BMJ Oncology

Whole-body integral dose and post-radiotherapy lymphocytopaenia in solid tumours

Nuradh Joseph ,[1] Lanka Alagiyawanna,[2,3] Thilina Ruwanpura,[4] Sanjeeva Gunasekera,[2,5] Lakitha Ruvinda,[6] Sampath Madushan,[6] Ananya Choudhury[7,8]

To cite: Joseph N, Alagiyawanna L, Ruwanpura T, *et al*. Whole-body integral dose and post-radiotherapy lymphocytopaenia in solid tumours. *BMJ Oncology* 2025;**4**:e000522. doi:10.1136/bmjonc-2024-000522

Received 01 July 2024
Accepted 14 January 2025

Linked

► https://doi.org/10.1136/bmjonc-2025-000745

Check for updates

[1]Clinical Oncology, District General Hospital, Matara, Sri Lanka
[2]Sri Lanka Cancer Research Group, Maharagama, Sri Lanka
[3]University Hospital Southampton NHS Foundation Trust, Southampton, UK
[4]Clinical Oncology, Royal Stoke University Hospital, Stoke-on-Trent, UK
[5]Clinical Oncology, Apeksha Hospital, Maharagama, Sri Lanka
[6]Medical Physics, Apeksha Hospital, Maharagama, Sri Lanka
[7]Clinical Oncology, The Christie NHS Foundation Trust, Manchester, UK
[8]Division of Cancer Sciences, The University of Manchester, Manchester, UK

Correspondence to
Dr Nuradh Joseph; nuradh@gmail.com

ABSTRACT

Objective Since modern radiotherapy techniques such as intensity-modulated radiotherapy (IMRT) pivot on a strategy of dose redistribution, it may increase integral dose and consequently worsening of lymphocytopaenia. In this study, our objective was twofold: first to validate the correlation between integral body dose and post-treatment lymphocytopaenia in a cohort of patients treated with curative-intent radiotherapy and second to validate its prognostic impact.

Methods and analysis Patients treated with curative intent radiotherapy with complete blood counts were included in the study. Data on the following variables were collected: treatment site, prescribed dose, use of concurrent chemotherapy, mean body dose, mean body volume, treatment technique and disease-free survival.

Results A total of 116 patients were included for analysis. There was a significant decline in lymphocyte counts after radiotherapy (2.2×10^9/L vs 0.8×10^9/L; p<0.001). Multivariate linear regression analysis of post-treatment lymphocytopaenia revealed a significant correlation with pretreatment lymphocyte counts, integral body dose, use of IMRT and use of concurrent radiosensitising chemotherapy. Univariate survival analysis was performed in 37 patients with squamous cell carcinoma of the head and neck. In the Cox proportional hazards model, post-treatment lymphocyte count was statistically significant as a continuous variable (Hazard Ratio=0.998, p=0.01) and as a dichotomous variable.

Conclusion The negative correlation between integral body dose and post-treatment lymphocytopaenia was validated, and post-treatment lymphocytopaenia is an adverse prognostic factor in patients with head and neck cancer treated with curative-intent radiotherapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Several studies have investigated dosimetric predictors of radiotherapy-induced lymphocytopaenia in addition to its prognostic impact.

WHAT THIS STUDY ADDS

⇒ In this work, we validated the negative correlation between whole-body integral dose and post-radiotherapy lymphocytopaenia. We further validated the adverse prognostic impact of post-treatment lymphocytopaenia in patients with squamous cell carcinoma of the head and neck treated with curative-intent radiotherapy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Strategies looking at keeping whole-body integral dose as low as reasonably achievable should be considered when treating patients with curative-intent radiotherapy. Post-radiotherapy lymphocytopaenia could help select patients for further consolidative treatment such as immunotherapy.

INTRODUCTION

Tumour-induced immune incompetence is recognised as a 'hallmark' of cancer.[1] Radiotherapy may both augment and diminish antitumour immunity through a number of mechanisms.[2] Since immune cells are highly radiosensitive, their depletion through apoptotic death following radiation could further impair the antitumor immune response.[3]

A large number of studies have shown that pretreatment lymphocytopaenia is an adverse prognostic biomarker, which may be a surrogate indicator of antitumour immune incompetence.[4] Post-treatment lymphocytopaenia has also been shown to predict poor survival in a number of tumours.[5 6] In lung cancer, it was shown that post-radiotherapy lymphocytopaenia was found to be correlated with whole-body integral dose.[7]

The importance of antitumour immunity in improving outcomes of patients was further confirmed by the results of the PACIFIC, which revealed that consolidative immunotherapy with the PD-L1 inhibitor durvalumab improves overall survival in patients with stage III non-small cell lung cancer treated with curative-intent chemoradiotherapy.[8]

Since modern radiotherapy techniques such as intensity-modulated radiotherapy (IMRT) pivot on a strategy of dose redistribution, it

may increase integral dose and consequently worsening of post-treatment lymphocytopaenia.[7 9]

Integral dose, defined as the product of mean body dose and body volume, is in effect the area under the curve of a differential dose-volume histogram. Since it captures the impact of high-dose and low-dose regions in a single parameter, it is a more robust predictor of lymphocytopaenia than other measures such as V5 (volume receiving at least 5 Gy).[7 10]

In this study, our objective was twofold: first to validate the correlation between integral body dose and post-treatment lymphocytopaenia in a cohort of patients treated with curative-intent radiotherapy and second to validate the prognostic impact of post-treatment lymphocytopaenia.

METHODS

Patients treated with curative-intent radiotherapy in the linear accelerator at the Apeksha Hospital, Maharagama, Sri Lanka, between 01 January 2016 and 31 June 2016 with pretreatment and post-treatment complete blood counts were included in the study.

This study was conducted as a retrospective secondary data analysis from outcome data collected as part of a service evaluation, and institutional approval was obtained from the Apeksha Hospital, Maharagama, Sri Lanka. The data were retrospectively collected and anonymised, and there was no involvement from patients or members of the public in the design, conduct or reporting of the study.

Data on the following variables were collected: treatment site, prescribed dose, use of concurrent chemotherapy, mean body dose, mean body volume, planning target volume (PTV), treatment technique (IMRT or three-dimensional conformal radiotherapy (3D-CRT)) pretreatment and post-treatment haemoglobin, white blood cell count, platelet count, lymphocyte count and neutrophil counts. In this context, body volume means the entire volume of tissues included in the simulation CT scan. This includes the PTV, contoured organs, and uncontoured normal tissues.

Post-treatment counts were done at the first post-treatment outpatient clinic review which often took place 1–2 weeks after completion of treatment. We feel this was a reasonable time point, as it allowed the full effect of the total treatment dose to have its impact before recovery of blood counts.

The significance of the difference between pretreatment and post-treatment blood counts was determined using the paired t-test. Univariate linear regression analysis was performed to determine the association with post-treatment lymphocytopaenia in a model incorporating the following variables: integral body dose (defined as the product of mean body dose and body volume), treatment technique, prescribed dose and use of concurrent chemotherapy. Variables significant on univariate analysis were carried forward to a multivariate regression analysis model.

Since a majority of patients were those with squamous cell carcinoma of the head and neck (SCCHN) treated with curative intent, survival analysis was limited to these patients. In patients with SCCHN, disease-free survival (DFS) defined as time to death, recurrence or loss to follow-up was determined. Univariate analysis of DFS in relation to lymphocytopaenia was performed using the Cox proportional hazards model incorporating absolute post-treatment lymphocyte count as a continuous variable and as a dichotomous variable dichotomised at median.

Table 1 Baseline characteristics of the study cohort

Age (median, range)	57 years (19–81)
Tumour site	Number (percentage)
Breast cancer	9 (8)
Central nervous system tumours	26 (22)
Squamous cell cancer of the head and neck	37 (31)
Prostate cancer	4 (4)
Rectal cancer	5 (4)
Cancer of the cervix	10 (9)
Lung cancer	3 (3)
Oesophagus	4 (3)
Other	18 (16)
Body volume (median, range)	12 L (1.7–52)
Mean body dose (median, range)	7.86 Gy (1.3–30.4)
Integral body dose (median, range)	87.3 L*Gy (15.1–652.3)
Prescribed dose to PTV (median, range)	55 Gy (40–72)
Treatment technique	
Intensity-modulated radiotherapy	60 (52)
Three-dimensional conformal radiotherapy	56 (48)

PTV, planning target volume.

RESULTS

Based on the inclusion criteria, 116 patients were included for analysis. The basic characteristics of the study population are depicted in table 1. SCCHN was the most common tumour followed by tumours of the brain. There was a near-even split between IMRT and 3D-CRT.

For concurrent chemoradiotherapy in patients with SCCHN and carcinoma of the cervix, the radiosensitising agent was intravenous cisplatin delivered at a dose of 40 mg/m^2 weekly. GCSF was usually not administered unless the patient developed grade 3 or more neutropaenia.

Table 2 describes the changes in the haematological parameters following treatment with radiotherapy. As evident from this table, except for median absolute neutrophil count, there was a statistically significant

Table 2 Changes in mean haematological parameters after radiation therapy

Parameter	Pretreatment	Post-treatment	Statistical significance
Absolute neutrophil count	4.9×10^9/L	4.8×10^9/L	p=0.77
Absolute lymphocyte count	2.2×10^9/L	0.8×10^9/L	p<0.001
Platelet count	270×10^9/L	225×10^9/L	p<0.001
Haemoglobin level	12 g/dL	11.4 g/dL	p<0.001

decline in median absolute lymphocyte counts, median platelet counts and median haemoglobin levels. Although the drop in median platelet count following radiotherapy (270×10^9/L to 225×10^9/L) was statistically significant, it was still above the lower limit of the normal range. Median haemoglobin level dropped marginally to 11.4 g/dL from 12 g/dL following treatment. However, the median absolute lymphocyte counts showed a substantial decline after radiotherapy (2.2×10^9/L vs 0.8×10^9/L).

Table 3 shows the results of the multivariate linear regression analysis of post-treatment lymphocytopaenia, and this revealed a statistically significant positive correlation with pretreatment lymphocyte counts and a negative correlation with integral body dose, use of IMRT and use of concurrent radiosensitising chemotherapy.

Univariate survival analysis was performed in 37 patients with SCCHN. In the Cox proportional hazards model, post-treatment lymphocyte count was statistically significant as a continuous variable (HR=0.998, p=0.01). Pretreatment lymphocyte count and pre- and post-treatment neutrophil counts were not significantly associated with survival.

When dichotomised at median absolute post-treatment lymphocyte count (0.72×10^9/L in the subgroup of patients with head and neck cancer), lymphocytopaenia was associated with poorer survival (log-rank test, p=0.05). This is illustrated in figure 1. One-year DFS was 36% in patients with lymphocytopaenia compared with 67% in patients without lymphocytopaenia.

DISCUSSION

While previous studies had shown a similar effect with thoracic radiotherapy, our study confirms the correlation between post-treatment lymphocytopaenia and whole-body integral dose across a wide range of tumours.[7] Although numbers were small, post-treatment lymphocytopaenia was a significant adverse prognostic factor in patients with head and neck cancer both as a continuous variable and as a dichotomous variable when dichotomised at median. Our findings are in conformity with the findings of a recent meta-analysis that confirmed the prognostic effect of post-treatment lymphocytopaenia in head and neck cancers.[11] Further work by El Houat *et al* across six tumour sites, including head and neck cancers, lung cancer, oesophageal and pancreatic cancers, also showed that post-radiotherapy lymphocytopaenia predicted for inferior survival.[12]

Price *et al* validated pretreatment lymphocytopaenia as a significant adverse prognostic factor in two large cohorts of patients with head and neck cancer treated with curative-intent radiotherapy.[13] However, it is noteworthy that pretreatment lymphocyte count was not associated with inferior survival in this cohort of patients.

Since post-treatment lymphocytopaenia could be a surrogate marker of antitumour immune suppression, our findings add to the weight of evidence that a competent immune response is important in consolidating the effects of radiotherapy when delivered as a curative treatment, which was confirmed by the results of the PACIFIC trial that revealed that consolidative immunotherapy with the Programmed Death-Ligand 1 (PD-L1) inhibitor durvalumab improves overall survival in patients with stage III non-small cell lung cancer. In a pooled analysis of patients treated with anti-PD-1/PD-L1 inhibitors and concurrent radiotherapy, it was shown that regression of lesions outside the irradiated field was significantly higher in patients with high post-radiotherapy lymphocyte counts.[14]

Through the release of damage-associated molecular pattern molecules, radiotherapy could stimulate the antitumour immune response by attracting antigen-presenting cells leading to the migration of CD8+ cytotoxic T cells to the tumour microenvironment.[15] However, apoptotic death of effector lymphocytes and other immune cells following radiation could further impair the antitumour immune response.[16]

Further work is needed to determine whether the post-treatment decline in cell count affects all lymphocyte subpopulations with equal effect. Since it has been shown that tumour infiltration with CD8 lymphocytes is prognostic in head and neck cancer, correlation between

Table 3 Linear regression analysis of correlation with post-treatment absolute lymphocyte count

Parameter	Univariate model	Multivariate model (standardised β)
Pretreatment lymphocyte count	p<0.001	p<0.001 (0.295)
Whole-body integral dose	p=0.002	p=0.003 (-0.253)
Use of concurrent chemotherapy	p<0.001	p=0.005 (-0.245)
Treatment technique	p=0.02	p=0.012 (0.213)
Prescribed dose	p=0.13	–

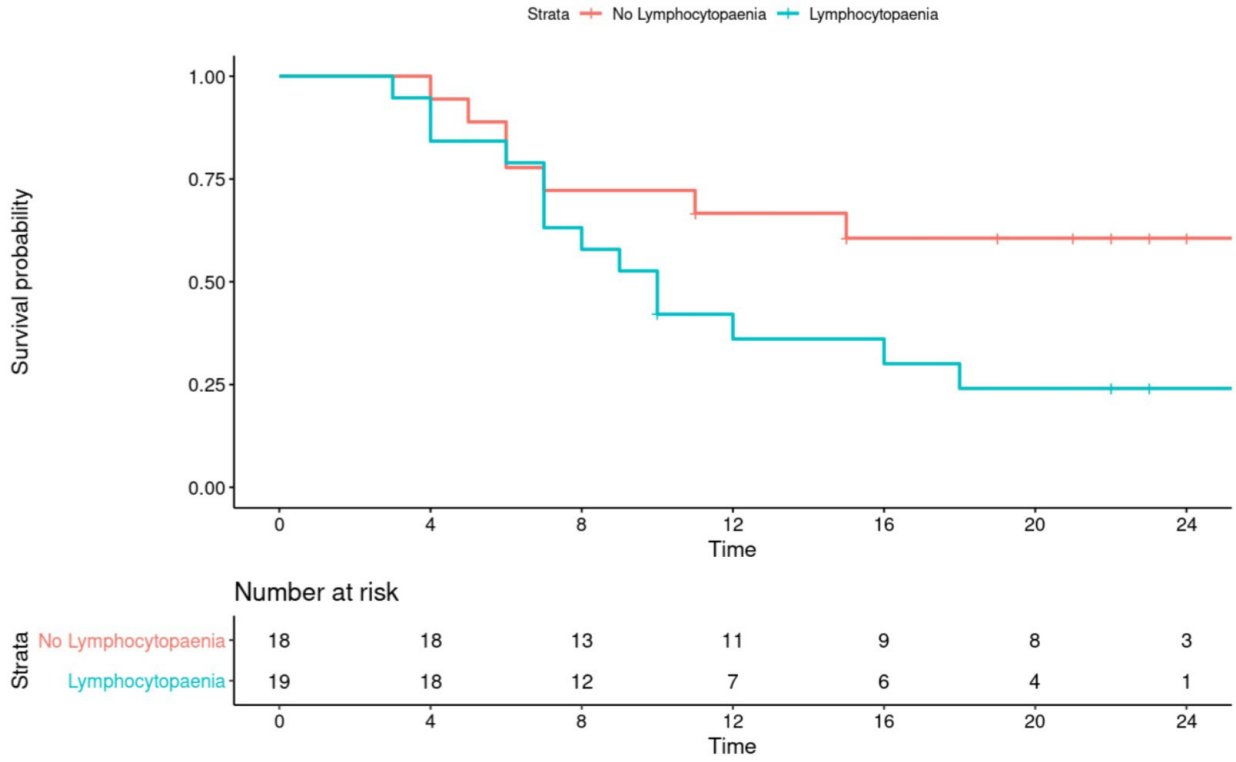

Figure 1 Impact of lymphocytopaenia on disease-free survival of patients with squamous cell carcinoma of the head and neck.

peripheral blood lymphocyte subpopulation counts and intratumoural lymphocyte counts needs further investigation.[13 17] Some previous studies in this space have analysed nadir counts during treatment as prognostic factors, and this is indeed a very interesting aspect to look into. Since this was a retrospective analysis, it was difficult to obtain consistent data on blood counts during treatment. However, since the dosimetric correlation was based on the total dose delivered, we believe the most relevant variable is post-treatment blood count.

Since integral dose is inevitably linked to the volume of irradiation, it is of vital importance that clinical target volume and PTV are defined with precision. In cancers of the head and neck and prostate cancer, patient selection and determining the appropriate extent of elective nodal irradiation must be done with prudence. Lin *et al* showed that lymphocytopaenia was worse in patients receiving bilateral neck irradiation in comparison with those receiving unilateral treatment.[18] In our study, patients receiving IMRT had a more profound decline in lymphocyte counts, suggesting that it is perhaps the 'low-dose bath' that impacts more on lymphocytopaenia.

The radiotherapy treatment protocols during the study period did not include specific dose constraints for lymphatic organs such as bone marrow, spleen and thymus gland. These structures were not contoured as organs and as such we could not analyse the dosimetric impact of individual lymphatic organs on post-treatment lymphocytopaenia. Integral body dose is the cumulative dose received by all tissues in the region of the simulated CT scan, which includes the PTV, contoured organs at risk as well as uncontoured normal tissues. We did not separately analyse correlation between lymphocytopaenia and PTV, since there is likely to be a strong correlation between PTV and integral dose leading to a confounding effect. Integral dose is often not considered a parameter of interest when setting dose constraints during inverse planning of IMRT. Our results suggest that more focus is needed on reducing the integral body dose, without compromising other dosimetric targets. The importance of sparing doses to lymphocyte-rich organs such as the heart, central blood vessels, bone marrow, spleen and thymus (in children) has been proposed by some investigators.[19] However, since lymphocytes are constantly circulating in peripheral blood, irradiation of any anatomic region is likely to result in apoptotic cell death. As such, whole-body integral dose is likely to have a more robust correlation with post-treatment lymphocytopaenia than the dose to the presumed lymphocyte-rich organs. Another alternative approach is the use of particle radiotherapy, such as proton beam therapy, which results in a lower integral body dose compared with photon beam therapy.[20]

Although our findings are consistent with previous work in this space, the absence of a validation cohort is a significant limitation of our work. Since SCCHN is the most common malignancy among males in Sri Lanka, this was reflected in our cohort as well.[21 22]

A major limitation of our work is that the sample size is relatively small. Obtaining data on all parameters including clinical, radiotherapy and follow-up, data were challenging since our hospital still maintains paper-based

records. Due to small numbers, we could not determine the prognostic effect of lymphocytopaenia in patients with other tumours. Even in head and neck cancers, the small sample size precluded a multivariate analysis of survival incorporating known prognostic factors. In addition, we did not determine if the dose to lymphocyte-rich organs mentioned above correlated with post-treatment lymphocytopaenia. A future prospective study is being initiated at our centre to analyse these aspects in greater detail.

Despite the small sample size, through this study, we have successfully validated the negative correlation between integral body dose and post-treatment lymphocytopaenia and the adverse prognostic effect of post-treatment lymphocytopaenia in patients with head and neck cancer treated with curative-intent radiotherapy.

X Nuradh Joseph @nuradh, Thilina Ruwanpura @ThilinaR_, Sanjeeva Gunasekera @Gunti777 and Ananya Choudhury @achoud72

Acknowledgements Prof. AC is funded by the Manchester Academic Health Science Network.

Contributors NJ and AC conceived the study. All authors were involved in the design of the study, data analysis, writing, editing and approval of the manuscript. NJ, LA, TR, LR, SG and SM were involved in data collection. NJ is the guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests AC: Editor-in-Chief of BMJ Oncology.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. This study was conducted as a secondary analysis of retrospective data collected for service evaluation and was approved by the Director of the Apeksha Hospital, Maharagama, Sri Lanka, where the study was carried out. This study was conducted as a secondary analysis of retrospective data collected for service evaluation.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD

Nuradh Joseph http://orcid.org/0000-0002-9519-210X

REFERENCES

1 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
2 Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol* 2009;10:718–26.
3 Yovino S, Kleinberg L, Grossman SA, *et al*. The etiology of treatment-related lymphopenia in patients with malignant gliomas: modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. *Cancer Invest* 2013;31:140–4.
4 Zhao J, Huang W, Wu Y, *et al*. Prognostic role of pretreatment blood lymphocyte count in patients with solid tumors: a systematic review and meta-analysis. *Cancer Cell Int* 2020;20:15.
5 Damen PJJ, Kroese TE, van Hillegersberg R, *et al*. The Influence of Severe Radiation-Induced Lymphopenia on Overall Survival in Solid Tumors: A Systematic Review and Meta-Analysis. *Int J Radiat Oncol Biol Phys* 2021;111:936–48.
6 Joseph N, Choudhury A. Lymphocytopenia and Radiotherapy Treatment Volumes in the Time of COVID-19. *Clin Oncol* 2020;32:420–2.
7 Joseph N, McWilliam A, Kennedy J, *et al*. Post-treatment lymphocytopaenia, integral body dose and overall survival in lung cancer patients treated with radical radiotherapy. *Radiother Oncol* 2019;135:115–9.
8 Antonia SJ, Villegas A, Daniel D, *et al*. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* 2017;377:1919–29.
9 Joseph N, Cicchetti A, McWilliam A, *et al*. High weekly integral dose and larger fraction size increase risk of fatigue and worsening of functional outcomes following radiotherapy for localized prostate cancer. *Front Oncol* 2022;12:937934.
10 Joseph N, Choudhury A. Dosimetric Predictors of Radiotherapy-Induced Lymphocytopenia in Lung Cancer. *J Thorac Oncol* 2021;16:e11–2.
11 Dai D, Tian Q, Shui Y, *et al*. The impact of radiation induced lymphopenia in the prognosis of head and neck cancer: A systematic review and meta-analysis. *Radiother Oncol* 2022;168:28–36.
12 El Houat Y, Massard C, Quillien V, *et al*. Meta-analysis and Critical Review: Association Between Radio-induced Lymphopenia and Overall Survival in Solid Cancers. *Adv Radiat Oncol* 2023;8:101038.
13 Price JM, Mistry HB, Betts G, *et al*. Pretreatment Lymphocyte Count Predicts Benefit From Concurrent Chemotherapy With Radiotherapy in Oropharyngeal Cancer. *J Clin Oncol* 2022;40:2203–12.
14 Chen D, Verma V, Patel RR, *et al*. Absolute Lymphocyte Count Predicts Abscopal Responses and Outcomes in Patients Receiving Combined Immunotherapy and Radiation Therapy: Analysis of 3 Phase 1/2 Trials. *Int J Radiat Oncol Biol Phys* 2020;108:196–203.
15 Liu Y, Dong Y, Kong L, *et al*. Abscopal effect of radiotherapy combined with immune checkpoint inhibitors. *J Hematol Oncol* 2018;11:104.
16 Pike LRG, Bang A, Mahal BA, *et al*. The Impact of Radiation Therapy on Lymphocyte Count and Survival in Metastatic Cancer Patients Receiving PD-1 Immune Checkpoint Inhibitors. *Int J Radiat Oncol Biol Phys* 2019;103:142–51.
17 Ward MJ, Thirdborough SM, Mellows T, *et al*. Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer. *Br J Cancer* 2014;110:489–500.
18 Lin AJ, Rao YJ, Chin RI, *et al*. Post-operative radiation effects on lymphopenia, neutrophil to lymphocyte ratio, and clinical outcomes in palatine tonsil cancers. *Oral Oncol* 2018;86:1–7.
19 Lambin P, Lieverse RIY, Eckert F, *et al*. Lymphocyte-Sparing Radiotherapy: The Rationale for Protecting Lymphocyte-rich Organs When Combining Radiotherapy With Immunotherapy. *Semin Radiat Oncol* 2020;30:187–93.
20 Durante M. Kaplan lecture 2023: lymphopenia in particle therapy. *Int J Radiat Biol* 2024;100:669–77.
21 Joseph N, Gunasekera S, Ariyaratne Y, *et al*. Clinical Oncology in Sri Lanka: Embracing the Promise of the Future. *Int J Radiat Oncol Biol Phys* 2019;105:466–70.
22 Rupasinghe T, Silva DC, Balawardena J, *et al*. Curative-Intent Radiotherapy for Squamous Cell Carcinoma of the Head and Neck in Sri Lanka: The Impact of Radiotherapy Technique on Survival. *Clin Oncol* 2021;33:765–72.