Identification and prioritization of tumor associated antigens for immunotherapeutic and diagnostic capacity in epithelial ovarian cancer: A systematic literature review

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Abstract

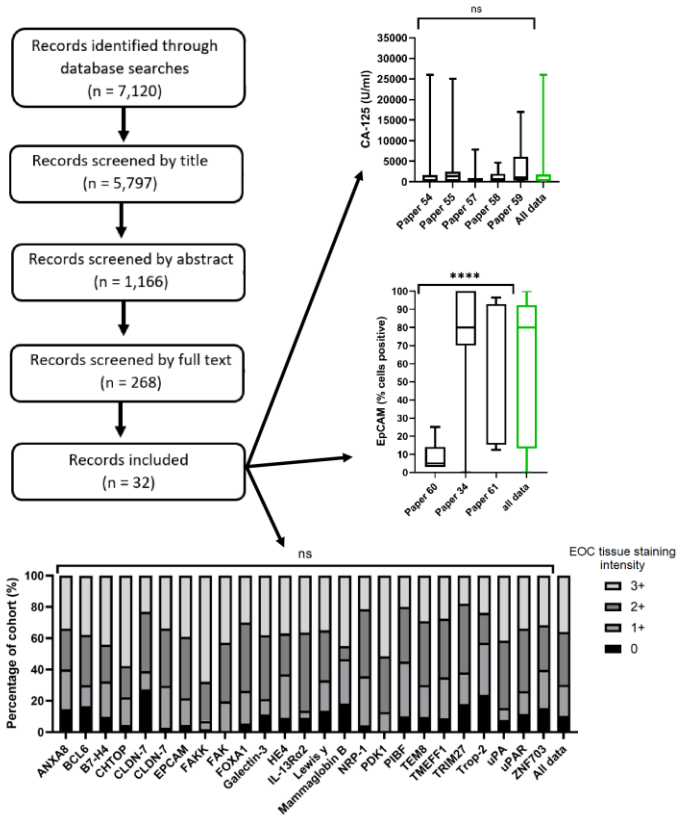
Epithelial ovarian cancer (EOC) is a prevalent carcinoma in the female population associated with poor prognostic outcomes, in part due to the late stage of disease at diagnoses. Aiming to identify tumor associated antigens (TAAs) with the potential to facilitate earlier detection and targeted therapy of EOC, five scientific literature repositories were systemically searched for primary literature sources reporting the expression of a TAA in the tissue or serum of adult females diagnosed with EOC and healthy women. We identified 7,120 articles of which 32 met our inclusion criteria and passed the bias-quality assessment. Subsequently data were collated on 29 TAAs whose expression had been analyzed in 2,181 patients and 589 healthy individuals. Reports of CA125 and EpCAM expression were numerous while tissue expression data were available for 28 TAAs. Data were segregated into three meta-cohorts for statistical scrutiny and their capacity for diagnostic and treatment targeting assessed. We showed that CA-125 was expressed homogenously in EOC patients while EpCAM was expressed heterogeneously. CA-125 was the most promising TAA target for both diagnosis and treatment, gaining a priority score of 12 (/12) while EpCAM gained a priority score of seven. Tissue expression of EOC TAAs was homogenous; 90% of the EOC population express any identified TAA while just 3% of healthy individuals will be positive for the same TAA. We suggest TAA profiling should be a fundamental aspect of EOC diagnosis, sitting alongside the FIGO framework, promoting reduced mortality and directing development of TAA targeted therapeutics.

Keywords: Epithelial ovarian cancer, tumor associated antigen, antigen directed therapy, antigen directed diagnosis

Summary

Profiling tumor associated antigens (TAA) should be universally harnessed in the clinic for quicker, less invasive diagnosis of epithelial ovarian cancer (EOC). CA-125 is the most robust target for EOC diagnosis and therapy while numerous other TAAs warrant further investigation.

Graphical_Abstract



Introduction

Epithelial ovarian cancer (EOC) is a non-communicable disease arising from malignant transformation in the fallopian tubes and resulting in the presence of tumors in the lining of the ovaries [1, 2]. Globally, ovarian carcinoma (OC) is the seventh most common cancer diagnosed and eighth most common cancer mortality within the female population [2, 3]. As with any carcinoma, the greatest risk factor for developing sporadic EOC is increasing age [4]. Risk begins to increase at age 40 when seven in every 100,000 women will be diagnosed with EOC, whilst most diagnoses occur in patients aged 55 to 64 [5].

Between 2002 and 2018, the number of women diagnosed with OC increased by 31% associated with a 2% increase in mortality [3]. The later EOC is diagnosed, the less likely patients will respond favourably to therapy meaning prognosis, and impact on fertility, is heavily dependent upon disease stage at diagnosis [6]. EOC presents symptomatically as abdominal bloating, reduced appetite and subsequent weight loss, atypical bowel movements, back pain and fatigue. [7]. Upon presentation, women will undergo invasive physical examinations including rectovaginal exploration, which is an attempt to feel for masses, alongside transvaginal ultrasonography for visualization of ovarian construction and vascularity, while any familial history of EOC will be recorded [8, 9]. Given the non-specific nature of EOC symptoms, many patients do not present until their condition has deteriorated over a period of three to six months, evidentiated by the fact that 66% of all EOCs are metastatic stage III or IV malignancies at the time of diagnosis [10, 11]. As such, 17% of women will die within 90 days of EOC diagnosis and in the case of emergency presentation, 56% of women will die within 12 months of diagnosis [12, 13].

Antigens proven to have expression profiles that are distinct in healthy versus diseased individuals can be used as biomarkers for diagnosis; During clinical investigation of EOC symptoms, clinicians may analyse the serum for the tumor associated antigen (TAA) CA-125, with 35U/ml recognized as the upper limit of detection in healthy individuals [5, 14]. Also known as mucin 16 (MUC16), this transmembrane glycoprotein is localized to the apical membrane of the epithelia under homeostatic conditions, acting to maintain hydration of the epithelial tracts and protect the surface from pathogen invasion and enzymatic incursion [15, 16]. During the development of EOC however, tumorigenic CA-125 localization broadens, spreading to the basolateral surfaces and micro-villi, and increasing in both density and frequency of expression [17]. Increased serum CA-125 levels have been branded a biomarker for EOC diagnosis in the literature since the mid-1980s however it is still not clinically recognised as a stand-alone diagnostic marker due to its elevated expression in other gynaecological presentations such as benign ovarian cysts and endometriosis [18-20].

The current first-line treatment for EOC consists of cytoreductive surgery, taxane- or platinum-based chemotherapy and antiangiogenic pharmaceuticals [2]. The effective removal of cancer stem cells, potentially chemoresistant cell populations and the auxiliary tumor microenvironment relies on access to a specialised gynaecologic oncology surgeon [21]. Thereafter, application of chemotherapeutics has increased efficacy, in theory, however in reality this patient group is plagued by primary and acquired chemotherapy resistance due to the various pathogenetic and aetiological pathways that lead to the disease, providing the cancer cell population protection from antineoplastic pharmaceuticals [22-24]. The addition of antiangiogenic bevacizumab to the standardised EOC intervention strategy has resulted in improved responses to overall treatment, however reliance on this cytotoxic cocktail does not prevent disease recurrence and is inherently systemic, resulting in toxicity and numerical adverse events [25, 26]. In fact, due to the harmful consequences of systemic therapies combined with the late stage of disease when most EOC is detected, 20% of EOC patients are too ill to tolerate treatment by the time they are diagnosed, leaving palliative care as the only option [12].

Molecular targeting therapies, however, aim to intervene in the specific molecular and genetic aetiology of an individual's EOC through highly specific treatment that targets the underlying pathogenesis resulting in considerably better efficacy [27]. Such interventions can interrupt metastasis and destroy malignant tissue whilst having minimal effect on healthy adjacent tissue, unlike systemic therapies.

At present, diagnosis of EOC is reactive rather than proactive and main-stay standard of care therapies lack disease-localised impact, resulting in a poor prognostic outlook for patients. With the recognised dystopia between the current gold standard detection and intervention strategies and the resultant stagnancy in mortality rates since the early 1980s, cancer diagnoses are expected to be the most dominant cause of death in the global population and the greatest impediment to increasing life expectancy scientists and healthcare professionals will face in the 21st century [3].

In the era of precision medicine, personalised therapeutics and clinically recognised diagnostic targets designed upon the wealth of TAA presentation data available in the literature are more realistic than ever before [6]. The objective of this systematic literature review was to identify and prioritise potential TAA targets for earlier detection and higher efficacy remedy of EOC. We aimed to identify and rank potential protein targets to advance the use of TAAs as reliable, functional and non-invasive diagnostic assets for use in the clinic. We examined the breadth of their applicability and the ability of such initiatives to benefit the EOC patient population. Further, we aimed to collate data on potential EOC targets that are being discussed in the literature, creating a catalogue of proteins that may warrant further laboratory investigation, before critically scrutinising their ability to perform as efficacious therapeutic targets. By systematically searching the literature for data on EOC TAA expression, the patient groups TAA expression profile could be defined and as such, antigen targets revealed.

Materials and methods

Systematic review

In accordance with **PICO**: The **p**articipants were adult EOC patients, **i**nterventions prior to antigen quantification were recorded, **c**omparators were the antigen expression levels in individuals with no evidence of disease (NED, i.e. individuals who present as clinically healthy) and the **o**utcome was quantification and cataloguing of TAA levels in the EOC patient group and prioritisation of these TAAs as targets for diagnosis and treatment.

Search strategy

Published manuscripts focusing on EOC TAAs were identified through the systematic searching of five scientific repositories (PubMed, Medline, CINAHL complete, the Cochrane library and Web of science) utilizing the following MeSH search terms and Boolean operators: Epithelial AND "ovarian cancer" OR "ovarian tumour" OR "ovarian tumor" OR "ovarian malignancy" OR "ovarian carcinoma" OR "ovarian metastasis" AND immunotherapy OR "immune target" OR "protein target" OR "antigen target" OR "expression target". Search terms were applied to the title, abstract and key word fields and no date restriction was applied.

Study selection

Following the PRISMA-P guidelines, primary literature sources written in the English language reporting directly quantified expression of a TAA in the malignant tissue or serum were selected for review [28]. To be included, papers must have numerically quantified the expression of a potential protein target in fresh or archival tissue samples collected from adult (18 years plus) females

diagnosed with EOC, along with quantification in fresh or archival tissue samples collected from individuals with NED. This would allow calculation of relative specificity. Excluded papers were those which non-numerically quantified the expression of a potential protein target in EOC and NED individuals (i.e. when expression was designated as positive or negative) due to the limited statistical analyses that could be conducted on such information. Additionally, those which did not quantify the expression of a potential protein target in EOC and NED individuals, those which relied upon cell line or animal models, and those which focused on any disease other than EOC, were excluded along with any protein quantification data obtained from adjacent, borderline, or benign tissues samples. Studies which did not satisfy the bias-quality assessment criteria were excluded. Duplicates were removed before manuscripts were scrutinized by title, then abstract, then full text against these criteria.

Bias-quality assessment

All papers reviewed by full text (n=268) were scrutinized against a specially developed bias-quality assessment matrix (available in supplementary material). Study design regarding selection of the patient and control cohorts, and quantification of the outcome were assessed. Choice of experimental protocol, scrutinizing the validity and appropriateness of the assay(s) including limit of detection and sensitivity, and ethical approvals were tracked to assess whether the research design and cohort recruitment were appropriate to address the stated aim(s) and if the declared conclusions were supported by the study data. In brief, we sought to identify papers which detailed distinct aims, and used methods based upon appropriate and clear background information, where the final conclusions were unambiguously supported by the presented data. The patient and control cohorts, which must have been present for the record to be considered for inclusion, must have been recruited from a clearly defined reference population following universally applied selection criteria. Informed, written consent must have been received by all participants. Assessment of whether the treatment or care provided to participants could have impacted the outcome were assessed by the reviewers and, to meet the bias-quality assessment criteria, any potential confounding variables must have been discussed alongside data interpretation in the literature in question. Papers which did not meet all of these criteria were excluded.

Data extraction

Number of patients in the cohort, the antigen of interest, quantification method, associated units and the antigen expression profile were extracted from all included papers and recorded in Excel. In addition, disease histology, grade or stage, and details of any cytoreductive therapy received prior to antigen quantification were extracted where disclosed.

Data analyses

For continuous data, antigen expression levels (median, range, lower and upper quartile) were quantified for each cohort. Non-continuous data were normalised to cohort size. Data from different manuscripts on the same TAA were pooled into meta-cohorts for statistical analysis. Meta-cohorts were analysed for normality before significant differences were identified using the appropriate parametric or non-parametric test. ROUT outlier identification (Q=1%) was applied to identify individual cohort data sets which did not fit the trend of their assigned meta-cohort. All analyses were performed using GraphPad Prism 9 software.

TAA prioritization as therapeutic and diagnostic targets

Based upon the principles outlined in the National Cancer Institute's prioritization criteria for cancer antigens, we designed three novel calculations to allow relative comparison of antigen suitability for diagnostic and therapeutic targeting [29]. For meta-cohorts containing continuous data, TAA level, range, median, lower and upper quartile were harnessed to calculate the relative specificity and

homogeneity of expression of the potential targets. Briefly, the smaller specificity A (%) and the higher specificity B (%), the better the antigen would be as a target as there is a larger difference between its expression profile in healthy verses diseased tissue, increasing the ability of targeted diagnostics to differentiate healthy versus diseased individuals, and the greater the likelihood that targeted therapeutics would localise to cancerous tissue. Further, the smaller the result of the expression homogeneity calculation (%), the more homogenous the expression in the patient group and the better the antigen would be as a target as any diagnostic or therapeutic strategies directed towards the protein could be more universally applied and broadly beneficial to the patient group.

Specificity A

$$\frac{Upper\ limit\ TAA\ expression\ NED}{Median\ TAA\ expression\ in\ metacohort} \times 100$$

Specificity B

$$\frac{Median\ TAA\ expression\ in\ metacohort\ -\ Upper\ limit\ TAA\ expression\ NED}{Median\ TAA\ expression\ in\ metacohort} \times 100$$

TAA expression homogeneity

$$\frac{TAA\ expression\ interquartile\ range\ of\ metacohort}{TAA\ expression\ total\ range\ of\ metacohort} \times 100$$

Results

Screened studies selected for systematic review

The search garnered 7,120 manuscripts of which 1,323 were duplicates. Of the 5,797 original titles screened, 4,613 were removed because they were not related to the expression of a TAA in adult females diagnosed with EOC. Of the 1,166 manuscripts eligible for abstract review, 991 were similarly excluded as were papers that relied solely on animal or cell line models. Of the 268 manuscripts screened by full text, 32 met the inclusion criteria (figure 1). Papers were excluded because they did not explicitly quantify the expression of an EOC TAA, antigen expression was quantified as positive or negative only, the full text record could not be accessed, or the manuscript did not meet the bias-quality criteria.

Published between 1989 and 2020, 38% of the studies were performed in centres based in China and 22% in the USA whilst the remaining studies were performed in the Netherlands, Turkey, Australia, Germany, Italy, Denmark, Korea, Canada or Japan. The papers were obtained from 24 different journals with four published in the International Journal of Gynecological Cancer, and Gynecological Oncology, two in Oncology Letters, the American Journal of Translational Research, and Cancer. Of the 32 papers, 85% reported patient histology, 79% reported stage or grade and 48% reported therapy received by patients prior to quantification of TAA expression. The most reported therapy was chemotherapy and 36% of studies chose to exclude patients who had received specified therapies from the cohort. Taking all records together, TAA expression levels were collated on 2,181 EOC patients and 589 individuals with NED and the profile of 29 TAAs catalogued. These data were split into three meta-cohorts for statistical analysis: Tissue staining, serum CA-125 and tissue EpCAM.

Tissue expression of TAAs is homogenous in EOC patients

Papers in the staining intensity (SI) meta-cohort were as follows: [30-53]. The literature search garnered tissue expression data from 24 manuscripts on 28 different TAAs. SI was determined

quantified as 0 (absent), 1 (low), 2 (moderate) or 3 (high) by immunolabelling techniques. These data were pooled into a meta-cohort for statistical analysis.

Raw data (Supplementary table 1) were extracted and catalogued before being normalized to the size of the individual cohort, allowing direct inter-cohort comparison. Once converted from number to percentage of patients in each SI group, the Shapiro-Wilk test qualified the meta-cohort data as normally distributed, before the one-way ANOVA identified no significant difference in the distribution of patients in each SI group between antigens (figure 2 Ai). Upon application of the ROUT outlier identification function (Q=1%), the staining distribution in four cohorts were identified as outliers from the rest of the meta-cohort: PPARβ, PEDF, ROR2 and SSEA4. As such, these data were removed from the meta-cohort before the Shapiro-Wilk and one-way ANOVA tests were repeated, again demonstrating that there was no significant difference in the number of patients with absent, low, moderate or high TAA SI, regardless of the antigen being quantified (figure 2 Aii). The SI of EOC associated antigens in patients was therefore homogeneous. These data were pooled indicating that 10% of the EOC patient population lack expression of any specified TAA, 20% express one or more TAA at low levels, 34% at moderate levels, and 36% at high levels (figure 2 Aii, all data).

The SI data in individuals with NED were similarly analysed. After conversion of the raw data (Supplementary table 1) to the percentage of individuals in each SI group, the Shapiro-Wilk test qualified the meta-cohort as not normally distributed. The non-parametric Kruskal-Wallis test was employed and identified no significant difference in the TAA SI between antigens in individuals with NED (figure 2 Bi). Further, application of the ROUT outlier function (Q=1%) recognized five TAA expression datasets as outliers from their assigned meta-cohort: PPARβ, PEDF, ROR2, SSEA4 and TRIM27. Four of these five antigens where also identified as outliers in the EOC SI meta-cohort. As such, these data were removed from the meta-cohort before reapplication of the Shapiro-Wilk normality test, qualifying the data as non-parametric, before the Kruskal-Wallis significance test determined that there was no significant difference in the number of NED individuals with absent, low, moderate or high TAA SI, regardless of the antigen being quantified (figure 2 Bii). The SI of EOC associated antigens in individuals with NED was therefore homogeneous indicating that 80% of healthy individuals lack expression of any specified EOC TAA, 12% express any TAA at low levels, 5% at moderate levels, and 3% at high levels (figure 2 Bii, all data).

Serum CA-125 levels are homogenous in EOC patients

Papers in the serum CA-125 meta-cohort were as follows: [54-59]. Expression of CA-125 in the serum of EOC patients was recorded in six of the included articles and combined into a meta-cohort containing data on a total of 78 patients (table 1). Raw data was analysed for normality using the Shapiro-Wilk test, classifying the data as non-parametric. The Kruskal-Wallis test then identified a significant difference in CA-125 serum level between patients from different cohorts (figure 3a). Box plot analyses were used to define the minimum, maximum, median, lower and upper quartile of CA-125 serum levels from the six cohorts (figure 3b) before the ROUT outlier identification function (Q=1%) was applied. The test determined that the expression profile quantified in paper 56 was an outlier from the rest of the meta-cohort. As such, the data on the eight patients from this paper were removed from the meta-cohort and the Shapiro-Wilk normality and Kruskal-Wallis tests repeated. Without cohort 56, there was no significant difference in CA-125 serum levels in the meta-cohort (figure 3c). The data therefore indicates that CA-125 serum levels in the EOC patient group was homogenous with a lower quartile of 135U/ml, a median of 416U/ml, and an upper quartile of 1710U/ml (figure 3d).

Further, serum CA-125 minimum, maximum, median, lower and upper quartile and interquartile range (IQR) of the meta-cohort, along with the defined maximum level in individuals with NED, were used to assign CA-125 a targeting priority score of 12 (out of 12) indicating its theoretical suitability for antigen directed diagnostic and therapeutic capacity (table 2).

Tissue EpCAM expression is heterogenous in EOC patients

Papers in the tissue EpCAM meta-cohort were as follows: [34, 60, 61]. Expression of EpCAM in the tumor tissue of EOC patients was recorded in three of the included papers that quantified expression as the percentage of positively stained cells (table 1). Raw data were analysed using the Shapiro-Wilk test which classified the data as non-parametric before the Kruskal-Wallis test identified a significant difference in the percentage of EpCAM positive tumor cells between the cohorts (figure 4a). Boxplot analysis of the three datasets were used to define the minimum, maximum, median, lower and upper quartile of EpCAM expression before the ROUT outlier identification function (Q=1%) was applied. The test did not identify any outliers in the meta-cohort illustrating EpCAM tissue expression in EOC patients was heterogenous (figure 4b). Box plot analyses of the meta-cohort indicated that the lower quartile of EpCAM expression in EOC patients was 17% of tumor cells positive, the median was 80% and the upper quartile was 91%, while the large interquartile range (IQR) of EpCAM expression supported its allocation as a heterogeneously expressed antigen (figure 4b).

These data along with the defined maximum expression of EpCAM in individuals with NED were used to assign the TAA a targeting priority score of seven (of a maximum of 12) indicating that it was theoretically unsuitable for antigen directed diagnostic and therapeutic targeting (table 2) [62].

Discussion

TAA diagnostic capacity

EOC disease progression occurs much faster than symptoms present, and given their non-specific nature, many patients present at the later stages of disease. Oncology bodies do not recommend screening for EOC in asymptomatic women, advising instead that investigative tests should only be applied if there is compelling clinical evidence of EOC, including increased pelvic mass [5, 63, 64]. Strikingly, this recommendation is contrary to the findings of numerable peer-reviewed screening trials; In a 30 year annual screening study of 46,101 of asymptomatic women aged 25 or above, 71 individuals were diagnosed with stage I (n=30), II (n=15) or III (n=26) EOC and 17 individuals with borderline premalignant lesions via transvaginal ultrasonography [65]. Accounting for just 0.2% of the monitored population, these 88 women all experienced significantly better 5-, 10- and 20- year survival rates, respectively, when compared to the rest of the EOC patient population. This was because their malignancy was diagnosed before the onset of symptoms and therefore before extensive progression [65]. Additionally, in a meta-analysis of three randomised controlled screening trials that harnessed routine CA-125 serum analysis accompanied by transvaginal ultrasonography, the screening meta-group were 1.18 times more likely to be diagnosed with EOC at an earlier stage, associated with a significant mortality reduction compared to the non-screening meta-control group [66].

Prevention and early detection is the cornerstone of reducing the number of lives lost to EOC. CA-125 could act as a valuable screening target for EOC in asymptomatic women. Before such initiatives could be harnessed in the clinic, the scientific community must work to justify the ability of CA-125 to robustly distinguish cancerous and control tissues. Further, to truly benefit the patient population, studies must validate the ability of CA-125 screening to detect EOC sooner. Understandably, ostensibly healthy women can be hesitant to participate in routine screening ventures given the invasive nature of the diagnostic procedures; Should CA-125 screening for EOC detection be clinically validated, willingness to undergo routine screening could be bolstered [67]. As only a relatively small proportion of the trial cohorts benefitted from screening, gynaecologic oncology bodies will not endorse routine EOC screening until a highly sensitive, specific and non-invasive option is available. This approach however fails to recognize the individuals whose lives were extended as a direct result of early detection [5, 68]. Our study provides further justification for the investigation of

whether CA-125 could fill the recognised gap in EOC screening of the asymptomatic population. With further laboratory investigation, advancement of CA-125 towards routine screening ventures could be authenticated.

Furthermore, it has been demonstrated that expression characteristics of patient tissue and serum can predict the progression profile of their pathogenesis along with how they respond to therapy. For example, let-7g, a serum-stable mircoRNA, has been proven to be expressed at significantly increased levels in chemo-sensitive high grade serous ovarian cancer (HGSC) patients [69]. Additionally, let-7g has been observed as a promoter of cell cycle arrest and barrier to epithelial to mesenchymal transition (EMT) in both wild-type and let-7g transfected ovarian cancer cell lines. A wider family of extracellular vesicle related micro-RNAs have also demonstrated an ability to reliably discriminate healthy individuals from EOC patients though study of their plasma expression, demonstrating statistically significant sensitivity and specificity capabilities [70, 71]. Such studies highlight the broad diagnostic capacity of serum stable proteins and micro-RNAs to include not only primary diagnosis, but prediction of appropriate therapeutics on an individual patient basis, which could intuitively improve patient outcomes.

All six of the papers included in the CA-125 meta-cohort quantified the TAAs expression in the context of assessing its application as a diagnostic marker for EOC [54-59]. Three also studied its expression in the context of using the antigen as a therapeutic target. Our analyses indicated that CA-125 expression is homogenous in EOC patients and is therefore predictive, with 75% of the population expressing the TAA at 135U/ml or above, 100U/ml over the limit of detection in individuals with NED. TAAs with homogeneous expression in the EOC patient group have a higher diagnostic capacity as the chance of a false-negative result is reduced [72]. Despite clinical oncology bodies not (yet) endorsing CA-125 as a reliable diagnostic marker, the literature and our own meta-analyses conclusively disagree. Given the predictable expression and well-studied behaviour of CA-125 in the EOC disease state versus homeostatic conditions, we suggest that CA-125 antigen screening should be performed routinely in the adult female population, especially for those at high-risk of developing EOC, promoting earlier diagnosis and improved mortality.

None of the papers identified through the systematic search assessed the capacity of EpCAM as a diagnostic target in EOC [34, 60, 61]. Further, we could not identify a single primary paper in the current literature that explicitly defines the heterogeneity of EpCAM expression in EOC patients. Papers 34, 60 and 61 analysed tumor samples from 23, 11 and seven patients respectively therefore any comment on expression heterogeneity would be weak due to the small cohort sizes. By combining these data into a meta-cohort we were able to define EpCAM expression as heterogenous in EOC patients. Given our analyses, and its relatively low specificity for diseased tissue, we would not recommend EpCAM as a diagnostic target in the EOC disease space with the current scientific understanding.

The ability of TAAs to be reliable diagnostic targets was bolstered by our SI meta-cohort analysis which established that 90% of the EOC population will express any TAA (of those 28 studied in the meta-cohort), whilst 70% of patients will score 2+ (moderate) or above regardless of the antigen in question. We suggest that TAA profiling of serum and/or tissue samples from the adult female population, alongside current smear testing practices, could offer a solution that minimises the patient discomfort that surrounds EOC diagnostic procedures, while supporting earlier diagnosis and consequent improved mortality.

TAA therapeutic capacity

Specificity is an important measure of TAA suitability for targeting as it indicates the capacity of any antigen directed therapy to be delivered to diseased tissue only [73, 74]. Our specificity calculations allowed a direct comparison of different TAAs and were defined based upon this principle. Specificity

A and B measure antigen targeting suitability; The smaller specificity A (%) and the higher specificity B (%), the better the antigen would be as a target as there is a larger difference between its expression profile in healthy verses diseased tissue. As such, antigens with a high prioritisation score in specificity A and B have a distinct ability to direct antigen guided drugs to diseased tissue, for example antibody-drug conjugates (ADC). Notably, the calculated data validates the need for two measures of antigen specificity to accurately prioritise antigens in this way as both CA-125 and EpCAM gained the highest prioritisation score of four against specificity B criteria, yet strikingly different prioritisation scores against specificity A criteria.

The higher the SI of an antigen within a patient population, the greater efficacy and wider applicability of any antigen-targeted therapies (and diagnostic strategies) as there are more sites for antigen-driven drug docking (and a higher likelihood of positive detection during diagnosis) [75, 76]. Of course, CA-125 has been suggested as a targetable TAA throughout the literature. Studies have proven that CA-125 overexpression supports the invasive phenotype of EOC by promoting P120 catenin translocation to the cytoplasm, subsequently activating Rho GTPase signalling responsible for cellular migration and proliferation [77, 78]. Additionally, through stabilisation of N-cadherin, CA-125 driven translocation of P120 catenin promotes EMT characteristic of stage III and IV EOC malignancies, further promoting the invasive and migratory phenotype [79, 80]. Therefore, it would make good sense to target this TAA, not only as a tissue-specific anchor for ADC docking as proven here, but also mechanistically to interrupt tumorigenic migration and proliferation pathways.

There is currently a phase three, double blind, placebo controlled, multicenter study in progress, involving 602 patients, to assess the safety and efficacy of a CA-125 targeted therapeutic agent to treat EOC [81]. Oregovomab is designed to target serum CA-125 and upon binding, it is thought that CA-125-Oregovomab complexes act to prime dendritic cells and provoke an immune response against the tumor [82]. As such, the drug is thought to obstruct the cancer hallmark ‘evasion of immune destruction’ [83]. Scheduled to conclude in 2027, the scientific and patient community eagerly awaits the study results, however it is predicted that when administered alongside chemotherapy, Oregovomab will increase progression free and overall survival rates in the patient group [84].

Elsewhere, immunotherapy initiatives for ovarian cancer have focused on cancer testis antigens (CTA), proven to have strong associations with clinicopathological features like tumor invasion and metastasis, including MAGE-A and NY-ESO-1, as they have significantly heightened expression in the diseased ovary compared to the healthy ovary [85-87]. Such CTA-targeted immunotherapies are able to shift responses of the established tumor microenvironment back towards homeostasis where immune orchestrators are able to identify and eliminate tumorigenic populations, re-establishing and preserving anti-tumor immune responses [88]. Researchers note, however, high expression of these TAAs is required to elicit efficacious responses from any CTA-targeted therapy, offering weight to our novel prioritization calculations [89]. Additionally, folate-receptor alpha (FRα), a membrane bound protein that coordinates DNA synthesis and repair mechanisms, has been suggested as a potential therapeutic target in EOC. The literature demonstrates up to 43% of EOC patients express the antigen in over 75% of their tumor tissue, making it a suitable therapeutic target for a considerable proportion of EOC patients [90]. Attempts to therapeutically target FRα in the EOC disease space have been numerable and consistently well tolerated by patients, however clinical responses are not consistent ranging from negligible to complete [90-93]. In the case of slight therapeutic response, clinicians theorize the consequences of heavy pre-treatment, such as chemo- and radiotherapies, offer an explanation for the unfavourable results, suggesting such initiatives (once advanced through full clinical validation) should be implemented in the earlier stages of EOC treatment [92]. Such data further supports the necessity of harnessing TAA profiling to direct meaningful clinical interventions.

All studies identified through the systematic literature review that quantified EpCAM expression in EOC patients did so as part of an assessment of the TAAs capacity to be a target for therapy [34, 60,

61]. Altogether, the three papers did little more than qualify and quantify EpCAM expression levels. All three papers recommended progression to clinical trial with an anti-EpCAM ADC, suggesting EpCAM targeted therapy may be beneficial in patients who fail to respond to current first line clinical strategies. However, with limited data authors in each study were reluctant to suggest that EpCAM targeted therapeutics could be the chosen treatment modality in the future. This suggests our assignment of EpCAM as a possible, yet low priority target was legitimate. Additionally, our classification of EpCAM as a heterogeneously expressed TAA suggests it may be a poor target for therapy due to its irregular pattern of expression within the EOC patient group.

Importantly, our SI meta-cohort analyses indicate that EOC TAAs can realistically be targeted for therapy, especially antibody driven therapies; It proves that the vast majority of the EOC patient population do express the studied TAAs at moderate (or higher) levels meaning any antigen targeted precision medicine would likely benefit a large proportion of EOC patients, providing a target with high specificity and homogeneity was chosen.

Systematic review limitations

A fundamental facet to performing a meaningful systematic literature review is comprehensive accumulation of appropriate literature directed by the constitution of bespoke search terms that are discrete to the subject area and exhaustive in capacity. At the review by title stage a substantial 4,613 papers were excluded as the research question did not include quantification of an EOC TAA, implying our search terms were too broad. Looking at the similarities between the 32 papers that did meet the inclusion criteria, three main antigen detection and quantification methods were used. In retrospect, we believe that adding “Immunoassay” OR “ELISA” OR “Immunohistochemistry” search terms and Boolean operators to the existing search expressions could have provided the necessary clarity and specificity to the search, potentially reducing the number of irrelevant papers identified and subsequently removed during screening. Additionally, a limiting factor to the statistical capacity of this review was restricted access to information. A substantial number of records were excluded because they quantified antigen expression as positive or negative only. Surprisingly, in the methodology and/or graphical presentations of these records it was indicated that more detailed (i.e. numerical) expression data were recorded, however this information was not disclosed to the reader. In fact, had more of the identified records disclosed full numerical data sets, many of the antigens in the SI meta-cohort may also have been eligible for scrutiny against our prioritization calculations, as was done for CA-125 and EpCAM. Just 48% of the included papers reported therapy received by patients prior to quantification of TAA expression. This limited our ability to assess the impact prior treatment had on protein expression profiles and subsequent suitability of the potential protein targets in treatment specific sub-groups. This remains an outstanding confounding variable in this review. We advocate information transparency in published works to allow the proliferation of primary research data into systematic review initiatives which have the power to statistically scrutinize multiple cohort data sets, less burdened by selection bias, and thus provide outcomes that include a wider range of studies.

Conclusion

Our analyses demonstrated that CA-125 would be a promising TAA target for both diagnosis and treatment in contrast to EpCAM. SI meta-cohort analyses concluded that targeting TAAs for diagnosis and therapy is scientifically sound, and demonstrated that such initiatives must be advanced towards routine clinical use. In today’s era of precision medicine, we can realistically target TAAs with cytotoxic agents to provide distinguished clinical interventions that lack systemic impact, enabling delivery of high drug payloads to malignant tissue. This can be performed whilst also harnessing routine TAA screening for earlier diagnosis and decreased mortality for EOC patients. We suggest TAA profiling should be a fundamental aspect of EOC diagnosis, sitting alongside the FIGO framework, to aid the development of targeted therapeutics and diagnostics, and facilitating effective personalised therapy. This data would not only allow clinicians to suggest the most appropriate care

for each patient, but meta-analyses of these data, as conducted here, would allow identification of new diagnostic biomarkers and therapeutic targets along with assessment of the applicability of multi-TAA treatment and diagnostic targeting.

Data availability statement

The original data presented in the study are included in the main text/supplementary material, further enquiries can be directed to the corresponding author.

Author contributions

The literature search was conducted and cross examined by L.W. and N.C. while B.G. directed its implementation. L.W. drafted the manuscript and all authors reviewed and revised the article, and provided approval of the manuscript prior to submission.

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Ethics statement

Ethical approval for this study was not required as the data employed did not reveal patient identifiers.

Supplementary material

Supplementary table 1 can be found at http://carcin.oxfordjournals.org/

Conflict of Interest Statement: None declared.

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Figure 1: Schematic of the systematic review detailing the record identification and screening process. Of the 7,120 records identified, 32 were included for meta-analysis.

Figure 2: A: The staining intensity of EOC associated antigens in individuals with EOC was homogeneous with 10% of the EOC patient population lacking expression of any specified TAA, 20% expressing any TAA at low levels, 34% at moderate levels, and 36% at high levels. **Ai:** Normalised data were data were qualified as non-parametric before significance testing indicated no significant difference in the TAA staining intensity between antigens. **Aii:** Application of the ROUT outlier function (Q=1%) reasoned removal of PPARβ, PEDF, ROR2 and SSEA4 expression profiles before reapplication normality and significance testing. There was no significant difference in the number of EOC patients with absent, low, moderate or high TAA staining intensity, regardless of the antigen being quantified. **B:** The staining intensity of EOC associated antigens in individuals with NED was homogeneous with 80% of healthy individuals lacking expression of any specified EOC TAA, 12% expressing any TAA at low levels, 5% at moderate levels, and 3% at high levels. **Bi:** Normalised data were qualified as non-parametric before significance tests identified no significant difference in the TAA staining intensity of the antigens in individuals with NED. **Bii:** Application of the ROUT outlier function (Q=1%) reasoned removal of PPARβ, PEDF, ROR2, SSEA4 and TRIM27 expression profiles from the dataset before reapplication of normality and significance tests. There was no significant difference in the number of NED individuals with absent, low, moderate or high TAA staining intensity, regardless of the antigen being quantified.

Figure 3: CA-125 was expressed homogenously in the EOC patient group **A:** CA-125 levels in the serum of EOC patients were extracted from six papers and analysed for normality and significance. *** p=0.001. **B:** Box plot analysis of the six papers were performed before extraction of the minimum, maximum, median, lower and upper quartile and application of the ROUT outlier identification function (Q=1%) which identified patient data collected from paper 56 as outliers from the rest of the meta-cohort. *** p=0.001. **C:** Upon removal of cohort 56, normality and significance tests were repeated. Without data set 56, there is no significant difference in the CA-125 levels detected in patient serum between the cohorts. **D:** Box plot analysis was repeated after removal of data set 56 and indicated that CA-125 serum levels were homogenous in the meta-cohort with a lower quartile of 135U/ml, a median of 416U/ml, and an upper quartile of 1710U/ml.

Figure 4: EpCAM was expressed heterogeneously in the EOC patient population. **A:** EpCAM tissue expression data were extracted from three papers and analysed for normality and significance which indicated there was a significant difference in the expression of EpCAM between the patient cohorts. **** p=<0.0001. **B:** Box plot analyses of the EpCAM expression data set were performed. The lower quartile of EpCAM expression was 17% of tumour cells positive, the median 80%, and the upper quartile 91%. **** p=<0.0001.

Table 1: Collation of the antigen expression data extracted from records 54 to 61, and record 34, on level or expression of CA-125 or EpCAM. The cohort profile details number of patients in each study (n), patient histology, disease stage or grade and treatment received by the patients before antigen levels were quantified, where available. The detection method used to quantify TAA presence in EOC patients and the associated units are provided. The expression profile median, range, lower and upper quartile (LQ and UQ) were calculated where possible. Abbreviations: S = Serous, Mu = Mucinous, E = Endometrioid, C = Clear cell, T = Transitional cell, Und = Undifferentiated, Unk = Unknown, FC = Flow cytometry, IHC = Immunohistochemistry, ND = not disclosed, DI – Data insufficient.

Table 2: Summary of the expression level data calculated from statistical analysis of the CA-125 and EpCAM expression meta-cohorts. Additionally, the upper limit of detection in the NED ovary was given. These values were employed to prioritise the antigens for diagnostic and therapeutic targeting based on relative specificity and homogeneity. The prioritisation criteria applied to CA-125 and EpCAM are stated (calculations detailed in methods) generating priority scores of 12 and 7 (out of 12) respectively.

Ref.	Cohort profile				Expression profile					
	n	Histology (n)	FIGO stage (n) or Grade, G (n)	Previous treatment	Protein target	Detection method (unit)	Median	Range	LQ	UQ
54	13	S (13)	FIGO III (11) IV (2)	Platinum chemotherapy	CA-125, Cancer antigen 125	Immunosorbent assay (U/ml)	360	51-26000	220	836
55	18	S (17) M (1)	G low (1) high (13) ND (4)	ND			1362	39-25000	218	2259
56	8	ND	FIGO I (3) III (4) IV (1)	Chemotherapy (7) Radiotherapy (2) Immunotherapy (2)			12048	873-31497	3636	16852
57	22	ND	FIGO I (4) II (1) III (15) IV (2)	ND			244	9-7830	99	819
58	7	S (5) M (1) Unk (1)	FIGO I (2) III (3) IV (1) Unk (1)	ND			259	1-4642	48	1361
59	10	S (10)	FIGO I (2) II (2) III (6)	ND			1074	60-16990	350	5108
60	11	ND	FIGO III (11)	ND	EpCAM, epithelial cell adhesion molecule	IHC (%)	5	3-25	4	12
34	23	ND	ND	ND			80	0-100	75	100
61	7	S (5) Unk (2)	FIGO I (1) III (3) IV (3)	Chemotherapy (7)		FC (%)	92	12-96	53	93

Expression		CA-125 (U/ml)	EpCAM (%)	Criteria	Assignment criteria (Result, priority score)	CA-125		EpCAM	
						Result (%)	Priority score	Result (%)	Priority score
NED	Upper limit ovary	35 [59]	27 [62]	Specificity A	Low (>50%, 1) Moderate (30-49%, 2) High (10-29%, 3) Very high (<10%, 4)	8	4	34	2
EOC	Minimum Lower quartile Median Upper quartile Interquartile range Maximum	9 135 416 1710 1575 3149 7	0 17 80 91 74 100	Specificity B	Low (<10%, 1) Moderate (10-29%, 2) High (30-49%, 3) Very high (>50%, 4)	92	4	66	4
				Homogeneity	Low (50%+, 1) Moderate (30-49%, 2) High (10-29%, 3) Very high (<10%, 4)	5	4	77	1
				TOTAL PRIORITISATION SCORE		**12**		**7**	

Figure 1

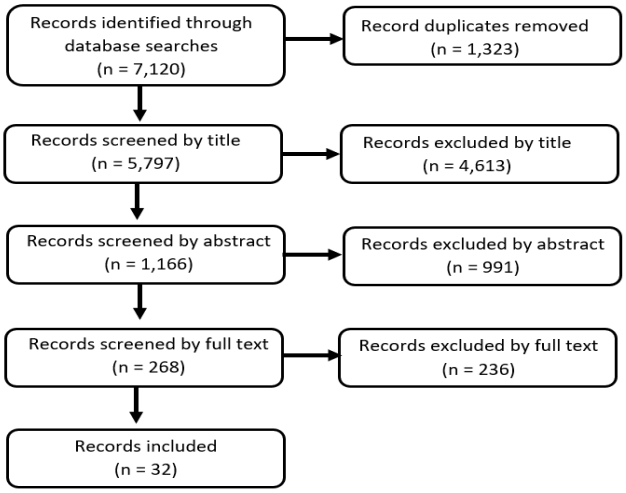

Figure 2

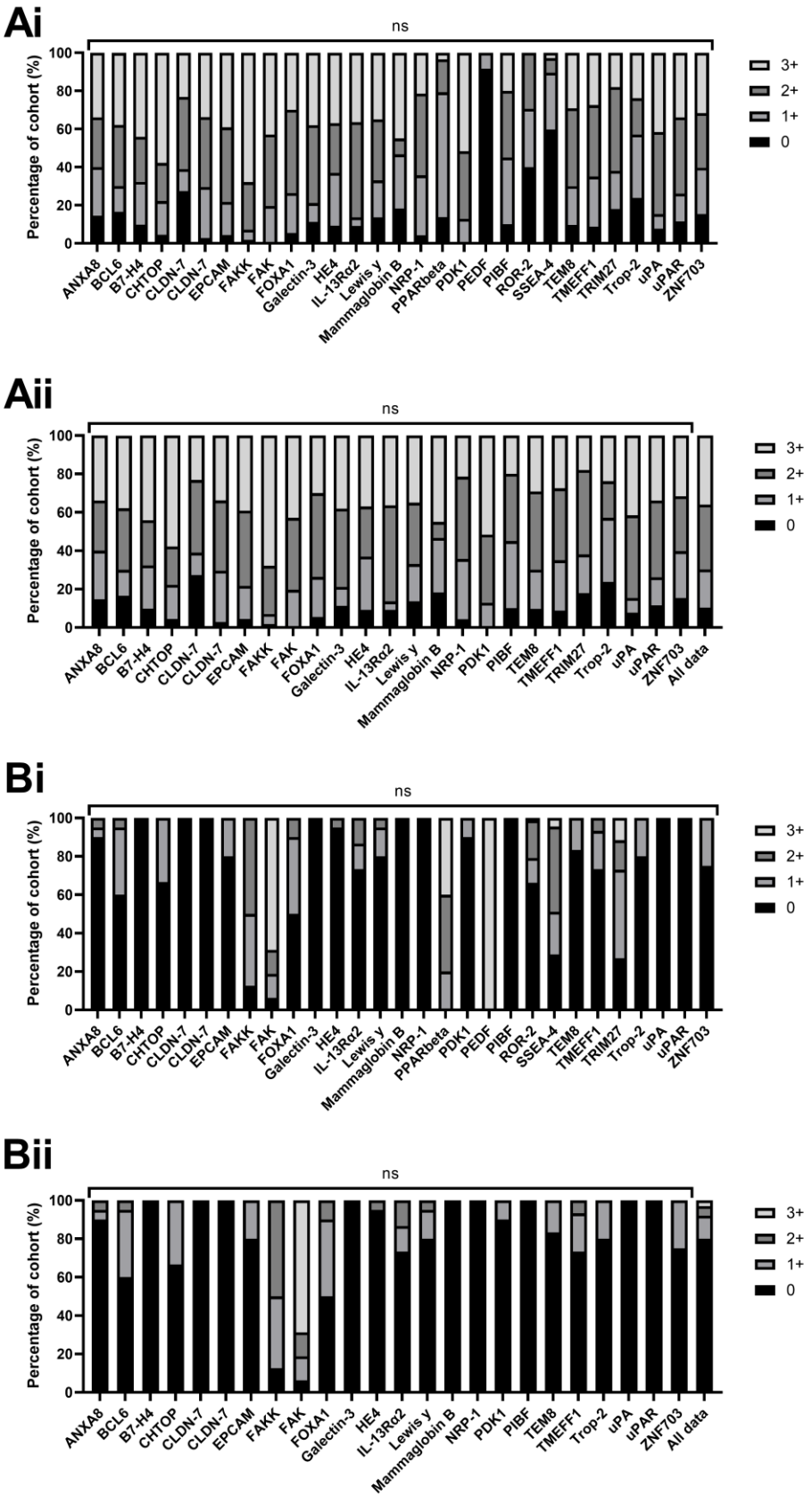

Figure 3

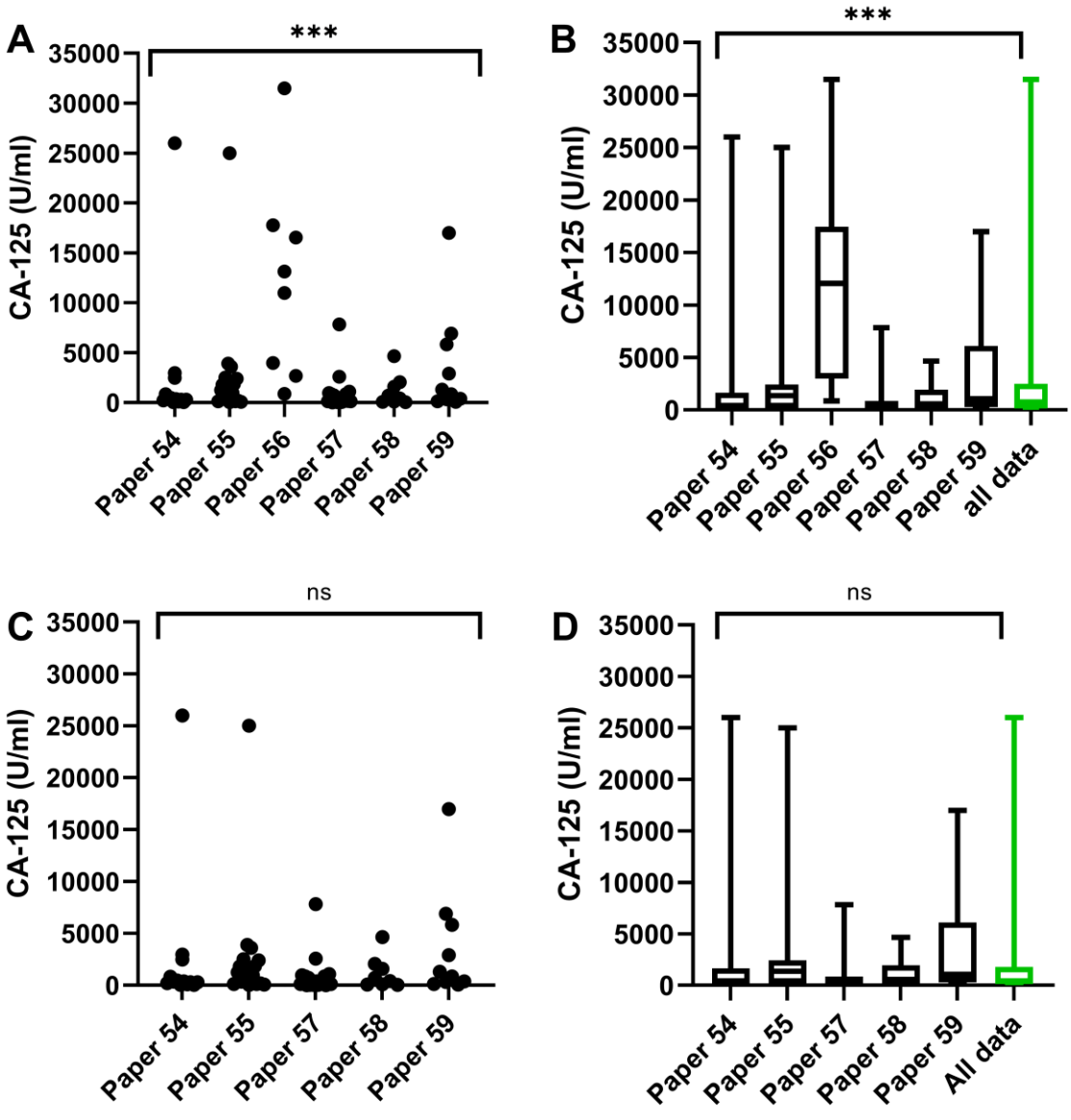

Figure 4