Pathological chemotherapy response score is prognostic in tubo-ovarian high-grade serous carcinoma: a systematic review and meta-analysis of individual patient data

Paul A. Cohen[1, 2, 3]§, Aime Powell[2, 3,]§, Steffen Böhm[4], C. Blake Gilks[5], Colin J.R. Stewart[6], Tarek M. Meniawy[7, 8], Max Bulsara[3], Stefanie Avril[9,10], Eleanor C. Brockbank[11], Tjalling Bosse[12], Gustavo Rubino de Azevedo Focchi[13], Raji Ganesan[14], Rosalind M. Glasspool[15], Brooke E. Howitt[16], Hyun-Soo Kim[17], Jung-Yun Lee[18], Nhu D. Le[19], Michelle Lockley[20,21], Ranjit Manchanda[22], Trupti Mandalia[23], W. Glenn McCluggage[24], Iain McNeish[25], Divya Midha[26], Radhika Srinivasan[27], Yun Yi Tan[28], Rachael van der Griend[29], Mayu Yunokawa[30], Gian F. Zannoni[31], The HGSC CRS Collaborative Network (Supplementary 1) and Naveena Singh[32].

1. Department of Gynaecological Oncology, Bendat Family Comprehensive Cancer Centre, St John of God, 12 Salvado Rd, Subiaco, Western Australia, Australia 6008.
2. Division of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, Australia 6009.
3. Institute for Health Research, The University of Notre Dame Australia, 32 Mouat Street Fremantle, Western Australia, Australia, 6160.
4. Department of Medical Oncology, Barts Health NHS Trust, West Smithfield, London, United Kingdom, EC1A 7BE.
5. Department of Anatomic Pathology, Vancouver General Hospital, 899 W 12th Ave, Vancouver, BC, Canada, V5Z 1M9.
6. Department of Histopathology, King Edward Memorial Hospital, 374 Bagot Road, Subiaco, Western Australia, Australia, 6008.

7. School of Medicine and Pharmacology, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia, Australia 6009.
8. Department of Medical Oncology, Sir Charles Gairdner Hospital, Gairdner Drive Nedlands, Western Australia, Australia, 6010.
9. Department of Pathology, School of Medicine, Case Western Reserve University. University Hospitals Cleveland Medical Center and Case Comprehensive Cancer Center, Wolstein Research Building, Room 6524, 2103 Cornell Road, Cleveland, Ohio, United States of America, 44106.
10. Institute of Pathology, Technische Universität München, Ismaninger Str. 22, Munich, Germany, 81675.
11. Department of Gynaecological Oncology, Barts Health NHS Trust, Whitechapel Rd, London, United Kingdom, E1 1BB.
12. Department of Pathology, Leiden University Medical Centre, Albinusdreef 2, PO Box 9600, 2333 ZA, Leiden, The Netherlands.
13. Department of Pathology, Federal University de São Paulo (UNIFESP), R Botucatu, 740 - São Paulo, SP, Brazil - CEP 04023-062.
14. Department of Cellular Pathology, Birmingham Women's NHS Foundation Trust, Mindelsohn Way, Birmingham, UK, B15 2TG.
15. Cancer Research UK Clinical Trials Unit, Glasgow, The Beatson West of Scotland Cancer Centre, University of Glasgow, 1053 Great Western Road, Glasgow, UK, G12 0YN.
16. Department of Pathology, School of Medicine, Stanford University, 300 Pasteur Drive, H2128E, Stanford, California, United States of America, 94305.
17. Department of Pathology, Severance Hospital, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul, Republic of Korea, 03722.

18. Department of Obstetrics and Gynecology, Institute of Women's Life Medical Science, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul, Republic of Korea, 03722.
19. Cancer Control Research, British Columbia Cancer Research Centre, 675 West 10th Ave, Vancouver, BC, Canada, V5Z1L3.
20. Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London, United Kingdom EC1M 6BQ.
21. University College London Hospital, 235 Euston Rd, Fitzrovia, London, United Kingdom NW1 2BU.
22. Department of Gynaecological Oncology, Barts Health NHS Trust, Royal London Hospital, 10th Floor, South Block, Whitechapel Road, London, UK, E1 1BB.
23. Department of Histopathology, Royal Devon and Exeter NHS Foundation Trust. Royal Devon and Exeter Hospital (Wonford), Old Pathology Building, Church Lane, Exeter, Devon, United Kingdom, EX2 5AD.
24. Department of Pathology, Belfast Health and Social Care Trust, Grosvenor Road Belfast, United Kingdom, BT12 6BA.
25. Division of Cancer, Department of Surgery and Cancer, Imperial College London, IRDB Building, Hammersmith Hospital, London UK W12 0NN.
26. Department of Pathology, Tata Medical Center, Kolkata. 14 MAR, Rajarhat, Kolkata, India, 700160.
27. Department of Cytology and Gynecological Pathology, Postgraduate Institute of Medical Education and Research, Sector 12, Chandigarh, India, 160012.
28. Department of Medical Oncology, Beatson West of Scotland Cancer Centre, 1053 Great Western Road, Glasgow, United Kingdom, G12 0YN.

29. Department of Anatomical Pathology, Canterbury Health Laboratories, 2 Riccarton Ave, Christchurch, New Zealand, 8011.
30. Department of Breast and Medical Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan.
31. Department of Pathology, Women and Child Health, Fondazione Policlinico Gemelli. Università Cattolica del Sacro Cuore. Largo F Vito 1. 00168 Roma Italy.
32. Department of Cellular Pathology, Barts Health NHS Trust, Whitechapel Rd, London, United Kingdom, E1 1BB.

§ PC and AP contributed equally.

KEY WORDS

Neoadjuvant chemotherapy; chemotherapy response score; prognosis; high-grade serous tubo-ovarian cancer.

CORRESPONDING AUTHOR

Associate Professor Paul Cohen
Department of Gynaecological Cancer Research
Level 5 Bendat Family Comprehensive Cancer Centre
St John of God Subiaco Hospital
12 Salvado Road
Western Australia 6008, Australia
(M) +61 406 888 339
(E) Paul.Cohen@uwa.edu.au

ABSTRACT

OBJECTIVE

There is a need to develop and validate biomarkers for treatment response and survival in tubo-ovarian high-grade serous carcinoma (HGSC). The chemotherapy response score (CRS) stratifies patients into complete/near-complete (CRS3), partial (CRS2), and no/minimal (CRS1) response after neoadjuvant chemotherapy (NACT). Our aim was to review current evidence to determine whether the CRS is prognostic in women with tubo-ovarian HGSC treated with NACT.

METHODS

We established an international collaboration to conduct a systematic review and meta-analysis, pooling individual patient data from 16 sites in 11 countries. Patients had stage IIIC/IV HGSC, 3-4 NACT cycles and >6-months follow-up. Random effects models were used to derive combined odds ratios in the pooled population to investigate associations between CRS and progression free and overall survival (PFS and OS).

RESULTS

877 patients were included from published and unpublished studies. Median PFS and OS were 15 months (IQR 5-65) and 28 months (IQR 7-92) respectively. CRS3 was seen in 249 patients (28%). The pooled hazard ratios (HR) for PFS and OS for CRS3 versus CRS1/CRS2 were 0·55 (95% CI, 0·45-0·66; P <0·001) and 0·65 (95% CI 0·50–0·85, P= 0·002) respectively; no heterogeneity was identified (PFS: Q=6·42, p=0·698, $I2$=0·0%; OS: Q=6·89, p=0·648, $I2$=0·0%). CRS was significantly associated with PFS and OS in multivariate

models adjusting for age and stage. Of 306 patients with known germline *BRCA1/2* status, those with *BRCA1/2* mutations (n=80) were more likely to achieve CRS3 (P = 0·027).

CONCLUSIONS

CRS3 was significantly associated with improved PFS and OS compared to CRS1/2. This validation of CRS in a real-world setting demonstrates it to be a robust and reproducible biomarker with potential to be incorporated into therapeutic decision-making and clinical trial design.

INTRODUCTION

Neoadjuvant chemotherapy (NACT) is increasingly used to treat women with tubo-ovarian high-grade serous carcinoma (HGSC) following the results of two randomised trials that demonstrated non-inferior overall survival (OS), and lower morbidity and mortality, compared to primary surgery in advanced disease.(1, 2) Interval debulking surgery (IDS) following NACT provides an opportunity to assess tumor response to antineoplastic treatments. Validated scoring systems provide prognostic information in patients with breast, esophageal, gastric and rectal cancers following neoadjuvant treatment, and are used to guide treatment decisions after surgery.(3-6) In 2015, a standardised scoring system for histological tumor regression in tubo-ovarian HGSC was proposed by Böhm and colleagues, who developed and validated a three-tier chemotherapy response score (CRS) that stratifies patients into complete/near-complete (CRS3), partial (CRS2), and no/minimal (CRS1) response based on omental examination.(7) Importantly, the CRS has been shown to be reproducible amongst pathologists.(8) The International Collaboration on Cancer Reporting (ICCR) subsequently recommended the use of the CRS to assess histological NACT effect in HGSC to enable standardised and objective reporting.(9) Single institution retrospective studies have since reported an association between CRS and progression-free survival (PFS) but not OS.(10-13) These studies are limited by small sample sizes, lack of power to detect associations between CRS and OS, heterogeneity in participants, and the number of NACT cycles and regimens used. In recognition of the precedent of insufficiently validated diagnostic tools that have previously been implemented in clinical trials prematurely(14) we formed an international collaborative network to analyse pooled retrospective patient level data from several centres. This collaboration enabled meta-analysis of individual patient data (IPD) with standardised inclusion criteria that would achieve greater statistical power to

investigate the prognostic role of the CRS, with the goal of providing a sufficient level of validation that may permit use of the CRS in clinical trials.

Our primary aim was to determine whether the CRS was prognostic in women with tubo-ovarian HGSC treated with NACT. Secondary objectives were to investigate whether i) the CRS correlated with macroscopic residual disease at completion of interval surgery, ii) the CRS predicted platinum-resistance (as conventionally defined by disease progression <6 months following last adjuvant chemotherapy cycle(15)), iii) a biochemical response in serum CA125 from diagnosis to pre-interval surgery was prognostic, and iv) patients with CRS3 had a higher frequency of pathogenic germline *BRCA1/2* mutations compared to those with CRS1 and CRS2.

MATERIAL AND METHODS

We performed a systematic review and meta-analysis based upon a Medline and PubMed search from August 31, 2015 to June 30, 2018, with no language restrictions. This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Ethical approval was obtained (St John of God Healthcare Human Research Ethics Committee Reference 1291) for transfer of de-identified individual patient data from participating sites for statistical analysis at the Institute for Health Research, University of Notre Dame, in Fremantle, Western Australia. Principal investigators at individual study sites obtained country-specific and local approvals.

SEARCH STRATEGY

We used the search terms “chemotherapy response score” AND “high-grade serous ovarian carcinoma”. A multi-centre research consortium that included 16 sites to access IPD from published and unpublished studies supplemented the search.

Published studies that reported the use of the CRS in patients with stage IIIC or IV ovarian, fallopian tube, or primary peritoneal HGSC, treated by NACT and IDS, were eligible for inclusion. After removing duplicates, two authors (PC and AP) independently examined titles and then abstracts of all studies identified according to the search strategy. The full texts of relevant abstracts were retrieved for further assessment. Uncertainties were resolved through discussion with a third author (NS). The Newcastle-Ottawa Scale and elements from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) quality assessment tool were used to assess risk of bias, with a low risk of bias considered a score of ≥7 or more.(16, 17)

Unpublished data were obtained from investigators who had previously published studies on prognostic importance of histological findings other than CRS(18-20), had presented data on CRS at international conferences, were known by the authors (NS, CBG, PC) to be from academic/tertiary referral centres and to be using CRS routinely in their clinical practice (NZ, NL, Canada, UK) and/or had expressed interest in contributing data to the meta-analysis through retrospective review and scoring of consecutive eligible cases from their centres (UK).

INCLUSION AND EXCLUSION CRITERIA

Study eligibility criteria were: patients with histologically confirmed International Federation of Gynecology and Obstetrics (FIGO) 2014 stage IIIC or IV ovarian, fallopian tube, or primary peritoneal HGSC, who had received 3-4 cycles of platinum-based NACT prior to IDS and had a minimum of 6 months follow up information. An additional criterion is implicit in the scoring system, which utilises the extent of disease in the single omental section showing the worst response to NACT, i.e. the maximum tumor load present; this is only valid in cases with documented omental disease prior to NACT. A standardised data collection tool was developed and disseminated to collect the following variables; age at diagnosis, date of first NACT cycle, date of last adjuvant chemotherapy cycle, serum CA125 values prior to the first NACT cycle and before IDS, number of NACT cycles administered, FIGO stage, residual disease (surgeon's visual assessment of completeness of the IDS categorised as no macroscopic residual disease 'R0', ≤1cm and >1cm), germline *BRCA1/BRCA2* mutation status, and date of disease progression, death or last known follow up. Clinical and laboratory data were collected through chart and tissue repository database review. Any discrepancies were resolved by consensus and arbitration by a panel of investigators (NS, PC, AP, SB, BG, MB, CS and TM).

Tumor regression scores were assigned by local gynecological pathologists at participating sites based on the omental section showing the least NACT response, as detailed in the original publication describing the CRS score (Supplementary Table 1). The original publication advised that CRS3 cases should be sub-divided into those with no residual tumor in the omentum and those with presence of residual microscopic omental tumour (Supplementary Table 1) at time of IDS.

STATISTICAL ANALYSIS

Statistical analysis was performed using Stata 15.0 (Stata Statistical Software Release 15; StataCorp LP, College Station, TX). Statistical significance was determined as a P value less than 0·05 for all hypothesis tests. Random IPD meta-analysis methods were used to assess PFS and OS. Hazard ratios (HR), odds ratios (OR) and their 95% confidence intervals (CI) were calculated and reported. Tests for heterogeneity were conducted and the $I2$ statistic was calculated to quantify the degree of heterogeneity between sites. Time-to-event analysis was performed using Cox proportional hazard regression models to investigate factors associated with PFS and OS. PFS was defined as the date of the first NACT cycle to disease progression, as per the Gynecologic Cancer Intergroup CA125 criteria(15) or radiological progression or death, whichever occurred first. OS was defined as the date of first NACT cycle to date of death or date of last known follow-up. In the presence of non-proportional hazards, a parametric Weibull regression model was used. Evidence of non-proportionality was assessed using PHTEST at the 5% level. PFS and OS for CRS3 were compared to CRS1/CRS2 combined[7]. Variables included in the models were age at diagnosis (years), disease stage, and completeness of IDS. The CA125 response and germline *BRCA1/2* mutation status were included in subsequent models. Violation of the proportional hazard assumption for the Cox model was tested using Schoenfeld residuals. The Harrell's C statistic was used to measure the performance of the survival models in discriminating overall PFS and OS to quantify the value of CA125 reduction (from baseline to pre-IDS) when assessed with clinicopathological factors.

Chi-square and Fisher exact tests were used to examine group differences between CRS and other categorical clinical variables. A multivariate logistic regression was performed to investigate the prognostic significance of CRS with surgical residual disease, platinum

resistance, defined as disease progression <6 months after the last chemotherapy cycle, and germline *BRCA1/2* mutation status.

RESULTS

We retrieved 6 published papers and 5 met the inclusion criteria (7, 10-13). 1 duplicate was removed (Figure 1). Risk of bias assessments are shown in Supplementary Table 2. Data were available for 1365 patients from 11 countries (Figure 1 and Supplementary Table 3). After exclusion of 488 patients who did not meet inclusion criteria, the final cohort comprised 877 patients (Figure 1). Patient characteristics, details of NACT and clinicopathological outcomes are presented in Table 1 and Supplementary Table 3. Of the sites that were able to provide complete data for CRS 3 cases (n = 202) information was available regarding anatomical site/presence of residual viable tumor after IDS for 100 cases; these were derived from 8 study sites, which collectively contributed 411 cases. Of these 32 (32%) were CRS 3 with no residual tumor in the omentum; notably only 11 of these cases (11/411; 2.7%) showed a complete pathological response (i.e. no residual tumor *at any site* based on histopathological assessment), as the remainder showed residual disease at sites other than the omentum. Frequencies of the CRSs reported by each country varied significantly (P <0·001).

677 of 877 (77·2%) patients developed recurrent disease. Median PFS was 14·9 months (IQR 5.4-65.2; Supplementary Table 3). The pooled hazard ratio (HR) for PFS (CRS3 compared to CRS1/CRS2) was 0·55 (95%CI, 0·45 - 0·66; P <0·001; Figure 2). No heterogeneity (statistical difference in reporting of CRS and PFS between countries) was identified (Q=6·42, p=0·698, $I2$=0·0%). In a Cox model adjusting for age, stage and residual disease at IDS, CRS and residual disease were significantly associated with PFS. CRS1/2 combined

were significantly associated with worse PFS compared to CRS3 (HR, 1·90; 95%CI, 1·58 - 2·28; P <0·001; Table 2). Patients with any residual disease were at increased risk of progression independent of CRS scores (Table 2, Supplementary Figure 1). A sub-group analysis of patients with CRS3 showed the presence of residual disease in the omentum vs. no residual omental disease to be associated with an increased risk of progression (HR, 1·94; 95%CI, 1·34 - 2·80; P <0·001; Supplementary Table 4, Supplementary Figure 3 and 4).

There were 407 deaths. The pooled HR for OS (CRS3 compared to CRS1/CRS2) was 0·65 (95%CI 0·50 – 0·85, P= 0·002; Figure 2). No heterogeneity was identified (Q=6·89, p=0·648, $I2$=0·0%). In a multivariate survival model that compared CRS3 with CRS1 and CRS2 combined, CRS1/2 were associated with significantly worse OS (HR, 1·73; 95%CI, 1·35 - 2·25; P <0·001; Table 2). Older age at diagnosis (P= 0·032) and residual disease at completion of IDS (> 0cm and ≤ 1cm *v* R0; HR, 1·49; 95% CI, 1·19 - 1·85; P <0·001; >1cm *vs.* R0; HR, 2.30; 95% CI, 1·71 - 3·08, P <0.001) were associated with worse OS (Table 3, Supplementary Figure 2). A sub-group analysis of patients with CRS3, showed the presence of residual disease in the omentum vs. no residual omental disease to be associated with worse OS (HR, 2·25; 95%CI, 1·31 - 3·87; P= 0·003; Supplementary Table 4, Supplementary Figure 3 and 4).

Because residual disease has consistently been shown to be the most important prognostic factor in women with tubo-ovarian HGSC, we performed a subgroup analysis of the 508 women debulked to R0 (Supplementary Table 5). In this group of patients CRS was significantly associated with PFS (CRS1/CRS2 *vs.* CRS3: HR, 1·81; 95%CI, 1·43 – 2·29; P <0·001; Supplementary Table 6) and OS (CRS1/CRS2 *vs.* CRS3: HR, 1·50; 95%CI, 1·08 – 2·09; P=0·017; Supplementary Table 6).

Data on CA125 response to NACT were available for 809 patients. Median pre-treatment levels were 1,073 kU/L (range, 4 – 52,785 kU/L). Overall, 7 (1.0%) patients did not show any reduction in their CA125 values from baseline to pre-IDS (4 had CRS1, 2 had CRS2 and 1 had CRS3). Two patients had CA125 values within the normal range at the start of treatment that did not alter (1 had CRS2 and 1 had CRS3). There were 774 patients who had a CA125 reduction of ≥50% and 565 patients who had a CA125 reduction of ≥90% from baseline to pre-IDS levels. CA125 response was not found to be a reliable prognostic factor for PFS (Harrell's C = 0·6092) or OS (Harrell's C = 0·6257) (Supplementary Table 7) and did not predict residual disease at completion of IDS (HR, 0·93; 95%CI, 0·69 – 1·29; P= 0·696).

80 patients had a germline *BRCA1/2* mutation (8 had CRS1, 39 had CRS2 and 33 had CRS3). 226 patients had no germline *BRCA1/2* mutation and *BRCA* status was unknown in 571 patients. Patients with *BRCA1/2* mutations were more likely to have a CRS3 compared to those who were *BRCA1/2* wild type (P = 0·027) and were less likely to have recurrence (P = 0.025, Supplementary Table 8) or to be deceased (p = 0.036, Supplementary Table 8).

The outcomes for residual disease at IDS by study are presented by CRS in Supplementary Table 5 (P<0·001). Complete resection (R0) was achieved in 72·6% of patients (178 of 245) with CRS3 and 53·6% (330 of 616) patients with CRS1/CRS2 combined (P<0·001; Supplementary Table 5). In a logistic regression model that adjusted for age, FIGO stage and CRS, residual disease was significantly more likely in patients with CRS1/CRS2 compared to those with CRS3 (HR, 2·36; 95%CI, 1·70 - 3·27; P <0·001).

206 patients recurred in the platinum-resistant timeframe; 85·4% had CRS1/CRS2 and 14·6% had CRS3 (P <0·001, Supplementary Table 9). A multivariate logistic regression model showed the likelihood of platinum-resistance was significantly higher in patients with CRS1/CRS2 compared with those with CRS3 (HR, 2·62; 95%CI, 1·62 - 4·22; P <0·001) and for those with residual disease >1cm (HR, 1·82; 95%CI, 1·05 - 3.16; P = 0·033).

DISCUSSION

This study showed that CRS was significantly associated with PFS and OS in multivariate analyses that adjusted for established ovarian cancer prognostic factors. Consistent with these findings, the CRS predicted surgical residual disease, platinum resistance, and germline *BRCA1/2* mutation status, which are all independently associated with survival. Despite the limitations of this study, discussed below, this is a real-world demonstration of the applicability and performance of CRS in routine clinical practice, outside the confines of a highly controlled clinical trial setting.

In terms of its prognostic significance the CRS system is a three tier score, with CRS3 characterizing a patient cohort with favourable outcomes. Analysis of CRS3 by absence of residual omental disease vs. presence of residual microscopic omental disease suggests that CRS3 separates into two prognostic sub-groups with the former being associated with improved PFS and OS as compared to the latter. Notably CRS3 with no residual disease in the omentum does not equate to what is generally considered a *complete pathological response*, i.e. no residual tumor at *any* site; only 11/32 (34%) of cases with no residual tumour in the omentum showed absence of tumour at all other sites. The differences observed

for PFS and OS between CRS1 and CRS2 were not statistically significant. The subdivisions of both CRS3 and this less favourable prognostic group of CRS1/CRS2 using more objective parameters than morphology alone, including genomics and assessment of immune cell infiltration, should be the subject of future studies.

Comparison of CRS scores between countries also demonstrates variability between proportions of cases showing CRS1/CRS2 versus CRS3. A previous study on reproducibility of CRS assignment between pathologists from different centres, and with different levels of experience, showed that training using the online tool and the original paper were sufficient to produce reproducible scoring of the same histological sections, with exceptionally high agreement in cases scored as CRS3 (kappa value 0.926).(7, 8) For this reason, we believe it is unlikely that the difference in proportion of CRS3 cases is related to interobserver variation in scoring. We chose not to include central review of cases because of the previous demonstration of reproducibility(8) and because our aim was to determine how well CRS performs as a prognostic biomarker in different centres worldwide *as used by local pathologists*, rather than with the incorporation of any centralised arbitration. The similarity in outcome prediction for CRS1/CRS2 *vs.* CRS3 across countries suggests that the scoring system is being applied as devised. A possible explanation for the observed difference between countries is variation in case selection at two decision points: the decision to offer NACT as opposed to primary surgery, and subsequently the decision to carry out IDS after 3-4 NACT cycles. Both are highly dependent on local surgical oncological practices, which vary widely.(1) Whilst it is probable that all patients given NACT who showed an excellent radiological and biochemical response would proceed to IDS, there would be some variation in the proportion of poor responders who would be offered IDS, based on the subjective assessment of likelihood of achieving complete or <1cm resection of all macroscopic disease.

Other possible explanations could be the proportion of cases excluded due to loss to follow-up, which could diminish the numbers of poor responders, and variations in chemotherapy schedule and dose intensity.

The CRS was associated with pathogenic germline *BRCA1/2* mutations, which validates *BRCA1/2* mutations as a predictive marker of platinum response.(24, 25) Importantly we observed a significant association between CRS1/CRS2 and disease progression within 6 months. The HGSC cases with CRS3 are enriched for *BRCA1/2* mutations, and likely for other homologous DNA repair pathway defects, and we hypothesise that those cases with CRS1/CRS2 will contain a higher proportion of *CCNE1*-amplified tumors of the C1 mesenchymal subtype, and characterized by fold-back inversions and other molecular markers of poor prognosis.(26) This would require confirmation in large prospective studies but suggests that CRS could be used to identify patients who might benefit from alternative therapeutic strategies.

It is notable that in the current meta-analysis CA125 response did not predict survival, CRS or surgical residual disease in patients who showed a sufficient response to NACT to undergo IDS.

Our study has several limitations that should be acknowledged. All included studies were retrospective cohorts and our multivariate analysis did not adjust for patient comorbidities and performance status. We did not monitor patient selection from contributing centres and this could have resulted in selection bias. There was no central pathology review and it is conceivable that subjective interpretation led to reported CRS values that might have misclassified some cases. Residual disease at IDS relied upon the surgeon's report, which is

notoriously unreliable and may have biased our findings.(27) Time from completion of NACT to initiation of post-operative adjuvant treatment has recently been shown to influence survival;(28) we did not collect this information, and it is possible that variation in this time interval introduced bias.

It is acknowledged that many factors contribute to the timing and pattern of disease relapse, such as the frequency of diagnostic procedures and follow-up intervals, diagnostic methods and tools used, residual disease volume and location, rate of tumor growth, differences in therapy and acquired platinum resistance. The evaluation of tumor response based only on omental disease does not take into account possible impact of tumor heterogeneity. These differences notwithstanding, the CRS provides an objective measure and biological readout of the response to NACT, which appears to encapsulate all of the aforementioned parameters and their complex interplay.

Strengths of our study are the large sample that included IPD from 16 centres in 11 countries and a meta-analysis that utilised published and unpublished studies with minimal heterogeneity. The main strength of this study is the demonstration of a strong and plausible association of CRS with NACT outcome and survival in a real-world, heterogeneous study population.

A Society of Gynecologic Oncology White Paper on an FDA Ovarian Cancer Clinical Trial Endpoints Workshop held in 2015 highlighted the potential of NACT response to act as a platform for biomarker discovery and regulatory approval of novel therapies.(29) However, despite strong support it was felt further work was required. The White Paper highlighted unanswered questions that included the true prevalence of complete pathological response in

patients treated by NACT, and whether pathological response should be a surrogate for PFS and/or OS. The current study provides provisional answers to these questions: the prevalence of CRS3 in 877 women treated by NACT who went on to IDS was 28% and CRS would appear to be a surrogate for both PFS and OS, independent of other known prognostic factors. In the publication by Böhm and colleagues that described and validated the CRS, histological regression in the primary adnexal tumor did not stratify patients into prognostic groups and adnexal response scores showed inferior reproducibility; in contrast, omental scores were prognostic and reproducible.(7) In the current study we were not able to assess histological regression in the adnexa or at other metastatic sites in all patients, and so it is uncertain whether our findings translate to all tissues and compartments such as visceral and diaphragmatic metastases, or retroperitoneal lymph nodes. Our results do however show that a complete or near complete pathological response in omental tumor alone (CRS3) is a biomarker for survival.

Our findings require prospective validation. However, based on our results we recommend that the CRS be incorporated as an endpoint in clinical trials of novel therapeutic agents that have a NACT arm, and that CRS3 continue to be further classified with respect to the presence or absence of microscopic residual disease in the omentum. If confirmed in prospective studies, the CRS represents an appealing primary endpoint in clinical trials as a surrogate for survival because it can be measured earlier. Of note, the CRS is the primary endpoint in iPRIME, an ongoing phase II study of Durvalumab plus Tremelimumab in combination with NACT in newly diagnosed women with HGSC (ACTRN12618000109202). Furthermore, the CRS offers an opportunity to personalise treatment and may transform future clinical trial design, by stratifying treatment according to CRS following IDS. Future research should focus on the development of a statistical model

to predict prognosis that incorporates the CRS with radiological and biochemical response, surgical outcome, tumor immune profile and molecular classification.

The CRS could provide clinically useful information to estimate a patient´s probability of early *vs.* late relapse. Most of the patients who will not relapse at five years show CRS3, making these women with no or minimal residual disease an attractive group for an additional adjuvant therapeutic agent such as poly (adenosine diphosphate–ribose) polymerase (PARP) inhibitors, that prolong PFS and could result in more cures, as shown in the recently published SOLO1 trial of maintenance Olaparib in epithelial ovarian cancer patients with *BRCA1/2* mutations.(30) In contrast, patients whose tumors are found to have CRS1/2 will likely experience recurrence within 5 years; given this poor prognosis these patients could enter immediately into trials of new therapy.

In summary, in this IPD meta-analysis of 877 patients, the CRS was significantly associated with PFS and OS in women with tubo-ovarian HGSC treated by NACT. This biomarker is now sufficiently validated that it can be incorporated into prospective clinical trial design to assess its potential to guide therapeutic decision-making.

Conflict of interest

The authors declare no conflict of interest.

Funding

No funding source to declare.

Availability of data and material

Supporting data is available on request to the corresponding author and its release subject to ethical approval.

Authors' contributions

PC and NS conceived the study

NS, BG, PC and AP identified studies and sites

AP created the data extraction forms

All authors extracted site-specific individual patient data

AP and MB did the statistical analysis

PC, AP, NS and BG wrote the manuscript

SB, TMM, CJRS, WGMcG, ECB, IMcN, NDL, RMG critically reviewed the manuscript

All authors reviewed the manuscript, approved the final version and are accountable for all aspects of the work.

REFERENCES

1. Vergote I, Trope CG, Amant F, Kristensen GB, Ehlen T, Johnson N, et al. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. The New England journal of medicine. 2010;363(10):943-53.

2. Kehoe S, Hook J, Nankivell M, Jayson GC, Kitchener H, Lopes T, et al. Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer

(CHORUS): an open-label, randomised, controlled, non-inferiority trial. The Lancet. 2015;386(9990):249-57.

3. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1999;17(2):460-9.

4. Mandard AM, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer. 1994;73(11):2680-6.

5. Becker K, Mueller JD, Schulmacher C, Ott K, Fink U, Busch R, et al. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. Cancer. 2003;98(7):1521-30.

6. Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. International journal of colorectal disease. 1997;12(1):19-23.

7. Bohm S, Faruqi A, Said I, Lockley M, Brockbank E, Jeyarajah A, et al. Chemotherapy Response Score: Development and Validation of a System to Quantify Histopathologic Response to Neoadjuvant Chemotherapy in Tubo-Ovarian High-Grade Serous Carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(22):2457-63.

8. Said I, Bohm S, Beasley J, Ellery P, Faruqi AZ, Ganesan R, et al. The Chemotherapy Response Score (CRS): Interobserver Reproducibility in a Simple and Prognostically Relevant System for Reporting the Histologic Response to Neoadjuvant Chemotherapy in Tuboovarian High-grade Serous Carcinoma. International journal of gynecological pathology

521 : official journal of the International Society of Gynecological Pathologists. 2017;36(2):172-
522 9.
523 9. McCluggage WG, Judge MJ, Clarke BA, Davidson B, Gilks CB, Hollema H, et al.
524 Data set for reporting of ovary, fallopian tube and primary peritoneal carcinoma:
525 recommendations from the International Collaboration on Cancer Reporting (ICCR). Modern
526 pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.
527 2015;28(8):1101-22.
528 10. Coghlan E, Meniawy TM, Munro A, Bulsara M, Stewart CJ, Tan A, et al. Prognostic
529 Role of Histological Tumor Regression in Patients Receiving Neoadjuvant Chemotherapy for
530 High-Grade Serous Tubo-ovarian Carcinoma. International journal of gynecological cancer :
531 official journal of the International Gynecological Cancer Society. 2017;27(4):708-13.
532 11. Ditzel HM, Strickland KC, Meserve EE, Stover E, Konstantinopoulos PA, Matulonis
533 UA, et al. Assessment of a Chemotherapy Response Score (CRS) System for Tubo-Ovarian
534 High-Grade Serous Carcinoma (HGSC). International Journal of Gynecological Pathology.
535 2018.
536 12. Lee JY, Chung YS, Na K, Kim HM, Park CK, Nam EJ, et al. External validation of
537 chemotherapy response score system for histopathological assessment of tumor regression
538 after neoadjuvant chemotherapy in tubo-ovarian high-grade serous carcinoma. Journal of
539 gynecologic oncology. 2017.
540 13. Singh AP, Kaushal V, Rai B, Rajwanshi A, Gupta N, Dey P, et al. Chemotherapy
541 Response Score is a useful histological predictor of prognosis in high grade serous
542 carcinoma. Histopathology. 2017.
543 14. Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in
544 Clinical T, Board on Health Care S, Board on Health Sciences P, Institute of M. In: Micheel

CM, Nass SJ, Omenn GS, editors. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US)
Copyright 2012 by the National Academy of Sciences. All rights reserved.; 2012.
15. Rustin GJ, Vergote I, Eisenhauer E, Pujade-Lauraine E, Quinn M, Thigpen T, et al. Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed by the Gynecological Cancer Intergroup (GCIG). International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2011;21(2):419-23.
16. Wells GA SB, O'Connell D et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2012. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed 10 October 2018).
17. Observational studies: getting clear about transparency. PLoS medicine. 2014;11(8):e1001711.
18. Petrillo M, Zannoni GF, Tortorella L, Pedone Anchora L, Salutari V, Ercoli A, et al. Prognostic role and predictors of complete pathologic response to neoadjuvant chemotherapy in primary unresectable ovarian cancer. American journal of obstetrics and gynecology. 2014;211(6):632 e1-8.
19. Ebata T, Yunokawa M, Yoshida H, Bun S, Shimoi T, Shimomura A, et al. The Prognostic Impact of the Pathological Response to Neoadjuvant Dose-Dense Therapy for Ovarian Carcinoma. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2017;27(9):1850-5.
20. Avril S. Histopathological markers of treatment response and recurrence risk in ovarian cancers and borderline tumors. Der Pathologe. 2017;38(Suppl 2):180-91.
21. Movahedi-Lankarani S KU, Bell DA, et al. Protocol for the examination of specimens from patients with primary tumors of the ovary, fallopian tube, or peritoneum. 2018.

570 http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution
571 Folders/WebContent/pdf/cp-ovary-fallopian-17protocol-1001.pdf (accessed 10 October
572 2018). .
573 22. Colombo N LJ. Special session: Summary of the ESMO-ESGO consensus conference
574 on ovarian cancer. ESMO Congress 2018-10-21. https://oncologypro.esmo.org/Meeting-
575 Resources/ESMO-2018-Congress/Special-session-Summary-of-the-ESMO-ESGO-
576 consensus-conference-on-ovarian-cancer.
577 23. Alonzo TA. Standards for reporting prognostic tumor marker studies. Journal of
578 clinical oncology : official journal of the American Society of Clinical Oncology.
579 2005;23(36):9053-4.
580 24. Hollis RL, Churchman M, Gourley C. Distinct implications of different BRCA
581 mutations: efficacy of cytotoxic chemotherapy, PARP inhibition and clinical outcome in
582 ovarian cancer. OncoTargets and therapy. 2017;10:2539-51.
583 25. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, et al. Association of
584 BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator
585 phenotype in patients with ovarian cancer. JAMA : the journal of the American Medical
586 Association. 2011;306(14):1557-65.
587 26. Wang YK, Bashashati A, Anglesio MS, Cochrane DR, Grewal DS, Ha G, et al.
588 Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer
589 histotypes. Nature genetics. 2017;49(6):856-65.
590 27. Chi DS, Ramirez PT, Teitcher JB, Mironov S, Sarasohn DM, Iyer RB, et al.
591 Prospective study of the correlation between postoperative computed tomography scan and
592 primary surgeon assessment in patients with advanced ovarian, tubal, and peritoneal
593 carcinoma reported to have undergone primary surgical cytoreduction to residual disease 1

cm or less. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25(31):4946-51.

28. Lee YJ, Chung YS, Lee JY, Nam EJ, Kim SW, Kim S, et al. Impact of the time interval from completion of neoadjuvant chemotherapy to initiation of postoperative adjuvant chemotherapy on the survival of patients with advanced ovarian cancer. Gynecologic oncology. 2018;148(1):62-7.

29. Herzog TJ, Ison G, Alvarez RD, Balasubramaniam S, Armstrong DK, Beaver JA, et al. FDA ovarian cancer clinical trial endpoints workshop: A Society of Gynecologic Oncology White Paper. Gynecologic oncology. 2017;147(1):3-10.

30. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. The New England journal of medicine. 2018.

FIGURE AND TABLE LEGENDS

Figure 1. Study selection.

Figure 2. Hazard ratio plots by country for a) PFS and B) OS adjusted for patient age at diagnosis, disease stage and residual disease status.

Table 1. Patient baseline characteristics, histological scoring of tissue, and surgical outcome at interval debulking surgery.

Table 2. Multivariate survival analysis of prognostic factors for PFS and OS (presents CRS 1 and 2 vs. 3) adjusted for patient age at diagnosis, disease stage, residual disease status and CRS.

Table 3. Multivariate survival analysis of prognostic factors for PFS (presents CRS 1, 2 and 3) adjusted for patient age at diagnosis, disease stage, residual disease status and CRS.

S1. *Supplementary 1 The High-Grade Serous Ovarian Cancer CRS Collaborative Network*.

S2. *Supplementary Table 1.* Criteria for three-tier chemotherapy response score (applicable to the omental section showing the least tumour response in cases with documented omental disease at start of treatment).

S3. *Supplementary Table 2.* Risk of bias assessment for published studies.

S4. *Supplementary Table 3.* Overview of participating countries.

S5. *Supplementary Figure 1.* Kaplan-Meier curves for progression free survival for CRS.

S6. *Supplementary Figure 2.* Kaplan-Meier curves for overall survival for CRS.

S7. *Supplementary Table 4.* Multivariate survival analysis of prognostic factors for PFS and OS. Model adjusted for patient age (categorical variable) at diagnosis, disease stage, residual disease status, CRS (CRS1/2, CRS3 with residual microscopic omental disease (3a) and CRS3 with no residual omental disease (3b).

S8. *Supplementary Figure 3.* Kaplan-Meier curves for progression free survival & overall survival for CRS3 with microscopic residual omental disease (3a) vs. CRS 3 with no residual omental disease (3b)

S9. *Supplementary Figure 4.* Kaplan-Meier curves for progression free survival & overall survival for CRS1/2, CRS3 – Partial Response vs. CRS 3 – Complete Response.

S10. *Supplementary Table 5.* CRS and outcome of interval debulking surgery (residual disease).

S11. *Supplementary Table 6.* Multivariate survival analysis of prognostic factors for PFS and OS for only patients with no residual disease (N = 532). Model adjusted for patient age (categorical variable) at diagnosis, disease stage, residual disease status, CRS (CRS1/CRS2 vs. CRS3) and country.

S12. *Supplementary Table 7*. Multivariate survival analysis for PFS and OS (N= 835) adjusted for patient age at diagnosis, CA-125 response (≥ 90% reduction or < 90% reduction), disease stage, residual disease status and CRS.

S13. *Supplementary Table 8.* Multivariate survival analysis for PFS adjusted for patient age at diagnosis, germline BRCA mutation status, disease stage, residual disease status and CRS.

S14. *Supplementary Table 9.* The CRS and primary platinum-resistant disease (N= 587).