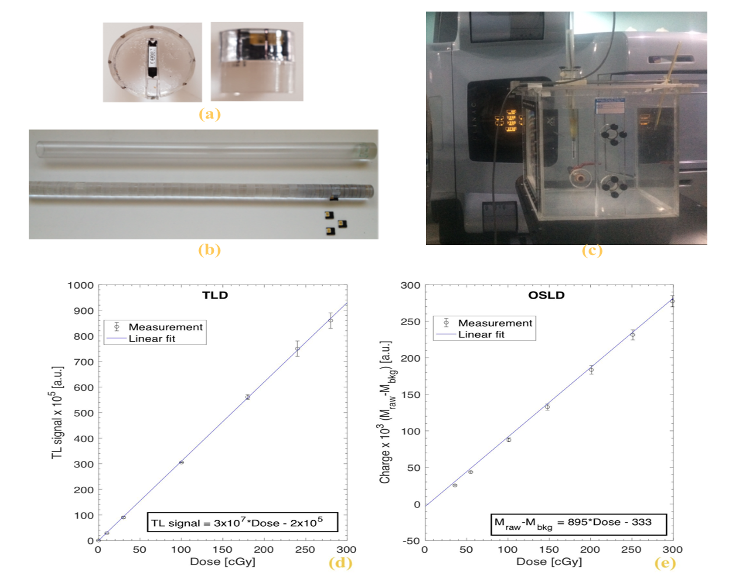
The manual dose calculations (Table 3.A) were performed by RadCalc v5.6 (LifeLine Software, United States) in a single point selected in the PTV. The tolerance level established was 5%.

The patient specific quality assurance for the plans was evaluated by portal dosimetry. The Novalis Tx is equipped with an amorphous silicon EPID (aSi‐1000 EPID). It has a 40 cm × 30 cm detecting surface with a matrix of 1024 × 768 pixels (0.392 mm pixel pitch). It is formed by a 1.0 mm copper layer, 0.34 mm scintillator phosphor (Gd2O2S:Tb), and 1.0 mm glass layer where electronic circuits are immersed.

Predicted images from EPID were obtained by the use of portal dose image prediction (PDIP) algorithm. PDIP is based on the pencil beam convolution algorithm, the TPS theoretical photon fluence matrix, collimator positions, and total monitor units [30, 31]. The PDIP algorithm was configured on Eclipse® (Varian Medical Systems, Palo Alto, CA) software using AIDA test, output factors, and beam intensity profile.

To evaluate the plan quality (Table 3.A), total arcs (120° arcs) were measured for VMAT treatments. Three gamma indices were evaluated (3%/2 mm, 3%/1 mm, and 2%/2 mm). The quality of the plans is valid and reliable as shown in previous work [32].

The quality of the plans was evaluated by the dose-volume histogram parameters such as conformity Paddick index (CI), gradient index (GI), V95%, V2% and Dmean for the three dose levels (Table 3.B).

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**Figure A**. (a) and (b) Polymethylmethacrylate (PMMA) devices to place the optically stimulated luminescence dosimeter (OSLD) in a water phantom. (c) Experimental setup for OSLD absolute dose calibration. Calibration curves for (d) thermoluminescent dosimeter (TLD) and (e) OSLD modified with permission from [8] in 6 MV photon beam.

**Table 3.** **A.** Quality assurance parameters (portal dosimetry and independent monitor units calculation) for the volumetric modulated arc therapy (VMAT) and Field-in-Field (FIF) techniques for phantom and patients. B. Dosimetric parameters: Paddick conformity index (PCI), gradient index (GI), mean dose (Dmean) and V95% for the three dose levels planning target volume (PTV).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **A. Quality assurance parameters** | | | | | | | | | |
| Patient | Arc | Monitor units [MU] | Portal dosimetry | | | | | | Independent MU calculation (RadCalc v6.3) [%] |
| Gamma criterion | | | | | |
| 3%/2mm Thr10% | | 3%/1mm Thr10% | | 2%/2mm Thr10% | |
| 1 | 1 | 377 | 98.5 | | 96.2 | | 94.7 | | 0.3 |
| 2 | 363 | 99.2 | | 96.6 | | 97.8 | |
| 2 | 1 | 302 | 98.4 | | 95.7 | | 93.9 | | 0.0 |
| 2 | 295 | 98.0 | | 94.3 | | 95.4 | |
| 3 | 1 | 342 | 98.3 | | 95.3 | | 95.0 | | 0.0 |
| 2 | 334 | 98.2 | | 94.9 | | 95.8 | |
| 4 | 1 | 278 | 98.3 | | 94.0 | | 92.0 | | 0.2 |
| 2 | 264 | 98.5 | | 95.8 | | 94.6 | |
| 5 | 1 | 345 | 99.4 | | 98.0 | | 98.4 | | 0.5 |
| 2 | 291 | 99.6 | | 98.3 | | 97.8 | |
| 6 | 1 | 355 | 97.2 | | 92.2 | | 94.3 | | 0.2 |
| 2 | 316 | 99.0 | | 96.0 | | 92.0 | |
| 7 | 1 | 145 | 98.3 | | 93.9 | | 96.4 | | 0.0 |
| 2 | 106 | 99.3 | | 96.8 | | 97.0 | |
| 3 | 131 | 99.3 | | 97.1 | | 97.5 | |
| 4 | 123 | 98.8 | | 95.2 | | 96.0 | |
| 8 | 1 | 353 | 99.0 | | 96.2 | | 92.0 | | 2.1 |
| 2 | 347 | 97.3 | | 93.2 | | 93.7 | |
| 9 | 1 | 256 | 98.6 | | 96.1 | | 94.5 | | 2.1 |
| 2 | 255 | 98.6 | | 95.9 | | 90.4 | |
| VMAT phantom | 1 | 115 | 99.5 | | 97.6 | | 97.3 | | -1.00 |
| 2 | 101 | 99.4 | | 97.8 | | 97.2 | |
| FIF phantom |  | 149 |  | |  | |  | | -1.60 |
| **B. Dosimetric parameters for PTV** | | | | | | | | | |
| Patient | PCI  5600 cGy | GI 5600 cGy | V95% 5600 cGy  [%] | V2% 5600 cGy  [%] | Dmean 5600 cGy  [cGy] | V95% 4600 cGy  [%] | Dmean 4600 cGy  [cGy] | V95% 4300 cGy  [%] | Dmean 4300 cGy  [cGy] |
| 1 | 0,763 | 0,896 | 96.6 | 106.0 | 5680 | 97.1 | 4760 | 96.9 | 4410 |
| 2 | 0,719 | 0,904 | 96.0 | 105.1 | 5630 | 98.9 | 4780 | 98.3 | 4450 |
| 3 | 0,882 | 0,912 | 98.0 | 106.2 | 5730 | 98.5 | 4770 | 98.6 | 4410 |
| 4 | 0,76 | 0,85 | 94.2 | 107.3 | 5690 | 96.4 | 4790 | 96.0 | 4450 |
| 5 | 0,839 | 0,896 | 97.8 | 107.6 | 5750 | 99.1 | 4830 | 98.6 | 4460 |
| 6 | 0,827 | 0,889 | 96.7 | 107.1 | 5760 | 100 | 4840 | 99.0 | 4470 |
| 7 | 0,791 | 0,896 | 96.6 | 106.6 | 5720 | 96.7 | 4740 | 97.4 | 4470 |
| 8 | 0,777 | 0,887 | 96.4 | 107.1 | 5680 | 96.7 | 4740 | 96.9 | 4400 |
| 9 | 0,794 | 0,888 | 96.2 | 105.7 | 5680 | 95.4 | 4690 | 94.8 | 4310 |
| VMAT Phantom | 0,789 | 0,883 | 95.0 | 105.7 | 5670 | 94.2 | 4690 | 97.0 | 4370 |
| FIF Phantom | 0,779 | 0,89 | 95.0 | 104.4 | 5610 | 96.6 | 4650 | 96.6 | 4290 |