Cytokine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

A novel cytokine profile associated with cancer metastasis to mediastinal and hilar lymph nodes identified using fine needle aspiration biopsy – A pilot study

Ali I. Saeed [a,f,*], Fares Qeadan [b], Akshay Sood [a], Dorothy J. VanderJagt [c], Shiraz I. Mishra [d], Deirdre A. Hill [e], Tobias Peikert [f], Mohan L. Sopori [g]

[a] Department of Internal Medicine, Pulmonary Critical Care and Sleep Medicine, University of New Mexico, Albuquerque, NM, United States
[b] Department of Internal Medicine, Division of Epidemiology, Biostatistics and Preventive Medicine, University of New Mexico, Albuquerque, NM, United States
[c] Department of Biochemistry and Molecular Biology, University of New Mexico, Albuquerque, NM, United States
[d] Departments of Pediatrics and Family and Community Medicine, University of New Mexico, University of New Mexico Cancer Center, Albuquerque, NM, United States
[e] Cancer Research and Treatment Center, Departments of Internal Medicine, Division of Epidemiology, University of New Mexico, Albuquerque, NM, United States
[f] Department of Internal Medicine, Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN, United States
[g] Lovelace Respiratory Research Institute, Albuquerque, NM, United States

ARTICLE INFO

Article history:
Received 9 January 2016
Received in revised form 10 August 2016
Accepted 14 August 2016
Available online xxxx

Keywords:
Lymphatic metastasis
Fine needle aspiration biopsy
Endobronchial ultrasound
Biomarker profile

ABSTRACT

Cancer metastasis to the lymph nodes is indicative of a poor prognosis. An endobronchial ultrasound-guided fine needle aspiration (EBUS-FNA) biopsy is increasingly being used to sample paratracheal lymph nodes for simultaneous cancer diagnosis and staging. In this prospective, single-center study, we collected dedicated EBUS-FNA biopsies from 27 patients with enlarged paratracheal and hilar lymph nodes. Cytokines were assayed using Bio-Plex Pro human cancer biomarker panels (34 cytokines), in a Bio-Rad 200 suspension array system. A mean cytokine value was taken from each subject with more than 1 lymph node station EBUS-FNA biopsies. Malignant and benign histologic diagnoses were established in 16 and 12 patients, respectively. An initial analysis using the Kruskal–Wallis test with Sidak correction for multiple comparisons, showed significant elevation of sVEGFR-1, IL-6, VEGF-A, Angiopoeintin-2, uPA, sHER-2/neu and PLGF in malignant lymph node samples compared to benign samples. The univariate logistic regression analyses revealed that 6 cytokines were significant predictors and 1 cytokine (PLGF) was marginally significant for discrimination between benign and malignant samples. The prediction power of these cytokines as biomarkers were very high according to the area under the ROC curve. Multiple logistic regression for subsets of the seven cytokine combined; provided an almost complete discrimination between benign and malignant samples (AUC = 0.989). For screening and diagnostic purposes, we presented the optimal discrimination cut-off for each cytokine: sVEGFR-1 (2124.5 pg/mL), IL-6 (40.2 pg/mL), VEGF-A (1060.1 pg/mL), Angiopoeintin-2 (913.7 pg/mL), uPA (248.1 pg/mL), sHER-2/neu (5010 pg/mL) and PLGF (93.4 pg/mL). For the very first time, a novel cytokine profile associated with cancer metastasis to the paratracheal lymph nodes were reported.

1. Introduction

Cancer metastasis to the lymph nodes is associated with poor prognosis, especially in patients with lung cancer [1]. Despite significant improvements in the management and treatment of cancer patients, most deaths are due to chemo-resistant metastasis [2]. The phenotypic drift [3,4] and intrinsic heterogeneity of cancer cells [5,6] contribute to the potential for metastasis and development of resistance to standard treatments; therefore, it is important to understand the pathogenesis of cancer metastasis on a cellular and molecular level with a goal to develop novel therapies directed towards prevention and treatment [7]. The clinical advantage for using cytokines as biomarkers in identifying a cancer metastasis status, relative to histopathological assessment, is the novel ability to identify discordant lymph nodes (elevated metastasis related cytokines with non-diagnostic or limited biopsy

* Corresponding author at: MSC 10-5550, 1 University of New Mexico, Albuquerque, NM 87131, United States.
E-mail address: asaeed@salud.unm.edu (A.I. Saeed).

http://dx.doi.org/10.1016/j.cyto.2016.08.011
1043-4666/

sample for histopathologic diagnosis). In clinical practice, early-stage lung cancer patients are serially followed after surgery for recurrence of cancer.

An increase in tumor size is associated with an increased incidence of metastasis to the regional lymph nodes [8]. Lymph node initially act as a barrier to the circulating cancer cells. Antigenicity of circulating cancer cells trigger the development of a complex immune reaction with histopathology indicative of follicular hyperplasia, sinus histiocytosis, lymph angiogenesis and foreign body granuloma formation [9]. The cross talk between cancer cells trying to seed into the lymph node changes over time and a nurturing microenvironment in the lymph node allows for growth of tumor cells [9]. The factors leading to this change are still unknown.

An endobronchial ultrasound allows for real time fine-needle aspiration (FNA) biopsy of mediastinal and hilar lymph nodes. Nakajima and colleagues reported expression of 2 cytokines in the FNA biopsy that correlated with ultrasound characteristics of the mediastinal lymph node [10,11]. Their work illustrated that measuring cytokines in the mediastinal lymph node aspirate using EBUS is feasible. The current study is based on the hypothesis that a cytokine signature obtained from FNA biopsy of a mediastinal or hilar lymph node with cancer metastasis differs from that of a benign lymph node. We aim to determine if we can reliably identify a comprehensive panel of cytokines from lymph node aspiration biopsy that can explain the changes in the lymph node microenvironment with cancer metastasis. In addition, we outline a standardized approach for collection, storage and processing of lymph node aspiration biopsy using multiplex technology.

2. Methods

2.1. Study design

In this prospective single-center pilot study, 28 patients consented for the study. We collected dedicated EBUS-FNA biopsies from 27 patients. In one patient, the EBUS bronchoscopy procedure was terminated for the patient's safety due to severe hypoxia; this patient was later diagnosed with metastatic lung cancer involving the pleural space.

2.2. Subjects

Patients were recruited using a convenience-sampling method from an outpatient specialized lung cancer clinic at the University of New Mexico Hospital (UNMH). These patients were found to have enlarged mediastinal or hilar lymph nodes (>10 mm) on computed tomography of chest (CT chest) or positron emission tomography scan (PET scan). The eligibility criteria included: the ability to communicate in English, at least 18 years of age, and enlarged mediastinal and hilar lymph nodes (>10 mm). Information regarding age and smoking history were obtained from each patient before the procedure. The physician performing the procedure obtained informed consent from each patient. The Human Research Review Committee of the University of New Mexico (UNM) Health Science Center and TRICORE Reference Laboratory Research Review Committee approved the study protocol.

2.3. Collection of aspiration biopsy samples

EBUS-FNA specimens (obtained at the third or fourth biopsy) were collected from mediastinal and hilar lymph nodes. The aspiration biopsy specimens were collected in sterilized 2-milliliter (mL) vials. The collection protocol required use of air in a 5 mL syringe to push the specimen out of the endobronchial aspiration biopsy needle, so as to avoid excessive dilution. If necessary, a metal stylet was used to push the specimen out of the biopsy needle. The specimens were immediately placed on ice and delivered within 2 h to the T1 (translational science) laboratory of the UNM Clinical and Translational Science Center (CTSC). These samples were stored at −72 °C until the day when cytokine analysis was performed.

2.4. Histopathology of aspiration biopsy

A staff pathologist at the UNMH made the histologic diagnosis from the lymph node aspiration biopsy. Patients with an FNA biopsy showing a polymorphous population of lymphocytes or inadequate samples were referred for a mediastinoscopy. If a mediastinoscopy was not performed or not offered due to co-morbidities, these patients were followed with a repeat contrast enhanced computed tomography scan of the chest in 3- to 6-month intervals to confirm stability or resolution of mediastinal and hilar lymph nodes. If the histopathology diagnosis was other than a malignancy, the respective sample was included for cytokine and data analysis as a benign lymph node. For lymph nodes with cancer metastasis, the primary sites of cancer were recorded for descriptive analysis.

2.5. Analysis of lymph node aspirates

A Bio-Rad Bioplex 200 suspension array system (Bio-Rad Laboratories, Inc., 171-000201) was used for the cytokine analysis. This bead based Luminex technology allows for multiple protein analyses in a single sample. The data are reported as median fluorescence intensity (MFI) and concentration (pg/mL). The concentration of unknown analytes bound to a fluorescent bead is proportional to the MFI of the reporter signal. The experimental protocol allows for simultaneous reporting of standards, controls, blanks and cytokines of interest in duplicate. This multiplex technology has multiple advantages over enzyme-linked immunosorbent assay (ELISA) as multiple protein analytes can be measured simultaneously in a smaller sample. A standard concentration experiment is performed simultaneously; thus, increasing the reliability of the experiment.

Efforts were made to process all specimens simultaneously according to the Bio-Plex human cancer biomarker assay instructions and experiment protocol. Bio-Rad Bioplex Data Pro software® (Bio-Rad Laboratories, Inc., 171-001513) was used for data analysis to identify the extreme values, data distribution and selection of range.

2.6. Statistical analysis

Statistical analyses were done with SAS software, version 9.4 (SAS Institute, Cary, NC). We used the median and interquartile range (25th and 75th percentiles) to describe the distribution of each cytokine within the benign and malignant groups. A mean cytokine value was taken for each subject with more than one lymph node EBUS-FNA biopsies. To compare the cytokine expression between the benign and malignant groups, non-parametric analyses were performed using the Kruskal–Wallis test with Sidak correction for multiple comparisons, uniformly a more powerful correction procedure than the Bonferroni correction [26]. We used univariate logistic regression modeling to assess the likelihood of having malignant cancer by each biomarker. Odds ratio (OR) estimates for each biomarker derived from the univariate models with two-sided 95% confidence intervals (CIs) that were based on Wald's statistic, such that Δ specifies the units of change for which the odds ratios were computed. The P values were provided as a measure of statistical significance, such that a P value of less than $\alpha = 0.05$ is an indication for statistical significance. We used the area under the ROC curve (AUC) as a measure of accuracy. To deter-

Please cite this article in press as: A.I. Saeed et al., A novel cytokine profile associated with cancer metastasis to mediastinal and hilar lymph nodes identified using fine needle aspiration biopsy – A pilot study, Cytokine (2016), http://dx.doi.org/10.1016/j.cyto.2016.08.011