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Prognostic value of blood-biomarkers related to hypoxia, inflammation, immune response and tumour load in non-small cell lung cancer – A survival model with external validation

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ABSTRACT

Aim: Improve the prognostic prediction of clinical variables for non-small cell lung cancer (NSCLC), by selecting from blood-biomarkers, non-invasively describing hypoxia, inflammation and tumour load.
Methods: Model development and validation included 182 and 181 inoperable stage I-IIIB NSCLC patients treated radically with radiotherapy (55.2%) or chemo-radiotherapy (44.8%). Least absolute shrinkage and selection operator (LASSO), selected from blood-biomarkers related to hypoxia [osteopontin (OPN) and carbonic anhydrase IX (CA-IX)], inflammation [interleukin-6 (IL-6), IL-8, and C-reactive protein (CRP)], and tumour load [carcinoembryonic antigen (CEA), and cytokeratin fragment 21-1 (Cyfra 21-1)]. Sequent model extension selected from alpha-2-macroglobulin (α2M), serum interleukin-2 receptor (sIL2r), toll-like receptor 4 (TLR4), and vascular endothelial growth factor (VEGF). Discrimination was reported by concordance-index.
Results: OPN and Cyfra 21-1 (hazard ratios of 3.3 and 1.7) significantly improved a clinical model comprising gender, World Health Organization performance-status, forced expiratory volume in 1 s, number of positive lymph node stations, and gross tumour volume, from a concordance-index of 0.66 to 0.70 (validation = 0.62 and 0.66). Extension of the validated model yielded a concordance-index of 0.67, including α2M, sIL2r and VEGF (hazard ratios of 4.6, 3.1, and 1.4).
Conclusion: Improvement of a clinical model including hypoxia and tumour load blood-biomarkers was validated. New immunological markers were associated with overall survival. Data and models can be found at www.cancerdata.org (http://dx.doi.org/10.17195/candat.2016.04.1) and www.predictcancer.org.

In the last three decades, lung cancer has been the leading cause of cancer deaths [1]. To increase the survival of lung cancer patients, we have witnessed an improvement of radiotherapy techniques and more effective (chemo)radiotherapy schemes (i.e., introduction of concurrent treatment) [2–4]. Attempts have been made to develop more accurate risk stratification for non-small cell lung cancer (NSCLC) patients, which would lead to more tailored, individualized and personalized care, avoiding over or under-treatment, by means of a radiation oncology based on multifactorial Decision Support Systems [5,6]. Therefore, the investigation of new prognostic parameters derived from, but not limited to, anatomic, molecular and functional imaging, genomics, and proteomics is warranted [7–9].

The analysis of biomarkers, including proteins, is a fast-developing, promising and challenging area of research, permitting the prediction or description of the evolution of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [10]. Oncoproteins are produced by tumour cells or in response to their presence, and may be released into the bloodstream of cancer patients. As tissue sampling is often not possible in lung cancer patients, blood sample collection by venipuncture is an attractive alternative, which is safe and easy to implement [10]. Blood-biomarkers reflect dissimilarities of the tumour microenvironment, are linked to disease prognosis and

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response to treatment. Blood-biomarkers, that can be measured in daily clinical practice and have been shown to be associated with treatment outcome were first identified in studies comprising large datasets. Based on this criterion, those which biological functions are related to processes of hypoxia [osteopontin (OPN) and carbonic anhydrase IX (CA-IX)]; inflammation [interleukin 6 (IL-6), IL-8, and C-reactive protein (CRP)], and tumour load [carcinoembryonic antigen (CEA) and cytokeratin fragment (CYFRA 21-1)], were analysed and externally validated [11–25]. As an exploratory step we investigated additional blood-biomarkers, including those related to immunological response, which could therefore be incorporated into immunotherapy assessment studies: alpha-2-macroglobulin (α2M), serum IL-2 receptor (sIL2R), toll-like receptor 4 (TLR4), and vascular endothelial growth factor (VEGF) [14,26–35].

Patients and methods

Development dataset

The development cohort included 195 stage I-IIIb NSCLC patients treated with (chemo)radiotherapy between October 2003 and October 2008. Clinical data and blood samples were prospectively collected to ensure standardization. Exclusion criteria included surgery or palliative treatment, and insufficient material to perform blood measurements (OPN, CA-IX, IL-6, IL-8, CRP, CEA, and CYFRA 21-1). All patients participated in the Biobank project (Clinical trials.gov identifiers: NCT00181519, NCT00573040, and NCT00572325) launched in 2003, and provided written informed consent. One hundred and eighty-two patients were treated according to dissimilar radiotherapy (RT) regimens, with a minimum dose of 50 Gy:

1. Forty-nine patients (26.8%) received the standard external beam radiation therapy (EBRT) protocol used until August 2005, of either 70 Gy (Stage I–II) or 60 Gy after induction chemotherapy (Stage III) in once-daily fractions of 2 Gy.
2. One hundred and one patients (55.2%) were treated with EBRT only according to the protocol as of August 2005, with an individualized dose delivered in fractions of 1.8 Gy twice daily, until normal tissue dose constraints were met (e.g., mean lung dose, or maximum dose to the spinal cord) [36].
3. Thirty-three patients (18%) received concurrent (chemo)radiotherapy with a total dose of 45 Gy, delivered in fractions of 1.5 Gy twice daily, followed by an individualized dose of 8 to 24 Gy delivered in fractions of 2.0 Gy once daily, again limited by the dose to surrounding organs at risk [37].

Validation dataset

The validation cohort consisted of 200 NSCLC patients with same characteristics as the development cohort, treated between March 2007 and September 2013. Measurements included the above mentioned blood-biomarkers plus VEGF, α2 M, TLR4 and sIL2R (Clinicaltrials.gov identifier: NCT01936571). One hundred and eighty-one patients received a minimum dose of 50 Gy and were treated as follows:

1. Sixty-eight patients (37.6%) received radiotherapy alone according to the protocol as of August 2005, with an individualized total dose delivered in fractions of 1.8 Gy twice daily, limited by the mean lung dose or the spinal cord dose [36].
2. One hundred and one patients (55.8%) received concurrent chemo-radiotherapy scheme for a prescribed dose of 45 Gy, followed by an individualized dose ranging from 8 to 24 Gy, delivered in fractions of 2.0 Gy once daily, again limited by the dose to surrounding organs at risk [37].
3. Twelve patients (6.6%) followed the Phase II Positron Emission Tomography (PET) boost trial (clinicaltrals.gov identifier NCT01024829), in which a dose escalation protocol was based on the Fluorine-18-Fluorodeoxyglucose distribution of the PET scans [38].

Radiation treatment

Patients were irradiated in accordance with local protocols and stage of the disease. No elective nodal irradiation was performed and irradiation was delivered 5 days a week [39]. Radiotherapy planning was performed on a XiO system (Computerised Medical Systems) until July 2012, using a convolution–superposition algorithm with inhomogeneity corrections and according to International Commission on Radiation Units & Measurements 50 guidelines. As of July 2012, radiotherapy planning was performed using RapidArc (Eclipse version 11.0), with a type B dose calculation algorithm (AcurosXB-10.0).

Endpoint

Study endpoint was overall survival (OS) calculated from start of RT until the date of death or last follow-up. Survival information was retrieved from "Gemeentelijke Basis Administratie" (GBA), the decentralized population registration system in the Netherlands. A patient who was alive at the end of the study was considered right-censored.

Blood-biomarker measurement

Blood-biomarkers measurements of the development dataset can be found elsewhere [40]. Measurements of the validation cohort were performed in a certified laboratory, using commercially available kits, in order to easily translate the results into clinical practice. For each patient, 3 aliquots of 0.5 ml of serum and 3 aliquots of 1.5 ml of plasma were available, which had been collected before the first fraction of radiotherapy, processed using standard protocols and finally stored in the institutional biobank. Measurements in plasma were performed using enzyme-linked immunosorbent assays for OPN (Quantikine Human Osteopontin Immuno assay; R&D Systems, Minneapolis, MN), CA-IX (Nuclea Diagnostics, Cambridge, MA), VEGF (R&D Systems), and TLR4 (MyBioSource, San Diego, CA). Measurements in serum for IL-6 and IL-8 were determined on Immulite XPi 2000 with a solid phase, enzyme labelled, chemoluminescence sequential immunometric assay (Siemens Medical Solutions Diagnostics, LA), for CRP on Cobas 8000 using an immunoturbimetric assay (Roche Diagnostics, Mannheim, Germany), for CEA on Immulite XPi using a solid-phase, two-site sequential chemoluminescent immunometric assay (Siemens Medical Solutions Diagnostics), for CYFRA 21-1 on Kryptor with a sandwich immuno-fluorescent assay (Brahms, ThermoFisher, Hennigsdorf, Germany), for α2M on BN ProSpec using immunonephelometric assays (Siemens Medical Solutions Diagnostics, LA, USA), and for sIL2R using an enzyme-linked immunosorbent assay (Diaclone, Basancon Cedex, France).

The analytes OPN, CA-IX, VEGF and TLR4 were assayed in plasma in duplicate using a Victor multilabel counter (Perkinelmer, Turku, Finland), while all other biomarkers were measured in singletons.

Descriptive statistics

Comparison of the development and validation datasets distributions was performed using a χ^2 test for categorical variables and a Student *t*-test for the continuous ones. Prior to this a variable transformation on the gross tumour volume (GTV) and blood

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