Biomarkers for Predicting Complete Debulking in Ovarian Cancer: Lessons to Be Learned

CARSTEN LINDBERG FAGÖ-OLSEN[1], BENT OTTESEN[1], IB JARLE CHRISTENSEN[2], ESTRID HØGDALL[3], LENE LUNDVALL[1], LOTTE NEDERGAARD[4], SVEND-AAGE ENGELHOLM[5], SOFIE LEISBY ANTONSEN[1], MAGNUS LYDOLPH[6] and CLAUS HØGDALL[1]

Departments of [1]Gynaecology, [4]Pathology and [5]Radiation Oncology, and [2]The Finsen Laboratory and Biotech Research and Innovation Center (BRIC), Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [3]Department of Pathology, The Danish Cancer Biobank, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark; [6]Department of Clinical Biochemistry and Immunology, Division of Microbiology and Diagnostics, Statens Serum Institute, Copenhagen, Denmark

Abstract. *Aim: We aimed to construct and validate a model based on biomarkers to predict complete primary debulking surgery for ovarian cancer patients. Patients and Methods: The study consisted of three parts: Part I: Biomarker data obtained from mass spectrometry, baseline data and, surgical outcome were used to construct predictive indices for complete tumour resection; Part II: sera from randomly selected patients from part I were analyzed using enzyme-linked immunosorbent assay (ELISA) to investigate the correlation to mass spectrometry; Part III: the indices from part I were validated in a new cohort of patients. Results: Part I: The area under the receiver operating characteristic curve (AUC) was 0.82 for both indices. Part II: Linear regression analysis gave an R^2 value of 0.52 and 0.63 for transferrin and β2-microglobulin, respectively. Part III: The AUC of the two indices decreased to 0.64. Conclusion: Our validated model based on biomarkers was unable to predict surgical outcome for patients with ovarian cancer.*

Primary debulking surgery (PDS), followed by adjuvant platinum and taxane-based chemotherapy, has long been considered the standard treatment for patients with ovarian cancer, and the correlation between residual tumour after PDS and survival is well-established (1). Recently, the use of neoadjuvant chemotherapy, followed by interval debulking surgery, has been suggested as an alternative if cytoreduction to no residual disease is impossible with PDS (2). However, a reliable and reproducible method to determine which patients can be completely debulked has not yet been established. Several studies have attempted to produce a valid model predicting the surgical outcome of PDS using a variety of different approaches, such as different diagnostic imaging techniques, laparoscopy, clinical characteristics, biological markers, or a combination of these (3-15). No method has yet been accepted in the clinical setting (16). There are several obstacles to overcome before a model can be said to be adequate in predicting surgical outcome (17). Most importantly, the method has to be reproducible by others, which means that external validation is mandatory. To date, only results from a laparoscopy-based model have been validated by others, whereas external validation of a computed tomography-based model have been attempted, with very inconsistent results (18, 19). The method should also preferably be relatively cheap, easy to conduct, and non-invasive. Furthermore, the model should limit the possibility of major intra- or inter-observer bias. A model that potentially fits all these requirements is one based on biomarkers from a blood sample.

The role of proteomic biomarkers in ovarian cancer is still a subject of research (20). From the Danish Pelvic Mass Study, two indices based on proteomic markers were found to correlate well with progression-free and overall survival, respectively (21), and the Danish Index showed promising results in discriminating between malignant and benign ovarian masses (22). Additionally, the ovarian cancer risk index (OvaRi) was investigated as a predictor for incomplete cytoreduction on PDS (23). The OvaRi was constructed with a diagnostic purpose, but nevertheless, predicted complete tumor resection with a 73% sensitivity at 70% specificity. These

Correspondence to: Carsten L. Fagö-Olsen, Department of Gynaecology, Section 7821, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, Copenhagen 2100, Denmark. Tel: +45 28784878, e-mail: carstenlo@gmail.com

Key Words: Ovarian cancer, primary debulking surgery, residual tumour, biomarkers, proteomics, diagnostic accuracy.

results indicate that some correlation between proteomic markers and dissemination of ovarian cancer exists.

We hypothesised that the proteomic profile of a tumor may reflect its intrinsic behaviour in regard to the tumor's capability to grow and metastasise in a pattern that impairs the probability of complete tumour removal–for instance, severe infiltrative growth.

The primary aim of our study was to construct a clinically-applicable model using proteomic biomarkers and other clinically-available characteristics to determine the probability of complete tumor debulking with PDS and secondly, to validate our model in a new cohort of consecutive patients.

Patients and Methods

The patients in this study were previously enrolled in the Danish Pelvic Mass Study. Details about the study and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) measurements have previously been reported in detail (21). Briefly, all patients were referred with suspicion of ovarian cancer between 2004 and 2012. Blood samples were collected within two weeks of surgery and handled according to biobanking guidelines. After initial examination, patients were referred for PDS with the goal of complete debulking. The presence of residual tumour was estimated by the operating gynaecologist. Exclusion criteria were pregnancy, previous cancer, and borderline pathology. The Danish Ethical Committee approved the protocol (KF01-22703 and KF01-143/04).

Our study consisted of three parts. In the pilot study (part I), quantitative data about seven biomarkers (β2-microglobulin, transferrin, apolipoprotein A1, cysteinylated transthyretin, hepecidin, internal fragment of inter-α-trypsin inhibitor IV, and connective tissue-activating protein) previously analyzed by SELDI-TOF-MS were investigated for their individual association with complete debulking. All biomarkers were log-transformed (log base 2). Biomarkers with statistically significant associations were then combined with age and Cancer antigen 125 (CA125) in order to construct an index for complete tumour debulking with PDS. Logistic regression analysis with complete tumour debulking as the dependent variable was applied with the biomarkers as explanatory covariates. The logistic models were reduced excluding nonsignificant covariates in a stepwise fashion. The results are presented as sensitivity, specificity and the area under the receiver operating characteristic curve (AUC). Goodness-of-fit was assessed using the Hosmer-Lemeshow test and 5-fold internal cross validation.

In part II, we randomly selected 29 patients from part I and analysed their sera using enzyme-linked immunosorbent assay (ELISA). In contrast to ELISA, SELDI-TOF-MS is a method for research and not applicable in a clinical setting, hence ELISA was preferred in order to make our model clinically relevant. The sera had been stored in a freezer at −20˚C at the Danish Cancer Biobank and were analysed using laser nephelometry (BN ProSpec; Siemens Healthcare Diagnostics, Deerfield, IL, USA) with the assays, N latex β2-Microglobulin and N Transferrin (Siemens Healthcare Diagnostics).

Linear regression analysis with SELDI-TOF-MS as explanatory variable and ELISA as dependent variable was applied in order to investigate the association between the two methods.

Table I. *Baseline and surgical data for patients in the pilot and validation study.*

	Part I–Pilot study (n=100)	Part III–Validation study (n=138)	*p*-Value
Median age (IQR), years	65 (58-73)	66 (55-74)	0.903
ASA score >2; % (n)	23% (23)	13% (18)	0.045
ECOG PS >2; % (n)	5% (5)	3% (4)	0.402
Median CA125 units, (IQR)	818 (364-2131)	522 (178-1210)	0.019
Stage, % (n)			
IIC	0	1% (1)	
IIIB	8% (8)	12% (17)	
IIIC	65% (65)	73% (101)	
IV	27% (27)	14% (19)	0.011†
Serous histology, % (n)	87% (87)	86% (119)	0.894
Residual tumor, % (n)			
0	21% (21)	43% (59)	<0.001
≤1 cm	46% (46)	66% (90)*	0.002
>1 cm	54% (54)	34% (47)*	

†Comparison of stage IV. *Data missing for one patient. ECOG PS: Eastern Cooperative Oncology Group performance status; CA125: Cancer antigen 125; IQR: interquartile range.

Part III was the validation study in which we tested our model in a new cohort of consecutive patients. Sample size was determined based on the criterion that the half-width of the confidence interval for the AUC of the model should be less than 10%, resulting in a desired sample size of 140 patients. All patients were treated at the Department of Gynaecology of the Rigshospitalet Copenhagen University Hospital, which is a tertiary referral centre, and the gynaecologists from our oncological section who performed the surgery were experienced and specialised in gynaecological cancer surgery. Storage and analysis of sera were identical in parts II and III.

Tests for differences in baseline data between the cohorts in parts I and III were carried out using Mann–Whitney *U*-tests and chi-square tests where appropriate. The validation set was analysed using the regression coefficients from the pilot study. The significance level was set at 5% and analysis was carried out using SPSS version 19.0 (IBM SPSS Inc., Chicago, Illinois, USA) and SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

In part I, data from 100 patients were available. Baseline and surgical data are listed in Table I. In the reduced model, only transferrin was significantly associated with complete tumor debulking (p=0.0014). However, since the association for β2-microglobulin was borderline significant (p=0.0565), we decided to construct two indices. In both indices, CA125 and age increased the prognostic value. Hence, one index consisted of CA125, age, and transferrin (CAT_{index}), and the other consisted of CA125, age, β2-microglobulin, and transferrin ($CABT_{index}$), and these two indices were selected for the validation study. Results are presented in Table II.

Table II. *Predicative values for assessing complete tumour removal with primary debulking surgery for patients with ovarian cancer.*

	Part I–Pilot study (n=100)				Part III–Validation study (n=138)		
	p-Value	Sensitivity at 80% specificity	Specificity at 80% sensitivity	AUC	Sensitivity at 80% specificity	Specificity at 80% sensitivity	AUC
TRF	0.0014	57	57	0.75	15	33	0.53
B2M	0.046	43	36	0.66	40	46	0.65
Age (per 10 years)	0.014	45	30	0.66	33	43	0.65
CA125	0.005	45	37	0.69	47	26	0.64
CAT	0.003	73	68	0.82	27	43	0.64
CATB	0.002	70	68	0.82	27	42	0.64

CA125: Cancer antigen 125; TRF, transferrin; B2M, β2-microglobulin; CAT_{index}, index of CA125, age and TRF; $CABT_{index}$, index of CA125, age, B2M and TRF; AUC, area under the receiver operating characteristic curve. In Part I, the *p*-values are for the univariate analyses of the association between TRF, B2M, age and CA125 and complete tumor debulking (yes *versus* no). Following, these four components were used in the reduced models to construct the CAT and CABT indices.

In part II, R^2 were 0.52 and 0.63 for transferrin and β2-microglobulin, respectively.

Data and sera from 138 patients were available in part III. Baseline and surgical data are listed in Table I, and the prognostic values of both indices are listed in Table II. A notable difference between the two cohorts and decrease in predicative values were observed.

Discussion

In the present study, we aimed to construct a clinically-applicable model to determine the probability of complete tumor removal by PDS for patients with ovarian cancer, and we were encouraged by the promising results from parts I and II. However, after validation in a new cohort, we found we were unable to reproduce the initial results of our model. There are probably several explanations for this. The main reason may be that the cohorts in parts I and III were not comparable (Table I).

All patients except for one in part I were recruited between 2004-2007. In contrast, 93% of the patients in part III were recruited between 2008-2012. Between these two periods, the proportion of patients with advanced-stage ovarian cancer that were not treated with PDS, but instead referred for neoadjuvant chemotherapy, increased approximately by 300% (24). This case selection is also reflected in the different proportions of patients without residual tumour following PDS. In our study, six patients were not treated at the Rigshospital Copenhagen University Hospital, and hence, 98% of all the patients were treated in the same Institution by virtually the same physicians, but clearly, the altered referral practice may have affected our results. Nonetheless, in studies like our own, we believe that validation in a new cohort of consecutive patients that reflects the background population and everyday practice in the clinic is essential in order to evaluate the true accuracy of the model. Hence, we believe that the validation study was carried out *lege artis*.

Our failure to reproduce our initial results reflects the difficulty of referring results from one Institution to another and may explain why validation studies of similar types of research are lacking in the literature. Different criteria for referral of patients for PDS or neoadjuvant chemotherapy, routines in the preoperative assessments, surgical skills, and attitude to performing extensive surgery may differ too greatly between different hospitals to make it possible to construct a model that would apply in general.

In our model, we selected complete tumour removal as our outcome since we consider that this outcome should be the ambition of PDS, is most relevant in regard to survival, and is least vulnerable to bias caused by postoperative visual estimation by the operating gynaecologists. However, we also tested our two indices for their ability to predict residual tumour of less than 1 cm (data not shown) since some institutions consider this endpoint relevant. The prognostic value increased slightly but was still far from relevant.

In conclusion, after the investigation of seven proteomic biomarkers, we were not able to establish a reproducible model to determine the probability of complete tumor removal by PDS.

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

The Danish Cancer Biobank is acknowledged for biological material and for information regarding handling and storage. The Danish Gynaecological Cancer Database is acknowledged for acquisition of all data regarding patients and treatment. The Civil engineer Bent Bogh, the Inge Bogh Foundation and The Torben and Alice Frimodts Foundation are acknowledged for financial support of the ELISA tests.

References

1 Du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I and Pfisterer J: Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: A combined exploratory analysis of three prospectively randomized phase III multicenter trials by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer *115(6)*: 1234-1244, 2009.

2 Vergote I, Tropé CG, Amant F, Kristensen GB, Ehlen T, Johnson N, Verheijen RH, van der Burg ME, Lacave AJ, Panici PB, Kenter GG, Casado A, Mendiola C, Coens C, Verleye L, Stuart GC, Pecorelli S, Reed NS and the European Organization for Research and Treatment of Cancer–Gynaecological Cancer Group; NCIC Clinical Trials Group: Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. N Engl J Med *363(10)*: 943-953, 2010.

3 Angioli R, Plotti F, Capriglione S, Aloisi A, Montera R, Luvero D, Miranda A, Cafà EV, Damiani P and Benedetti-Panici P: Can the preoperative HE4 level predict optimal cytoreduction in patients with advanced ovarian carcinoma? Gynecol Oncol *128(3)*: 579-583, 2013.

4 Bristow RE, Duska LR, Lambrou NC, Fishman EK, O'Neill MJ, Trimble EL and Montz FJ: A model for predicting surgical outcome in patients with advanced ovarian carcinoma using computed tomography. Cancer *89(7)*: 1532-1540, 2000.

5 De Jong D, Eijkemans MJ, Lie Fong S, Gerestein CG, Kooi GS, Baalbergen A, van der Burg ME, Burger CW and Ansink AC: Preoperative predictors for residual tumor after surgery in patients with ovarian carcinoma. Oncology *72(5-6)*: 293-301, 2007.

6 Everett EN, Heuser CC, Pastore LM, Anderson WA, Rice LW, Irvin WP and Taylor PT: Predictors of suboptimal surgical cytoreduction in women treated with initial cytoreductive surgery for advanced-stage epithelial ovarian cancer. Am J Obstet Gynecol *193(2)*: 568-574, 2005.

7 Fagotti A, Ferrandina G, Fanfani F, Ercoli A, Lorusso D, Rossi M and Scambia G: A laparoscopy-based score to predict surgical outcome in patients with advanced ovarian carcinoma: A pilot study. Ann Surg Oncol *13(8)*: 1156-1161, 2006.

8 Ferrandina G, Sallustio G, Fagotti A, Vizzielli G, Paglia A, Cucci E, Margariti A, Aquilani L, Garganese G and Scambia G: Role of CT scan-based and clinical evaluation in the preoperative prediction of optimal cytoreduction in advanced ovarian cancer: A prospective trial. Br J Cancer *101(7)*: 1066-1073, 2009.

9 Gerestein CG, Eijkemans MJ, Bakker J, Elgersma OE, van der Burg ME, Kooi GS and Burger CW: Nomogram for suboptimal cytoreduction at primary surgery for advanced stage ovarian cancer. Anticancer Res *31(11)*: 4043-4049, 2011.

10 Kang S, Kim TJ, Nam BH, Seo SS, Kim BG, Bae DS and Park SY: Preoperative serum CA-125 levels and risk of suboptimal cytoreduction in ovarian cancer: A meta-analysis. J Surg Oncol *101(1)*: 13-17, 2010.

11 Kebapci M, Akca AK, Yalcin OT, Ozalp SS, Calisir C and Mutlu F: Prediction of suboptimal cytoreduction of epithelial ovarian carcinoma by preoperative computed tomography. Eur J Gynaecol Oncol *31(1)*: 44-49, 2010.

12 Risum S, Hogdall C, Loft A, Berthelsen AK, Hogdall E, Nedergaard L, Lundvall L and Engelholm SA: Prediction of suboptimal primary cytoreduction in primary ovarian cancer with combined positron emission tomography/computed tomography--a prospective study. Gynecol Oncol *108(2)*: 265-270, 2008.

13 Risum S, Høgdall C, Loft A, Berthelsen AK, Høgdall E, Nedergaard L, Lundvall L and Engelholm SA: Standardized FDG uptake as a prognostic variable and as a predictor of incomplete cytoreduction in primary advanced ovarian cancer. Acta Oncol *50(3)*: 415-419, 2011.

14 Stashwick C, Post MD, Arruda JS, Spillman MA, Behbakht K, Davidson SA and Kelly MG: Surgical risk score predicts suboptimal debulking or a major perioperative complication in patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer. Int J Gynecol Cancer *21(8)*: 1422-1427, 2011.

15 Testa AC, Ludovisi M, Mascilini F, Di Legge A, Malaggese M, Fagotti A, Fanfani F, Salerno MG, Ercoli A, Scambia G and Ferrandina G: Ultrasound evaluation of intra-abdominal sites of disease to predict likelihood of suboptimal cytoreduction in advanced ovarian cancer: A prospective study. Ultrasound Obstet Gynecol *39(1)*: 99-105, 2012.

16 Ibeanu OA and Bristow RE: Predicting the outcome of cytoreductive surgery for advanced ovarian cancer: A review. Int J Gynecol Cancer *20(Suppl 1)*: S1-11, 2010.

17 Kang S and Park SY: To predict or not to predict? The dilemma of predicting the risk of suboptimal cytoreduction in ovarian cancer. Ann Oncol *22(Suppl 8)*: viii23-viii28, 2011.

18 Axtell AE, Lee MH, Bristow RE, Dowdy SC, Cliby WA, Raman S, Weaver JP, Gabbay M, Ngo M, Lentz S, Cass I, Li AJ, Karlan BY and Holschneider CH: Multi-institutional reciprocal validation study of computed tomography predictors of suboptimal primary cytoreduction in patients with advanced ovarian cancer. J Clin Oncol *25(4)*: 384-389, 2007.

19 Brun JL, Rouzier R, Uzan S and Darai E: External validation of a laparoscopic-based score to evaluate resectability of advanced ovarian cancers: Clues for a simplified score. Gynecol Oncol *110(3)*: 354-359, 2008.

20 Van GT, Cadron I and Vergote I: The utility of proteomics in gynecologic cancers. Curr Opin Obstet Gynecol *23(1)*: 3-7, 2011.

21 Høgdall E, Fung ET, Christensen IJ, Yip C, Nedergaard L, Engelholm SA, Risum S, Petri AL, Lundvall L, Lomas L and Høgdall C: Proteomic biomarkers for overall and progression-free survival in ovarian cancer patients. Proteomics Clin Appl *4(12)*: 940-952, 2010.

22 Høgdall C, Fung ET, Christensen IJ, Nedergaard L, Engelholm SA, Petri AL, Risum S, Lundvall L, Yip C, Pedersen AT, Hartwell D, Lomas L and Høgdall EV: A novel proteomic biomarker panel as a diagnostic tool for patients with ovarian cancer. Gynecol Oncol *123(2)*: 308-313, 2011.

23 Risum S, Høgdall E, Engelholm SA, Fung E, Lomas L, Yip C, Petri AL, Nedergaard L, Lundvall L and Høgdall C: A proteomics panel for predicting optimal primary cytoreduction in stage III/IV ovarian cancer. Int J Gynecol Cancer *19(9)*: 1535-1538, 2009.

24 Fagö-Olsen CL, Ottesen B, Kehlet H, Markauskas A, Mosgaard BJ, Ottosen C, Sogaard CH, Søgaard-Andersen E and Høgdall C: Neoadjuvant chemotherapy as ovarian cancer treatment: ever more used with major regional differences. Dan Med J *59(8)*: A4477, 2012.

Received December 12, 2013
Revised January 17, 2014
Accepted January 17, 2014