Setup error of electronic portal image device in IMRT for thoracic tumors and its influence

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Abstract. – OBJECTIVE: The aim of this study was to analyze the setup error of the electronics portal image device (EPID) in intensity-modulated radiation therapy (IMRT) for thoracic tumors and the influence on the outward expansion distance of the target area.

PATIENTS AND METHODS: A total of 202 patients with chest tumors admitted to our hospital from March 2016 to March 2018 were selected as the observation subjects. All patients were treated with IMRT. The original plan was developed based on the SM_{90} obtained by the planning target volume (PTV) expansion method, and the new plan was obtained by shifting the isocenter coordinates of the treatment plan according to the positioning error value obtained by EPID. Before the treatment, EPID scans were performed. The electronic radiation field images (ERIs) were registered with the digitally reconstructed radiographic images (DRRs) generated by the treatment planning system using the image registration software, and the setup errors in the X, Y, and Z directions were further measured. The PTV was developed according to ERIs, and the setup error was simulated to obtain the PTV with 95% internal target volume (ITV) reaching the prescribed dose under the condition of a setup error. The outward expansion distance of clinical target volume (CTV) → PTV was calculated.

RESULTS: In this experiment, the setup errors in X, Y, and Z directions were (-2.00±1.16) mm, (0.16±1.14) mm, and (-0.55±1.16) mm, respectively. The systematic error in the Z direction was -3.00 mm, and the random error in the X direction was 3.30 mm. The CTV → PTV outward expansion distance was set as 7, 8 and 7 mm in the X direction, Y direction and Z direction, respectively. At this time, under the presence of setup error, the PTV D_{95} and the ITV V_{100} in the new plan were (62.23±3.85) Gy and (97.51±1.56) %, respectively, effectively ensuring that 95% ITV of 90% patients reached the prescribed dose. In contrast, the ITV D_{95} and ITV V_{100} in the presence of setup error were (56.11±5.26) Gy and (90.15±3.12) %, respectively, at a CTV → PTV outward expansion distance of 5 mm, which could not guarantee that 95% ITV of 90% patients reached the prescribed dose. In the presence of a setup error, the double-lung 5 Gy irradiation of the total heart volume (V_5), the double-lung 20 Gy irradiation of the total heart volume (V_{20}), mean lung dose (MLD), mean heart dose (MHD), and $D_{1\ cm^3}$ of the new plan increased by 0.89%, 0.29%, 0.13%, 0.06%, and 5 Gy, respectively, compared with the original plan.

CONCLUSIONS: In general, the first treatment of radiotherapy in thoracic tumors mostly has a certain degree of setup error, which is most evident in the X direction. When the CTV → PTV outward expansion distance is set at 7, 8, and 7 mm in the X direction, Y direction, and Z direction, respectively, it can effectively ensure that 95% ITV reach the prescribed dose in 90% of patients in the presence of a setup error. EPID helps to achieve the desired effect of radiotherapy, improves the efficacy of radiotherapy, and reduces the side effects caused by radiotherapy errors.

Key Words:
Electronics portal image device, Intensity-modulated radiation therapy, Setup error, Outward expansion distance of target area.

Introduction

The chest is a high-incidence area of malignant tumors. Frequently occurring tumors in the chest include lung cancer and esophageal cancer, which not only endanger the life and health of patients but also bring heavy medical and economic burdens to families and society. Due to the atypical early symptoms of lung cancer, esophageal cancer, and other chest malignancies, most patients are in the middle and late stage when they are diagnosed, thus missing the optimum opportunity for surgical treatment. For such patients, radiation therapy is the commonly selected strategy[1,2]. Radiation therapy is one of the main methods for the treatment of malignant tumors. The key to its success is to accurately measure and distribute the target area while avoiding radiation damage to adjacent normal tissues and endangered organs. Currently, the commonly used radiation therapy in clinics includes

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intensity-modulated radiation therapy (IMRT) and total body radiation (TBI)[3]. However, in the course of radiotherapy, setup errors, and target changes commonly occur during each treatment. These errors will not only lead to the loss of target irradiation but also transfer the high-dose area to the dangerous organ area, resulting in serious complications or sequelae.

Research[4,5] shows that the accuracy of radiotherapy is closely related to the effect of radiotherapy. With the rapid development of medical equipment, the acceptable dose of the treatment target area gradually increases, and the impact on the surrounding normal tissue is becoming smaller and smaller[6]. Therefore, how to ensure that the treatment position is confirmed before treatment and whether it is consistent with the planned position has become the focus of current research. An electronic portal image device (EPID) is an imaging system that uses a detector matrix to acquire images in the beam direction of the emission field. At present, it is widely used to verify the location of the target area and the shape of the radiation field in radiotherapy patients, helping to check whether the center position, the shape of each irradiation field, the angle of incidence, and the size of the field are correct, which can minimize the radiation error, improve the treatment effect, while reducing the occurrence of adverse reactions, and is well used in bone marrow transplantation and in the treatment of prostate cancer[7,8]. However, there are few related studies on the impact of the outward expansion distance of the target area.

In this study, 202 patients with chest tumors admitted to our hospital from March 2016 to March 2018 were selected as the observation subjects to analyze the setup error of EPID in IMRT for thoracic tumors and the influence on the outward expansion distance of the target area.

Patients and Methods

The present study was a retrospective study. A total of 202 patients with thoracic tumors admitted between March 2016 and March 2018 were selected based on inclusion and exclusion criteria. All patients underwent enhanced CT scanning before treatment with IMRT. Electronic radiation field images of the patients were collected. Radiological parameters were recorded and compared between the original plan and the new plan. The flow diagram of this study is shown in Figure 1.

General Materials

A total of 202 patients with chest tumors admitted to our hospital from March 2016 to March 2018 were selected. Inclusion criteria: (1) All patients were diagnosed with thoracic

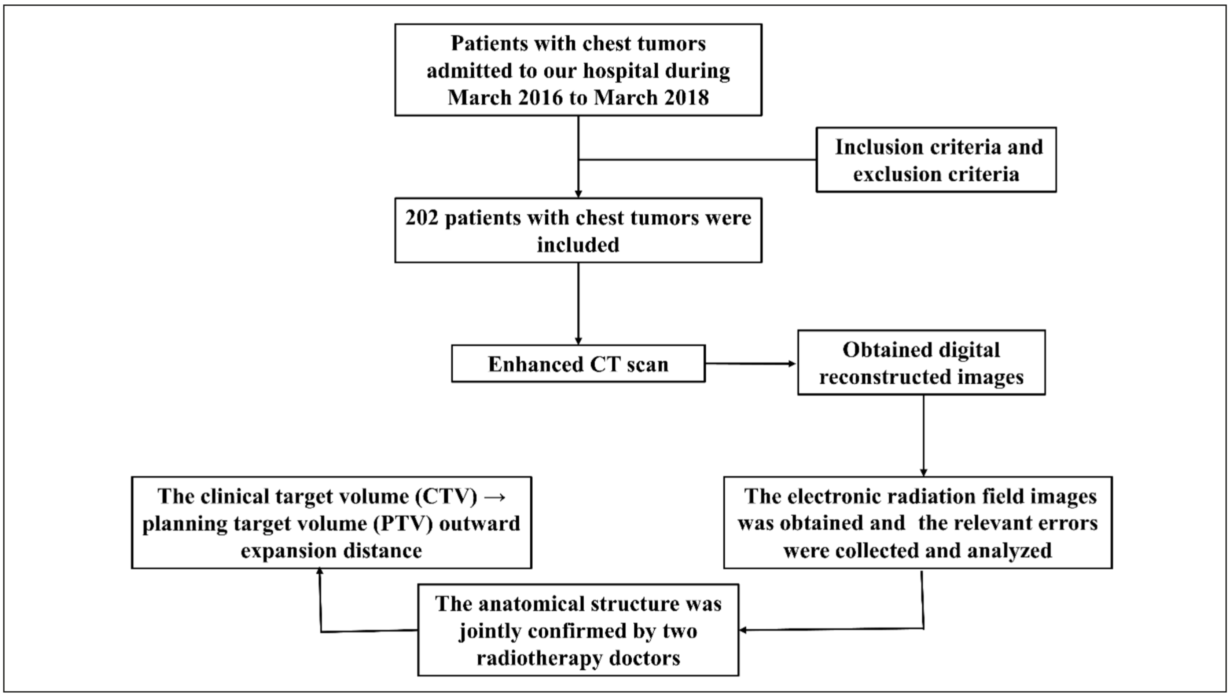

Figure 1. The flow diagram of this study.

tumors by pathology. (2) All patients received IMRT. (3) All patients and their families were informed and had good compliance and signed the informed consent. Exclusion criteria: (1) Patients who had multiple malignancies; (2) patients who had severe dysfunction in important organs; (3) patients who had a severe chronic disease or patients who could not tolerate the treatment. Among the 202 patients, 91 were males and 111 were females, with an average age of (57.95±12.17) years. Among them, there were 28 cases of lung cancer, 95 cases of esophageal cancer, 40 cases of left breast cancer, 38 cases of right breast cancer and 1 case of thymic cancer. The operation of this experiment was approved by the Ethics Committee of our hospital.

Methods

All patients underwent enhanced CT scans before IMRT treatment. The patients were instructed to take the supine position. The head was held with both hands, and the position was fixed with a thermoplastic membrane. The reference level was determined under the conventional simulator, and the marker pen drew the corresponding horizontal and vertical marking lines, according to the three-dimensional laser light. Enhanced CT scanning using Siemens SOMATOM Force dual-source CT (Siemens AG, Berlin, Germany) was performed on the patient while they maintained calm breathing. The scanning range was from the cricothyroid membrane to the lower edge of the diaphragm, with the layer spacing and thickness of both 5 mm. The scanned images were transmitted to the CMS FOCUS2.6.1 3D treatment planning system [CMS Xio Elekta (Beijing) Medical Devices Co., Ltd., Changping District, Beijing, China]. After the target area was delineated and the radiotherapy plan was designed, the positive and lateral digital reconstructed images (DRRs) were automatically generated. Before the first treatment, the front, back field and lateral field images were collected according to the planned isocentric marker, and then the electronic radiation field images (ERIs) of the positive position (rack angle of 0°) and the lateral position (rack angle of 270°) were photographed by EPID every week. The EPI shooting condition was set to a large field area of 20 cm × 20 cm, a small field area of 10 cm × 10 cm, and an accelerator hop of 2-3 MU. Dual-exposure shooting was used to photograph, and the relevant errors were collected and analyzed. Using the artificial registration function of the iView GT workstation [Elekta (Beijing) Medical Devices Co., Ltd., Changping District, Beijing, China], DRRs were used as a reference image, and the trachea, bulge or vertebral body, spinous process, or sternum of the thoracic spine were used as reference markers, and two radiotherapy doctors jointly confirmed the anatomical structure. Through EPI and DRR registration, the swing error value was obtained. The swing error in the direction of X (left and right), Y (front and back), and Z (up and down) was measured, and the systematic error and random error in the three directions were recorded, of which the systematic error was the average, and the random error was the standard deviation.

Outcome Measures

The manual registration function of iView GT workstation was applied. Taking the DRRs as the reference images and the trachea, carina or vertebral body, spinous process, or sternum of the thoracic vertebrae as the reference marks, the anatomical structures were jointly confirmed by two radiologists. Through EPI and DRR registration, the setup error value was obtained. The setup error in the X (left and right), Y (front and back), and Z (up and down) directions were measured, and the systematic error and random error in the three directions were recorded. Therein, the systematic error was the mean, and the random error was the standard deviation.

The clinical target volume (CTV) → planning target volume (PTV) outward expansion distance

CTV of patients with thoracic tumors was expanded by 2, 3, and 2 mm in the X, Y, and Z directions to form the internal target volume (ITV). The external expansion was carried out 10 times using the PTV external expansion method, and the plan was made according to PTVs of different sizes. The isocenter coordinates were modified according to the positioning error measured by ERIs. The minimum ITV → PTV outward expansion distance when 95% ITV volume reached the prescribed dose was recorded. The ITV → PTV outward expansion distance SM_{90} was recorded when 95% ITV of 90% of patients reached the prescribed dose in the presence of a setup error. The original plan was developed according to SM_{90} obtained by the PTV expansion method, and the new plan was obtained by shifting the isocenter coordinates of the treatment plan according to the positioning error value obtained by EPID.

The new plan was obtained by automatically modifying the size of the field based on the original plan without changing the conditions such as the angle of the field, the dose weight of the field, the direction and angle of the wedge plate and the prescribed dose. A dose-volume histogram (DVH) was plotted to record two sets of treatment indicators, including 95% of the planned dose, 90% of the target volume received (D_{95}), and 100% of the prescribed dose (V_{100}). The indicators, including the lung volume of the lungs receiving more than 5 Gy irradiation of the total lung volume percentage (V_5), the volume of the lungs receiving more than 10 Gy irradiation of the total lung volume percentage (V_{10}), the volume of the lung receiving more than 20 Gy irradiation of the total lung volume percentage (V_{20}), the lung receiving more than 30 Gy irradiation of the total lung volume percentage (V_{30}), the lung receiving more than 40 Gy irradiation of the total lung volume percentage (V_{40}), heart volume of heart irradiation exceeding 25 Gy of the total heart volume (V_{25}), heart volume receiving more than 30 Gy irradiation of the total heart volume (V_{30}), heart volume receiving more than 40 Gy irradiation of the total heart volume (V_{40}), mean lung dose (MLD), mean heart dose (MHD), and the acceptable dose of spinal cord ($D_{1\ cm3}$) were compared. The formula for swing error expansion was $MPTV=2.5\sum+0.7\sigma$, where the $\sum$ is the systematic error and the σ is the random error. The edge expansion formula of the setup error is shown in Figure 2.

Statistical Analysis

The experimental data were analyzed using Statistical Package for the Social Sciences (SPSS 20.0, IBM Corp., Armonk, NY, USA) software. The measurement data, including the double-lung V_5, double-lung V_{20}, MLD, MHD, and $D_{1\ cm3}$, were in line with the normal distribution and were expressed as ($\bar{x}\pm s$) and were compared using a *t*-test. $p<0.05$ indicated that the statistical results were statistically significant.

Results

Clinical Data of 202 Patients with Thoracic Tumors

Among 202 patients with thoracic tumors, there were 28 cases of lung cancer, 95 cases of esophageal cancer, 40 cases of left breast cancer, 38 cases of right breast cancer, and 1 case of thymic cancer. The detailed general materials are shown in Table I.

Analysis of Setup Error of Each Patient in Three Directions

202 patients were included in this experiment. The setup errors in X, Y, and Z directions were (-2.00±1.16) mm, (0.16±1.14) mm, and (-0.55±1.16) mm, respectively. The systematic error was maximum in the Z direction, which was -3.00 mm. The random error was maximum in the X direction, which was 3.30 mm (Table II and Figure 3).

Analysis of Target Area's Outward Expansion Distance

The ITV → PTV outward expansion distance, which could guarantee that 95% ITV of each patient could reach the prescribed dose, was obtained through the PTV expansion method. It was found that the outward expansion distance of ITV → PTV was mostly 2-4 mm, accounting for 82.18%.

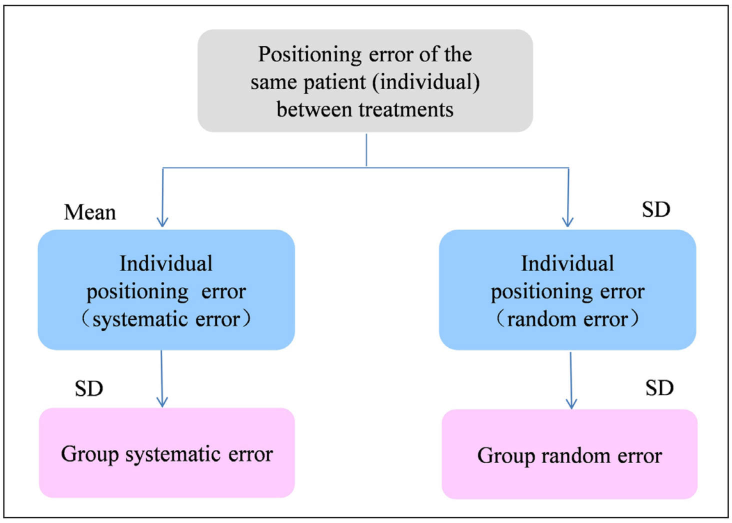

Figure 2. The edge expansion formula of the setup error.

Table I. Analysis of clinical data of patients with thoracic tumors.

General materials		Lung cancer (n=28)	Esophageal cancer (n=95)	Breast cancer (n=78)
Gender	Male	19 (67.86)	70 (73.68)	1 (1.28)
	Female	9 (32.14)	25 (26.32)	77 (98.72)
Age (year)		58.32±9.10	65.63±9.92	48.50±8.62
Body mass index		23.01±4.28	25.46±4.23	24.55±3.83
Tumor stage	Stage II	8 (28.57)	23 (24.21)	7 (8.97)
	Stage III-IV	20 (71.43)	72 (75.79)	71 (91.03)
Tumor volume (cm^3)		169.23±86.35	36.32±17.15	5.96±3.15

Table II. Analysis of setup error of each patient in three directions (mm).

Directions	Mean	Standard deviation	Systematic error	Random error
X direction	-0.20	1.16	-2.50	3.30
Y direction	0.16	1.14	-2.60	3.00
Z direction	-0.55	1.16	-3.00	2.70

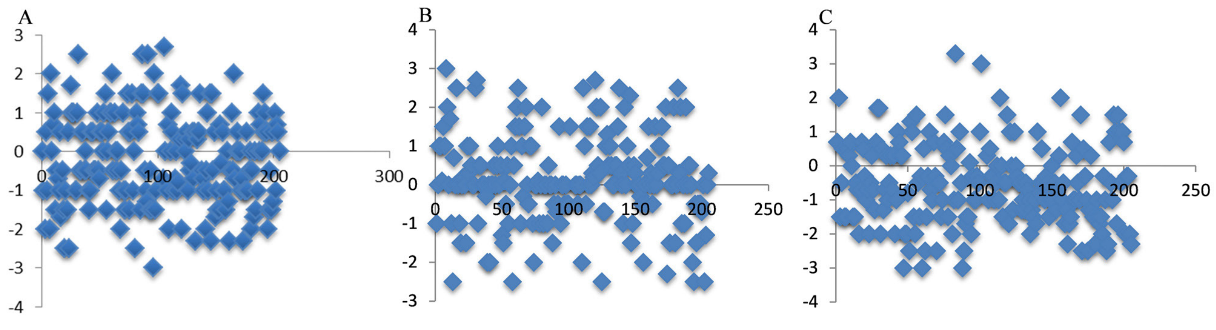

Figure 3. The setup error of each patient in three directions. **A**, The setup error in the X direction; (**B**) The setup error in the Y direction; (**C**) The setup error in the Z direction.

In order to ensure that 95% ITV in 90% of patients could reach the prescribed dose in the presence of a setup error, the outward expansion distance SM_{90} of ITV → PTV should be set as 5 mm (Table III).

Comparison of Dose Parameters Between the New Plan and the Original Plan for Radiation Target Areas of Patients with Chest Tumors

The outward expansion distance of CTV → ITV was set to 2, 3 and 2 mm in the X direction, Y direction and Z direction, respectively, and the outward expansion distance of ITV → PTV was 5 mm; the outward expansion distance of CTV → PTV was 7, 8 and 7 mm in the X direction, Y direction and Z direction respectively. Under the presence of a setup error, the PTV D_{95} and PTV V_{100} in the new plan decreased by 5.06% and 9.59 Gy, respectively, compared with the original plan. The new plan with PTV D_{95} of (62.23±3.85) Gy and ITV V_{100} of (97.51±1.56) %, respectively, could ensure that 95% ITV of 90% of patients reached the prescribed dose. In contrast, the ITV D_{95} and ITV V_{100} of (56.11±5.26) Gy and (90.15±3.12) %, respectively, in the presence of a setup error could not guarantee that 95% ITV of 90% of patients reached the prescribed dose (Table IV).

Comparison of Dose Parameters in the New and Original Plans for the Target Areas of Double Lung, Heart and Bone Marrow of Each Patient

In the presence of a setup error, the double-lung V_5, the double-lung V_{20}, MLD, MHD, and $D_{1\ cm3}$ of the new plan increased by 0.89%, 0.29%, 0.13%, 0.06%, and 5 Gy, respectively, compared with the original plan. However, there was no significant difference in the indicators as

Table III. Analysis of target area's outward expansion distance (cases, %).

External expansion distance	The number of cases (case)	The percentage (%)
1 mm	20	9.90
2 mm	48	23.76
3 mm	80	39.60
4 mm	38	18.81
5 mm	16	7.92

Table IV. Comparison of dose parameters between the new plan and the original plan for radiation target areas of each patient ($\bar{x}$±s).

Dose parameters	Original plan (%)	New plans (%)	*t*	*p*
PTV D_{95} (Gy)	60.18±3.96	55.12±3.56	13.4506	<0.001
PTV V_{100} (%)	94.85±0.12	85.26±5.26	25.906	<0.001
ITV D_{95} (Gy)	63.86±8.56	62.23±3.85	2.468	0.014
ITV V_{100} (%)	99.85±0.68	97.51±1.56	19.543	<0.001

PTV: planned target area; ITV: internal target volume; D_{95} was the dose received by 95% target volume (Gy); V_{100} was the percentage of target volume included in 100% prescribed dose (%).

double-lung V_5, double-lung V_{10}, double-lung V_{20}, double-lung V_{40}, heart V_{25}, heart V_{30}, heart V_{40}, MLD, and MHD between the original plan and the new plan (p>0.05). The difference in $D_{1\ cm3}$ between the original plan and the new plan was statistically significant (p<0.05) (Table V).

Discussion

The results of this study indicate that there is a certain degree of setting error in the initial radiotherapy of chest tumors, with the X direction being the most obvious. EPID helps to achieve the expected radiotherapy effect, improve the radiotherapy efficacy, and reduce the side effects caused by radiotherapy errors.

The chest is a part of the body that is prone to developing malignant tumors. Previous statistical reports[9] showed that among the 10.9 million new malignant tumors worldwide in recent years, lung cancer accounted for about 1.35 million with a high incidence rate and high mortality. Esophageal cancer is the fourth highest mortality malignant tumor in China[10]. The mortality and complication rate of surgical treatment of thoracic tumors are relatively high due to the large difference in the surface, thickness, and tissue density of the body parts and the proximity to the spinal cord. Moreover, distant metastasis and extensive infiltration occurred in more

Table V. Comparison of dose parameters in the new and original plans for the target areas of double lung, heart and bone marrow of each patient.

Dose parameters	Original plan (%)	New plans (%)	*t*	*p*
Double lung V_5 (%)	31.26±10.56	32.15±14.56	0.703	0.482
Double lung V_{10} (%)	25.02±11.64	25.31±11.49	0.252	0.801
Double lung V_{20} (%)	14.56±6.23	14.85±6.78	0.448	0.655
Double lung V_{30} (%)	8.60±4.15	8.63±4.40	0.071	0.944
Double lung V_{40} (%)	4.25±2.14	4.36±2.44	0.482	0.630
Heart V_{25} (%)	13.98±14.12	13.67±14.42	0.218	0.827
Heart V_{30} (%)	11.41±11.67	11.02±11.75	0.335	0.738
Heart V_{40} (%)	7.09±8.14	6.45±7.79	0.807	0.420
MLD (Gy)	7.85±2.86	7.98±2.58	0.480	0.632
MHD(Gy)	8.63±8.15	8.69±8.07	0.0744	0.941
$D_{1\ cm3}$ (Gy)	34.56±2.89	39.56±5.21	11.928	<0.001

V_5 was the percentage of the volume receiving 5 Gy irradiation of the total volume, V_{10} was the percentage of the volume receiving 10 Gy irradiation of the total volume, V_{20} was the percentage of the volume receiving 20 Gy irradiation of the total volume, V_{25} was the percentage of the volume receiving 25 Gy irradiation of the total volume, V_{30} was the percentage of the volume receiving 30 Gy irradiation of the total volume, V_{40} was the percentage of the volume receiving 40 Gy irradiation of the total volume.

than half of the patients at the time of diagnosis. Thus, the curative effect of surgical treatment is not ideal. Therefore, radiotherapy has become the first measure for thoracic tumor treatment[11,12]. Radiation therapy technology has developed from two-dimensional radiation therapy to three-dimensional conformal radiation therapy and intensity-modulated radiation therapy, which greatly reduces the dose received by normal tissues and increases the tumor dose, thus improving the local control rate and survival rate. However, this requires more and more higher positioning accuracy.

IMRT treatment is a common, precise radiotherapy method that integrates medical imaging, computer technology, and quality assurance measures. Targeted high-dose radiation to the tumor focus can effectively reduce the radiation damage to the surrounding tissues and has a good curative effect. However, IMRT treatment requires accurate setup, and the setup error during the treatment process has received widespread attention[13]. The setup error is the difference between the position of the actual treatment and the reference position of the simulated positioning and treatment plan, including systematic error and random error. Among them, the systematic error is the error caused by the simulated positioning system, laser lamp, accelerator, and other instruments before the patient's treatment, and the randomization error is the difference in the patient's repetitive position with each treatment[14]. The setup error leads to changes in the actual treatment position, which not only affects the radiation dose of the tumor focus but also makes the radiation dose of some target areas insufficient, so it is impossible to obtain a satisfactory high dose. In addition, radiation with inaccurate positioning may also damage normal tissues and organs because some areas of the surrounding normal tissues receive additional irradiation, which leads to a reduction in the local control rate of tumors, an increase in the proportion of recurrence and metastasis, and an aggravation of the complications and sequelae of radiotherapy. Therefore, reducing the setup error during IMRT treatment is the key to improving the efficacy[15]. EPID is a two-dimensional radiotherapy image guidance technology that has the advantages of large space, high tissue resolution, simple operation, fast imaging, and low price. EPID can replace the traditional verification film technology by evaluating the setup error online, and there are many applications for precise localization of chemotherapy for lung cancer[16]. A total of 202 patients were included in this experiment. The average error values in the X, Y, and Z directions were -2.00 mm, 0.16 mm, and -0.55 mm, respectively, of which the random error was the largest in the X direction, suggesting that EPID could accurately measure the setup error. The maximum random error in the X direction may be related to the patient's breathing, chest movement, and arm-holding posture. Early studies[17] believe that respiratory control is a common way to control the displacement of thoracic tumor targets in IMRT. By using thermoplastic membranes and abdominal briquettes to limit the amplitude of the respiratory body surface, the setup error caused by respiratory movements can be reduced. In this study, the thermoplastic film was used to fix the position, which could help reduce the setup error.

EPID can not only measure the setup error but also help to determine the CTV → PTV expansion distance. Esposito et al[18] analyzed IVD results from 2002 patients and 32,276 tests and found that EPID IVD is effective in intercepting important errors and improving the efficacy of radiotherapy. According to previous reports[19,20], CTV → PTV outward expansion distance was set to be 7, 8 and 7 mm in X direction, Y direction and Z direction, respectively. PTV D_{95} and ITV V_{100} in the new plan were (62.23±3.85) Gy and (97.51±1.56) %, respectively, in the presence of setup error, which could effectively ensure that the 95% ITV of 90% of the patients reached the prescribed dose. However, when CTV → PTV was expanded to 5 mm, the 95% ITV of 90% of the patients was not able to reach the prescribed dose. At the same time, because the anatomical position of the thoracic tumor is close to the important organs such as the lungs, heart and spinal cord, the large setup error may induce radiation injury to the heart and other important organs. In the case of a setup error, the double-lung V_5, double lung V_{20}, MLD and MHD in the new plan increased by 0.89%, 0.29%, 0.13% and 0.06%, respectively, compared with the original plan, but the difference was not statistically significant. The results suggested that EPID could not only ensure the effect of radiotherapy, but also effectively reduce the radiation damage of other surrounding normal organs with high safety. $D_{1\ cm^3}$ increased by 5 Gy, and the difference was statistically significant. It might be related to the maximum systematic error in the Z direction and the large spinal cord dose. However, the spinal cord dose was still within the tolerable range and had little impact on the spinal cord. Further, it was shown that EPID could help the precise positioning of radiotherapy and improve its efficacy, in line with the results of Alharthi et al[21]. In this experiment, the influence of related factors that might affect random and systematic errors, including organ movement, patient movement, body shape change, and technician placement techniques, was reduced, and the feasibility of this result was confirmed.

However, due to the limited study time, the long-term efficacy in the patients has not been followed up, and there may exist some other dose errors during the whole treatment process. In the future, the research time will be expanded and the proportion of various dose deviations in the treatment process will be measured for further exploration.

Conclusions

In general, the first treatment of radiotherapy in thoracic tumors mostly has a certain degree of setup error, which was most evident in the X direction. When the CTV→PTV outward expansion distance is set at 7, 8, and 7 mm in the X direction, Y direction, and Z direction, respectively, it could effectively ensure that 95% ITV reaches the prescribed dose in 90% of the patients in the presence of a setup error. EPID helps to achieve the desired effect of radiotherapy, improve its efficacy, and reduce the side effects caused by radiotherapy errors.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All procedures carried out for patients were in accordance with the ethical standards of the Ethics Review Committees of the Affiliated Hospital of Putian University (Ethical Approval No. 2 02306).

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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Conflict of Interest

The authors declare that they have no competing interests.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

QSC: guarantor of integrity of the entire study, study concepts, study design, definition of intellectual content, literature research, manuscript review, clinical studies and experimental studies and data analysis.
JKX, XC, YHZ: clinical studies and experimental studies, data acquisition, data analysis, statistical analysis and manuscript preparation and editing.

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