Gynecologic Oncology 124 (2012) 192–198

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Prognostic significance of tumor-infiltrating T cells in ovarian cancer: A meta-analysis

Wei-Ting Hwang [a,b,1], Sarah F. Adams [a,1], Emin Tahirovic [b], Ian S. Hagemann [c,2], George Coukos [a,*]

[a] Ovarian Cancer Research Center, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104, USA
[c] Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

ARTICLE INFO

Article history:
Received 29 July 2011
Accepted 28 September 2011
Available online 29 October 2011

Keywords:
Tumor-infiltrating lymphocytes
TIL
Ovarian cancer
Prognosis
Meta-analysis

ABSTRACT

Objective. The presence of T cells within the epithelial component of tumors, as histologic evidence of anti-tumor immunity, has been associated with a survival advantage in multiple studies across diverse patient cohorts. We performed a meta-analysis of studies evaluating the prognostic value of tumor-infiltrating lymphocytes (TIL) on survival among women with ovarian cancer and to investigate factors associated with variations in this effect, including patient characteristics, surgical outcomes, tumor histology, and study protocols.

Method. Published studies that evaluated the association between TIL and patient survival were identified. Descriptive statistics, outcome data, and study quality were extracted from studies that met inclusion criteria. Hazard ratios and 95% confidence intervals were pooled across studies using the random-effects model. Publication bias was investigated using a funnel plot and heterogeneity was assessed with subgroup analysis and I^2 statistics.

Results. Ten suitable studies comprising 1815 patients with ovarian cancer were analyzed. Our results demonstrate that a lack of intraepithelial TILs is significantly associated with a worse survival among patients (pooled HR: 2.24, 95% CI; 1.71–2.91). Variations in the prognostic value of TIL status based on debulking status, scoring method, and geographic regions were identified.

Conclusions. Intraepithelial TILs are a robust predictor of outcome in ovarian cancer and define a specific class of patients, whose distinct tumor biology should be taken into account in devising appropriate therapeutic strategies.

Introduction

Epithelial ovarian cancer is a heterogeneous disease, with significant variation in both the presentation and response to therapy. Prognosis is affected by patient factors, such as age or genetic background, as well as tumor characteristics, including stage, grade, histologic subtype, and chemotherapy sensitivity [1,2]. Recent studies have also identified immunologic biomarkers of prognosis, with longer survival times documented among women with histologic evidence of an anti-tumor immune response. Although T cells are present in the stroma of most tumor specimens, a survival advantage has been associated specifically with the presence of T cells in epithelial tumor islets (intraepithelial tumor-infiltrating lymphocytes, TILs) [3]. In addition to correlations with clinical outcome, evidence favoring an active role for TILs in tumor clearance is provided by data demonstrating that these are oligoclonal T cell populations that recognize tumor antigens *ex vivo* and secrete cytokines characteristic of effector cells [4–7]. With the emergence of immunotherapeutic strategies for the treatment of ovarian cancer, it will be important to validate immunologically relevant tumor biomarkers to optimize patient selection for clinical trials and to prospectively track responses to immunotherapeutics [8,9].

Although all studies of patients with ovarian cancer have described a prognostic advantage associated with intraepithelial TILs, differences in the measurement and characterization of TILs have limited the clinical utility of this biomarker. Questions remain as to whether inconsistencies in results derive from differences in study methodology or whether variable outcomes among diverse patient cohorts illustrate underlying biologic or environmental modifiers of anti-tumor immunity. For example, while some reports have quantified all $CD3^+$ T cells as TILs, others have focused specifically on cytotoxic $CD8^+$ T cells. Additionally, the criteria used to score tumors as TIL-positive or TIL-negative have not been consistent across studies. It is also unclear whether associations between TIL status and survival varied according to the standard prognostic factors, such as age, stage, histology, or surgical outcomes.

The objective of this study is to review the prognostic significance of intraepithelial TILs for overall survival across diverse cohorts of women with ovarian cancer using meta-analytical tools. Our secondary objective is to identify patient, tumor, or methodological characteristics that may explain the variations in the published findings.

* Corresponding author at: Ovarian Cancer Research Center, University of Pennsylvania, Room 1315, Biomedical Research Building II/III, 421 Curie Blvd, Philadelphia, PA, 19104-6160 USA. Fax: +1 215 573 5129.
E-mail address: gcks@mail.med.upenn.edu (G. Coukos).
[1] Contributed equally to this work.
[2] Present Address: Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110, U.S.A.

0090-8258/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2011.09.039

Methods

We followed guidelines for the design, analysis, and reporting of meta-analyses of observational studies published by the MOOSE group [10].

Search strategy

Studies published before December 2010 were identified in PubMed using the following search terms: "ovarian cancer" and "TIL" "lymphocytes", and "T cell". There was no language restriction. The references of all publications were reviewed to identify additional relevant studies.

Study selection

Studies that met the following criteria were included in the meta-analysis: studies must have (1) been published as original articles; (2) evaluated human subjects; (3) investigated CD3 and/or CD8 lymphocytes in ovarian cancer; (4) reported disease-specific or overall survival; and (5) contained the minimum information necessary to estimate the effects (*i.e.*, hazard ratio) and a corresponding measure of uncertainty (*i.e.*, confidence interval, P-values, standard errors or variance). As an additional criterion, when a single population was reported in multiple reports, only the report with the most complete data was included to avoid duplication.

Data extraction

Using a predefined form, data on study cohorts, methodology and results were extracted. The author, year of publication, and region where each study was conducted were noted. The collected patient or tumor characteristics including the number of women in each cohort, the duration of follow-up (mean or median), the ages at the time of surgery (mean, median), and the surgical outcomes (optimal or suboptimal cytoreduction), distributions of stage, grade, and histologic subtype, were recorded. Methodology characteristics analyzed included the markers used (CD3 or CD8), scoring protocols to identify TILs. The number or distribution of TIL-positive or TIL-negative cases, and results of univariate and/or multivariate survival analyses (*e.g.*, log rank test, Cox proportional hazards model) were extracted. We did not contact authors for additional data.

Measures

The endpoint used in this meta-analysis is overall survival. In the absence of overall survival data, disease-specific survival was substituted because these two measures are expected to be similar for ovarian cancer patients. For CD3 and/or CD8 TIL, study-defined binary variables indicating either the presence (*versus* absence), positive (*versus* negative), or high (*versus* low) marker expression were used and described as "TIL-positive" or "TIL-negative" for this meta-analysis.

Assessment of study quality

Study quality was independently rated by two coauthors (WH, ET). Because there is no validated instrument to measure study quality for prognostic marker studies in an observational setting, we adapted the Newcastle–Ottawa Scale and the framework suggested by Altman [11,12]. Briefly, this instrument assesses the quality of studies based on study population (three criteria), prognostic variables (four criteria), outcome measures (two criteria), study duration (one criteria), and statistical analysis (two criteria). Each of the criteria was rated on a three-level scale; zero (no report or criterion not met), one (criterion partially met), or two (criterion was met). Scores from individual criteria were summed and divided by the maximum possible score to produce a total score between zero and one, where higher scores denote greater study quality. The final quality ratings were based on the averaged score (95% limits of agreement: −0.22, 0.11).

Statistical analysis

The hazard ratio (HR) was used as a measure of the prognostic value, and defined as the hazard of death for women with TIL-negative tumors over the hazard of death for women with TIL-positive tumors, so that a hazard ratio >1 indicated an elevated risk of death in cases lacking intraepithelial TIL. Following the method described in Parmar et al. [13], the log-hazard ratio and its standard error for each study were derived. All but one study reported results of a Cox regression analysis; for the remaining study, the log-hazard ratio and its standard error were estimated indirectly based on the reported P-value for the log rank test and the number of deaths observed in the study. If results of both univariate and multivariate Cox regression analyses were reported, we used estimates from the multivariate Cox regression model for a more direct estimate of the effect of TIL after controlling for potential confounding variables. In two studies where results for both CD3 and CD8 were reported, the estimates based on CD8 markers were used for the primary analysis. To account for heterogeneity among studies, random effects models were used to estimate pooled HRs [14]. The 95% confidence interval (CI) for the pooled HR was reported. Homogeneity of effects across studies was assessed using I^2 statistics [15]. This statistic describes the percentage of total variation across studies that are due to heterogeneity rather than chance (25% low heterogeneity, 50% medium, 75% high).

In secondary analyses, pooled HRs were estimated by specific TIL markers (CD3, CD8). One study which did not distinguish between CD3 and CD8 was included in the CD8 analysis. Subgroup analyses were carried out to investigate potential sources of between-study heterogeneity and to assess whether conclusions were sensitive to restricting studies to subgroups that might have different prognostic effects. Subgroups were defined according to TIL scoring algorithm (zero *versus* >0), specimen processing (paraffin-embedded, tissue micro-arrays (TMA), cryosection), debulking status (optimal only *versus* mixed), histology (serous only *versus* mixed), stage (III/IV only *versus* mixed), grade (>75% grade three, *versus* less), and by geographic region (North America, Europe, Japan). Tests for effects-subgroup interaction were performed. Publication bias was evaluated by inspecting the symmetry of the funnel plot and formally tested with Begg's adjusted rank correlation test [16,17]. Statistical analysis was conducted with Stata version 11 (College Station, Texas) and Review Manager Version 5·0 (The Cochrane Collaboration).

Results

Study selection

Of 18 potentially eligible articles, ten met the inclusion criteria and were evaluated further. Fig. 1 provides a summary of the selection process [3,18–32]. The research quality among the selected studies was high; with median quality score of 0.86 (range 0.75 to 0.92).

Patient cohorts

Characteristics of patient cohorts from the analyzed studies are shown in Table 1. The median number of women evaluated per study was 142 (range 70 to 500), with a total of 1815 subjects across all studies. The mean age in all cohorts was similar, ranging from 55 to 62 years. The percentage of women with stage III–IV disease varied from 16.8% to 100%, with four studies including only advanced cases [3,19,31,32]. Most patients had serous tumors, with two studies focusing exclusively on women with serous cancers [19,31], and 41% to 100% of