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Brief communication

Correlation between radio-induced lymphocyte apoptosis measurements obtained from two French centres

Corrélation des valeurs d'apoptose radio-induite des lymphocytes obtenues par deux centres français

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ABSTRACT

Purpose of the research. – In the era of modern treatment delivery, increasing the dose delivered to the target to improve local control might be modulated by the patient's intrinsic radio-sensitivity. A predictive assay based on radio-induced lymphocyte apoptosis quantification highlighted the significant correlation between CD4 and CD8 T-lymphocyte apoptosis and grade 2 or 3 radiation-induced late toxicities. By conducting this assay at several technical platforms, the aim of this study was to demonstrate that radio-induced lymphocyte apoptosis values obtained from two different platforms were comparable.
Materials and methods. – For 25 patients included in the PARATOXOR trial running in Dijon the radio-induced lymphocyte apoptosis results obtained from the laboratory of Montpellier (IRCM, Inserm U1194, France), considered as the reference (referred to as Lab 1), were compared with those from the laboratory located at the Institut de cancérologie de Lorraine (ICL, France), referred to as Lab 2. Different statistical methods were used to measure the agreement between the radio-induced lymphocyte apoptosis data from the two laboratories (quantitative data). The Bland–Altman plot was used to identify potential bias.
Results. – All statistical tests demonstrated good agreement between radio-induced lymphocyte apoptosis values obtained from both sites and no major bias was identified.
Conclusions. – Since radio-induced lymphocyte apoptosis values, which predict tolerance to radiotherapy, could be assessed by two laboratories and showed a high level of robustness and consistency, we can suggest that this assay be extended to any laboratories that use the same technique.

Mots clés :
Test de quantification de l'apoptose radio-induite des lymphocytes
Tolérance à la radiothérapie
Validation de plateforme

RÉSUMÉ

Objectif de l'étude. – Même si les nouvelles techniques de radiothérapie permettent une escalade de dose en épargnant les tissus sains, certains patients souffrent de toxicité radio-induite grave. Il est nécessaire de sélectionner ces patients « radiosensibles » afin de leur proposer une prise en charge adaptée. Une étude préliminaire a démontré que l'apoptose radio-induite des lymphocytes circulants était corrélée avec une toxicité tardive de grade 2 ou 3. Plusieurs essais prospectifs concernant des patients atteints d'un cancer du sein, de la prostate ou encore de la sphère ORL sont en cours. Afin de développer cette technique de détection de la quantification de l'apoptose radio-induite des lymphocytes sur plusieurs plateformes, l'objectif de cette étude était de tester les valeurs quantifiées par deux plateformes.

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Matériels et méthodes. – À partir de prélèvements sanguins de 25 patients recevant un traitement pour un cancer de la sphère ORL, les résultats de la quantification de l'apoptose radio-induite des lymphocytes obtenus au laboratoire référent de Montpellier (IRCM, Inserm U1194, France : Lab1) ont été comparés à ceux du laboratoire de l'Institut de cancérologie de Lorraine (ICL, France : Lab2). Une série de méthodes statistiques a mesuré la concordance des valeurs mesurées en insu par les deux laboratoires. La présence de biais potentiels a été évaluée par un graphe de Bland–Altman.
Résultats. – L'ensemble des tests statistiques démontrent une concordance des valeurs de la quantification de l'apoptose radio-induite des lymphocytes obtenues par les deux sites sans biais majeurs.
Conclusions. – La quantification de l'apoptose radio-induite des lymphocytes, facteur prédictif de la tolérance des patients aux radiations ionisantes, peut être pratiquée par différents laboratoires puisque cette étude démontre de façon robuste des résultats équivalents d'un test portant sur un même échantillon.

1. Introduction

The outcome of radiation therapy is balanced between the probability of tumour control and the risk of injury to normal tissue. In the era of modern treatment delivery, increasing the dose delivered to the target to improve local control might be modulated by the patient's intrinsic radio-sensitivity. In other words, patients who are not identified as "hyper-reactive" may receive higher localized dose to improve local control.

Widely accepted international recommendations, such as QUANTEC publications recommend dose constraints for each organ at risk to limit radio-induced toxicity [2]. There is wide variation among patients in normal tissue tolerance and therefore in the frequency and intensity of radio-induced late toxicity. About 5% of patients receiving radiotherapy developed abnormally severe late toxicity [3,5,7]. Consequently, there is a growing interest in personalized radiotherapy by selecting patients with excessive radio-sensitivity before treatment.

In 1996, a predictive assay based on radio-induced lymphocyte apoptosis quantification was developed [6,11]. In 2005, the first prospective study, which included 399 patients treated with curative-intent radiotherapy, was published and highlighted the significant correlation between CD4 and CD8 T-lymphocyte apoptosis and grade 2 or 3 radiation-induced late toxicity [10]. Overall, test values greater than 16% were significantly associated with a very low risk of grade ≥ 2 late toxicity. Inversely, test values below 10% were strongly associated with severe late complications. Several trials confirmed these data for various tumour locations (breast, prostate, head and neck, etc.) [12]. Recently, Foro et al. confirmed the significant correlation between radio-induced apoptosis of CD4+ T-lymphocytes and genitourinary toxicity in 214 patients with prostate cancer treated with radiotherapy [8].

This test was used as a stratification factor in a randomized trial and confirmed its ability to identify patients at high risk of developing radiation-induced late toxicity [1].

In all of these studies, radio-induced lymphocyte apoptosis were evaluated at one laboratory. It is of great interest to develop this assay at several technical platforms in order to extend its use to a larger number of patients before radiotherapy.

In this study involving 25 patients, we compared the radio-induced lymphocyte apoptosis results obtained from the laboratory of Montpellier (IRCM, Inserm U1194, France), considered as the reference (referred to as Lab 1), with those from the laboratory located at the Institut de Cancérologie de Lorraine (ICL, France), referred to as Lab 2. Our goal was to demonstrate that test values obtained from these two platforms were comparable.

2. Materials and methods

2.1. Patients

Between June 2010 and September 2014, 25 patients with head and neck carcinoma and enrolled in an ancillary study (PARATOXOR) of a prospective quality-of-life trial participated in this agreement analysis. The PARATOXOR protocol was approved by the institutional ethics committees and all patients had provided written informed consent.

Before starting radiotherapy, two samples of blood (7 mL) were collected in heparinized tubes. Samples were sent at room temperature using a specialized carrier (Transporteo, France) and were delivered within 18 hours to both laboratories.

2.2. Radiation-induced apoptosis

Details of the assay have been described elsewhere [11]. Briefly, blood was diluted 1:10 in RPMI 1640 medium containing 20% foetal bovine serum, irradiated at 0 Gy (sham samples) or 8 Gy using a linear accelerator (Saturne 42, Varian in Montpellier and Clinac iX, Varian for ICL). After 48 hours of incubation, cells were labelled using fluorescein isothiocyanate (FITC)-conjugated anti-CD8 monoclonal antibodies (Becton Dickinson, France), red blood cells were lysed, and the DNA was stained with propidium iodide. Samples were measured in duplicate using a flow cytometer (FACS scan for Montpellier, and FACScalibur for ICL), and data analysis was performed using Cell Quest software for the two sites (Becton Dickinson, San Jose, CA). Apoptotic CD8 T-lymphocytes were defined as positively selected FITC-labelled cells (FL2 greater than 10^1) and presenting a DNA content lower than 1*n*.

Each apoptosis evaluation was carried out in triplicate. The mean values for each patient were described in supplementary data Table. The radio-induced lymphocyte apoptosis measurements were expressed as mean percentages after 8 Gy minus mean percentages in non-irradiated controls (0 Gy).

2.3. Statistics

Different statistical methods were used to measure the agreement between the radio-induced lymphocyte apoptosis data from the two laboratories (quantitative data).

Graphical methods with scatter plots were used to visualize how the data from the two laboratories deviated from the line of perfect agreement. The Bland–Altman plot was used to identify potential bias. In order to face with the interchangeability of measurement methods, we used an alternative approach based on estimation of the mean and standard deviation of differences between measurements [4].