Identification of biomarkers for the diagnosis and targets for therapy in patients with clear cell ovarian cancer: a systematic literature review

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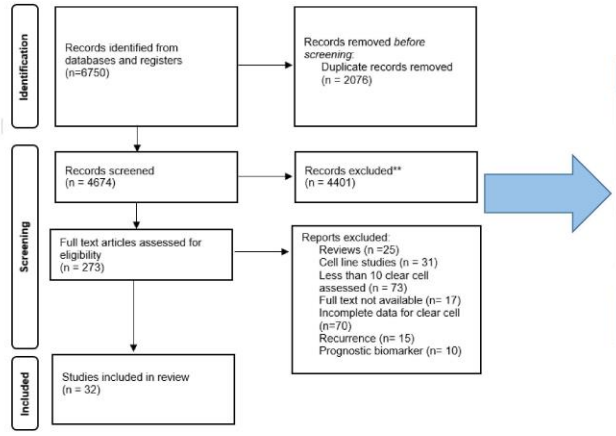
ABSTRACT

Clear cell ovarian cancer (CCOC) is a rare type of epithelial cancer often resistant to platinum-based chemotherapy. Biomarkers for the diagnosis of CCOC, and targets for immunotherapy, both have the potential to improve outcomes for patients. Our review aims to determine whether any antigens already identified in the literature could fulfil this remit. PubMed, Medline, Web of science, Scopus, Cochrane, CINAHL and EMBASE were searched and included all reported studies up until August 2021. Primary research articles on human adult females including at least 10 CCOC patients were included. Quality assurance was carried out using a modified version of the QUADAS-2 tool. Sensitivity, specificity and AUC were extracted from each included study by two independent reviewers. Twenty three articles were included which identified 19 gene transcripts/proteins and one antibody, with reported sensitivities between 21-100% and specificities between 0-100% for expression in CCOC and differentiation from other epithelial ovarian cancer subtypes, benign gynaecological disease or normal tissue. 12 studies identified biomarkers with a sensitivity and specificity above 80%. A panel of biomarkers consisting of IMP3, napsin A and HNF-1β achieved the highest AUC of 0.954. This review demonstrates that there are promising candidate biomarkers for the diagnosis of CCOC, some of which are highly specific, and have the potential to act as targets for therapy. However, larger cohort studies are needed to validate these biomarkers and their potential use in clinical practice.

Graphical_Abstract



Biomarkers achieving a sensitivity & specificity >90%

Author	Biomarker	Sensitivity	Specificity
Kandalaft et al	Napsin A	100%	96.5%
Li et al	Dual NHF1β and Napsin A	97.4%	91.2%
Rehki et al	Napsin A	100%	90.9%
Sugita et al	IGFBP-1	90.3%	94.2%

Lu et al. achieved an AUC of 0.954 using IMP3/HNF1β/Napsin A

INTRODUCTION

Clear cell ovarian cancer (CCOC) is a rare subtype of epithelial ovarian cancer (EOC) which accounts for less than 5% of all ovarian malignancies and 3.7-12.1% of EOC cases (1) with serous being the most prevalent histological subtype (2). CCOC is more prevalent in Asian populations, accounting for up to 20% of all ovarian cancer cases in Japan (3). The main predisposing factor for the development of clear cell is a history of endometriosis (4), other risk factors include: early menarche, late menopause, nulliparity, infertility, BRCA1 and BRCA2 mutations (5).

Whilst clear cell is often diagnosed at an early stage (6), patients frequently present with non-specific symptoms, making it difficult to diagnose EOC; therefore, there is a need to identify novel biomarkers for EOC that can be used for the differential diagnosis of women presenting with non-specific gynaecological symptoms. Serum biomarkers could provide a method of the earlier detection of EOC and the current serum biomarker used to aid the diagnosis of ovarian cancer is CA125. CA125 is a membrane protein, however it is not very specific for EOC (7) and can be even less specific for CCOC. The gold standard for conclusive diagnosis is post-operative histological examination (8) so specific immunohistochemical markers for CCOC can also aid diagnosis. Additionally, late stage CCOC carries a poor prognosis due to its resistance to platinum based chemotherapy (9) so new, more effective treatment strategies need to be developed. CCOC has a unique immune-suppressive microenvironment (10) and therefore immunotherapy could be an attractive treatment option in this cancer.

In this review, we aimed to evaluate the current biomarkers identified in existing literature that are the most sensitive and specific for CCOC to allow differentiation from other subtypes of EOC and ovarian tumours of other origins. We also wanted to identify possible candidates for targeted therapy, in particular immunotherapy, to discern other treatment options for this subtype of EOC. There has been limited literature identifying targets for the therapy of CCOC

and therefore our systematic review will help to provide a summary of existing literature on this matter and enable us to identify where further research is needed.

METHODS

A systematic literature review was performed to identify primary studies which identified biomarkers to aid the diagnosis of CCOC, that have a high sensitivity and specificity, and allow the differential diagnosis of clear cell from other subtypes of epithelial cancer. We then looked at candidate biomarkers that could be used as future targets for therapies such as immunotherapy. This review was performed and reported according to The Preferred Reporting Items for Systematic Review (PRISMA) guidelines and registered with PROSPERO (CRD42021259078) (11).

Article Identification

Articles were identified through a search of PubMed, Medline, Web of science, Scopus, Cochrane, CINAHL and EMBASE and included all reported studies up until August 2021. The search terms used can be found in **Appendix S1.** Articles were restricted to English language, there were no restrictions on setting, geographical location or year published. In Scopus, the document type was restricted to "Article" and the source type was restricted to "Journal" in order to yield more relevant results that match the inclusion criteria.

Article Selection

Articles were subjected to the screening process to remove irrelevant literature. Two authors, HB and OS, screened titles, abstracts and full text articles against inclusion and exclusion criteria independently. Any discrepancies were resolved by consensus between HB and OS at each stage. The inclusion criteria were primary research articles on human adult females including at least 10 CCOC patients. Exclusion criteria included: review; case reports; paediatrics; animal studies; cell line studies; clear cell recurrence; metastasis from another primary cancer and prognostic biomarker studies. Initially case series were excluded in the protocol but due to the rare nature of clear cell we decided to include them at the screening criteria stage to assess all possible biomarkers for clear cell.

Quality Assessment and Data Extraction

Quality assurance was performed independently by HB and OS to assess the applicability of all articles using the QUADAS-2 tool (12) which removed all lower quality studies. Any disagreements were resolved by consensus.

Data extraction was performed by HB and OS for the genes, proteins and antibodies that were shown to contribute to the pathogenesis of CCOC **(Supplementary Table 1)**. The data extracted from each full text article included: author, year, article title, country, study design, patient characteristics (age, background medical history), inclusion and exclusion criteria, study outcomes, number of controls, number of healthy donors, total number in the study,

total number analysed, International Federation of Gynecology and Obstetrics (FIGO) staging, methods, biomarker examined, bodily fluid or tissue examined, sensitivity, specificity, area under the curve (AUC), statistical analysis undertaken and follow up. Specificity was calculated for the studies which did not directly present this in their results. We analysed the biomarkers based on the sensitivity, specificity and AUC, where this information was provided, for all included articles. Due to the heterogeneity between the study designs and methods and the limited raw data available in the articles, it was decided a meta-analysis calculating receiver operating curves for each of the biomarkers could not be performed and therefore we conducted a narrative synthesis of the data available (sensitivity, specificity and AUC) for each biomarker.

RESULTS

Selected Articles

The database search initially identified 6750 papers. There were 2076 duplicates, which were removed. In the following step, 4401 articles were excluded based on title and abstract, and the remaining 273 articles were assessed for eligibility based on the full texts. 241 articles were excluded after full-text screening (**Figure 1**). In totality, 32 studies were identified that matched the inclusion criteria. After quality assurance was performed (**Supplementary Table 2)**, which removed nine low quality studies, 23 articles were identified for data extraction and further analysis.

In the 23 studies, 19 genes/proteins and one antibody were identified that were expressed and were involved in the pathogenesis of CCOC **(Supplementary Table 1).**

Study Characteristics

The studies included varied in study design; studies used either case-control, cohort or case series design to investigate the expression of biomarkers for CCOC. The majority of studies included a comparison of other EOC subtypes, other ovarian malignancy, benign gynaecological disease or normal tissue. Studies were conducted in a variety of countries including: Japan (11 studies), USA (seven studies), China (two studies), Turkey (one study), India (one study) and one cross-country study. The sample sizes of CCOC cases varied across the included studies, ranging from 10-1033 cases.

Serum Biomarkers

Two articles identified candidate serum biomarkers for the diagnosis of CCOC; Arakawa et al. (13) identified tissue factor pathway inhibitor 2 (TFPI2) as a diagnostic serum biomarker to differentiate CCOC from other ovarian disease including other EOC subtypes, benign tumours and endometriomas. Diagnosis was confirmed by pathology after resection. They developed an ELISA for this biomarker and set the cut off at 345 pg/ml to differentiate CCOC from ovarian disease. The training set showed a sensitivity and specificity of 79.3% and 91%, respectively, which was independently validated delivering a sensitivity and specificity of 71.4% and 91.9%, respectively. AUC was reported as 0.893 for the training set and 0.817 for the validation set demonstrating TFPI2 as a good differentiator between CCOC and other

ovarian disease. TFPI2 outperformed CA125 in diagnosing CCOC with CA125 showing a sensitivity and specificity of 79.3% and 41.8% respectively, with an AUC of 0.595 in the training group and an AUC of 0.795 in the validation group. This indicates that TFPI2 could be a more accurate diagnostic biomarker for CCOC than CA125.

Furthermore, Mikami et al. (14) found the candidate A2160 which is a fully-sialylated alpha-chain of complement 4-binding protein as a potential serum biomarker for CCOC. They used mass spectrometry and used one healthy woman who showed a normal healthy pattern among controls as a normalising standard for the peak areas. They established a cut off of 1.6 U/ml to differentiate early stage CCOC from endometriomas. At this cut off the sensitivity was 87% and specificity was 94% with an AUC of 0.92, outperforming CA125 which showed a sensitivity of 71% and specificity of 50% with an AUC of 0.67; therefore indicating A2160 could be a more specific biomarker for CCOC once validation has been performed.

Immunohistochemical Markers and Targets for Therapy

Firstly, all of the following biomarkers were identified as being expressed in CCOC using IHC on Formalin-Fixed Paraffin-Embedded (FFPE) tissues with the exception of Ueda et al. (15) who used frozen tissue specimens for biomarker analysis. All cases diagnosed as CCOC in each study were confirmed by pathologists as true clear cell tumours to provide a reference standard for the accuracy of each biomarker examined.

A promising target for immunotherapy is programmed cell death ligand 1 (PD-L1) which is a transmembrane protein which is upregulated on antigen-presenting cells in a normal immune system. Howitt et al. (16) reported expression of PD-L1 in a subset of CCOCs with Microsatellite Instability and a high level of mutations (MSI-H), at a sensitivity of 100% and specificity of 55.5% against microsatellite stable CCOCs. However only three of the 30 CCOCs were MSI-H making this a very small sample for the determination of biomarker expression. Of the microsatellite stable CCOCs, 44.4% expressed PD-L1. Willis et al. (17) also reported CCOCs expressing PD-L1 compared to endometrial clear cell carcinoma with a sensitivity and specificity of 74% and 9.52%, respectively. There was low specificity when compared to endometrial clear cell carcinoma due to the similarities in histopathologic features, however this demonstrated the possibility of using PD-L1 as a target for all clear cell gynaecological tumours. Additionally, two studies used NCI markers to assess MSI in CCOC. Cai et al. (18) evaluated MSI using matched pairs of CCOC and normal tissue and found six of the 42 CCOCs were MSI-H with an overall sensitivity and specificity of 67.7% and 94.4%, respectively. Similarly, Ueda et al. (15) found 44.4% of MSI-H CCOCs had MSH3 abnormalities with 100% specificity for the NCI marker; however, this study used no controls and therefore the results need independent validation to determine whether MSH3 abnormalities specifically occur in malignant tissue rather than healthy tissue or other benign conditions.

Hampras et al. (19) discovered the gene TGFBR2 had the most significant association with CCOC, reporting a p value of 0.001 for the gene burden test in the regulatory T cell (Treg) pathway. Compared to healthy controls TGFBR2 increased the risk of developing CCOC by 21% (OR=1.21) indicating that this would be an attractive target for therapy.

Napsin A was the most studied biomarker, in eight studies in total, and five studies examined napsin A alone (20-24). Napsin A has previously been identified as a biomarker for lung adenocarcinoma (25). All eight studies reported napsin A as having a specificity greater than 80% and all studies, except for Lu et al. (26), reported a sensitivity of more than 80% compared to other EOC subtypes or other gynaecological tumours. The study with the best diagnostic accuracy was Kandalaft et al. (24) that demonstrated a sensitivity of 100% for napsin A and a specificity of 96.5% when compared with expression in other EOC subtypes. A further five studies identified single diagnostic biomarkers that could be possible targets for therapy. Hagemann et al. (27) investigated mammaglobin, which is a known biomarker for breast cancer, as a biomarker for CCOC. However, the sensitivity was low at 21% and had a specificity of 78.98% compared to other EOC subtypes and normal tissue. Therefore mammaglobin may be a less attractive target for therapy due to its low expression in CCOC. Furthermore, Maeda et al. (28) identified glypican-3 as a target for therapy and a biomarker for CCOC. The control group included normal tissue and EOC subtypes with some of these samples expressing glypican 3; therefore there was a reported specificity of 86.5% against all controls but 92.4% against EOC subtypes and a sensitivity of 44%. Lowery et al. (29) conducted a case-control study investigating BAF250a loss in CCOC in comparison to a healthy control group and found 41% of clear cell cases had loss of this protein. The specificity was 52.3% due to endometrioid EOC demonstrating a higher loss in comparison, but demonstrated its potential as a promising therapeutic target. Kato et al. (30) identified hepatocyte nuclear factor 1 beta (HNF-1β) as a potential biomarker for clear cell with a high sensitivity of 100% and specificity of 86.2% compared to other EOC subtypes, showing a good diagnostic accuracy in the cases examined. Finally, Okamoto et al. (31) identified the antibody for epithelial antigen, Ber-EP4, as a candidate biomarker for CCOC. It expressed a sensitivity of 90%, however, due to the high expression of Ber-EP4 in other subtypes the specificity was 7.69% indicating a less specific target for clear cell. However when differentiating EOC from normal tissues the sensitivity and specificity increased to 100% and 90% respectively, demonstrating this could be a target for the therapy of all EOC subtypes.

Six additional studies investigated multiple biomarkers that are expressed in CCOC. Firstly, Tsukioka et al. (32) identified glucose transporters as potential biomarkers and targets for therapy with the control group consisting of EOC subtypes. GLUT1, GLUT3 and GLUT4 were identified as being expressed in clear cell with GLUT1 showing the highest sensitivity at 95.9%. However, these biomarkers showed a low specificity due to the fact that they were also expressed in other EOC subtypes, therefore whilst these would provide a useful target for the therapy of EOC, they were less specific with regards to clear cell. Yamamoto et al. (33) identified a potential target for therapy with platelet derived growth factor receptor alpha and beta (PDGFR-α and PDGFR-β). The control group used in this study included patients with endometriosis where some samples were shown to express these biomarkers. Both PDGFR-α and PDGFR-β showed a sensitivity of 97%, however PDGFR-α was superior with a specificity of 85.7%, indicating its potential as a candidate for future therapy. Sugita et al. (34) identified IGFBP-1, is a member of the insulin-like growth factor binding proteins, and found it was a specific marker for clear cell compared to other EOC subtypes with a sensitivity of 90.3% and specificity of 94.2%. They also investigated expression of HNF-1β in these cases which demonstrated a good diagnostic value with a sensitivity and specificity of 96.8% and 84.1% respectively. Similarly, Fadare et al. (35) investigated multiple biomarkers

including napsin A, AMACR and HNF-1β compared to other EOC subtypes and other ovarian tumours. Napsin A and AMACR showed the highest specificity of 99%, while HNF-1β showed the highest sensitivity at 92%. Two of these studies used a panel of biomarkers to improve the specificity of the diagnostic test. Li et al. (36) investigated HNF-1β and napsin A as dual biomarkers and found the sensitivity and specificity was 97.4% and 91.2% respectively compared to other EOC subtypes. Lu et al. (26) used a combined panel consisting of the proteins IMP3, HNF-1β and napsin A which had the highest specificity of all biomarkers assessed of 98.63% and an AUC of 0.954 indicating that these biomarkers were good classifiers able to distinguish CCOC from other EOC subtypes. However, HNF-1β alone showed the highest sensitivity of 89.39%.

DISCUSSION

Main Findings

Our systematic review identified biomarkers from the existing literature that are expressed in CCOC and have the theoretical potential to act as immune or molecular targets for therapy. Of the 23 included studies, 12 reported biomarkers that achieved a sensitivity and specificity above 80% while only four of these studies reported a sensitivity and specificity above 90% (21,24,34,36) with a CCOC diagnosis being confirmed by a pathologist. The sensitivity of all biomarkers ranged from 21%-100% and specificity ranged from 0-100% compared to other EOC subtypes, gynaecological disease or normal tissue indicating a large difference in expression for the biomarkers included. AUC ranged from 0.817 to 0.954 with the highest AUC achieved by Lu et al. (26) using a panel biomarker including IMP3, napsin A and HNF-1β which showed a high specificity for CCOC and is therefore a reliable and promising diagnostic test for clear cell.

Interpretation

Due to the rare nature of CCOC, there have been few systematic reviews conducted focusing on CCOC; therefore this review provides novel insights into the diagnostic biomarkers that could be used for CCOC with some being highly sensitive and specific for CCOC compared to other EOC subtypes or benign gynaecological diseases. The two serum biomarkers identified in this review both outperformed CA125 when diagnosing CCOC, indicating the potential for more sensitive and specific serum biomarkers are available for early diagnosis of CCOC. These are vital in ensuring more effective treatment and a better prognosis for clear cell, when this disease is diagnosed at an earlier stage, whilst also providing a cost-effective test for CCOC. This is also supported by a systematic review performed by Hulstaert et al. (37) who investigated RNA serum biomarkers for the diagnosis of EOC and found multiple of these biomarkers outperformed CA125 in diagnostic accuracy. Kobayashi et al. (38) identified genetic markers that have the potential to act as serum biomarkers for CCOC and therefore show the capacity to further outperform CA125 as a widespread diagnostic tool in clinical practice when used to specifically diagnose CCOC. They identified genes and proteins that were also identified in the present study including HNF-1β, IGFBP-1, napsin A, glypican 3 and TFPI2, demonstrating that further research into these biomarkers could identify them as serum biomarkers and/or targets for therapy. As

some of these biomarkers have been found in cancers of other origin or benign gynaecological disease, their individual use in clinical practice may not be as useful in diagnosing CCOC but we have highlighted the usefulness of a panel of biomarkers to increase the specificity of each individual biomarker for CCOC diagnosis.

Targeted therapy is an attractive treatment option for CCOC due to its frequent resistance to platinum based chemotherapy (8) which carries an even poorer prognosis due to the increased risk of recurrence and worse survival rates (39). Targeted therapy aims to deliver a drug directly to a particular gene or protein that is specific for cancer cells to reduce the side effect profile of therapies (40). In particular immunotherapy has emerged as a newer strategy to treat cancer and is often combined with other conventional therapies (41) to enhance the effectiveness of treatment and reduce future recurrence; therefore indicating an ideal treatment option for CCOC. Trials of immune checkpoint therapy using PD-L1 inhibitors have been carried out and have so far shown a modest response in EOC (42), however better responses occur with higher PD-L1 expression. Howitt et al. (16) showed a high expression of PD-L1 in MSI-H CCOCs indicating that immune checkpoint therapy may be of more benefit in this subset of CCOCs. Additionally, Amano et al. (43) identified HNF-1β and ARID1A as potential targets as reported in our review and a current trial is investigating the sensitivity of CCOC to ATR inhibitors (44). Therefore, our study highlights multiple biomarkers that could be used as therapeutic targets, that are both specific for CCOC and can provide an effective treatment option, when the tumours are resistant to chemotherapy.

Limitations

There were multiple limitations in our study. Firstly, due to the experimental nature of the included studies they often had small sample sizes with the majority including less than 50 CCOC cases. Most of the studies examined biomarker expression compared to other EOC subtypes with a smaller number comparing expression to healthy donors or benign gynaecological disease so further research needs to investigate the expression of these biomarkers in comparison to normal tissues to determine whether these biomarkers could be used as therapeutic targets. Also in most of the studies there was a risk of overfitting due to the lack of validation in an independent cohort; therefore in order to truly assess the diagnostic accuracy of these biomarkers and their specificity for CCOC further research needs to be performed to validate these findings. Each study classified positive staining for the biomarkers at different levels which introduces bias into the results and therefore in future validation studies a standard staining level should be used for each biomarker to accurately investigate expression in CCOC. Furthermore, this study was limited to English language which may have excluded some biomarkers and targets for therapy. The impact of searching databases by title, abstract and keywords limited the number of irrelevant studies where the search term might have only appeared in the main text of the study but may also have led to the exclusion of some relevant articles.

CONCLUSIONS

In conclusion, we have reviewed 20 biomarkers, including genes, proteins and antibodies that were identified from existing literature and look promising for the diagnosis and treatment of CCOC. There is a growing need to find new methods of diagnosing and treating EOC; therefore, larger independent studies need to be conducted to validate the findings of these experimental studies to assess their potential use in future clinical practice.

List of Abbreviations

AMACR - alpha-methylacyl-CoA racemase

AUC - area under the curve

CCOC - clear cell ovarian cancer

ELISA - enzyme linked immunosorbent assay

EOC - epithelial ovarian cancer

FIGO - International Federation of Gynecology and Obstetrics

HNF-1β - hepatocyte nuclear factor 1 beta

IHC- immunohistochemistry

MSI- microsatellite instability

PD-L1 - programmed death ligand 1

PDGFR - platelet derived growth factor receptor

TFPI2 - tissue factor pathway inhibitor 2

Availability of data and materials

Data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Contributions

B.G. designed the study, H.B. and O.S. performed the systematic literature review, analysed the data and made the figures. H.B. and B.G. wrote the paper. All authors read and approved the final manuscript.

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Figure 1