Accepted Manuscript

British Journal of General Practice

CA125 test result, test-to-diagnosis interval and stage in ovarian cancer: a cohort study using electronic health records

Funston, Garth; Mounce, Luke; Price, Sarah; Rous, Brian; Crosbie, Emma; Hamilton, William; Walter, Fiona

DOI: https://doi.org/10.3399/BJGP.2020.0859

To access the most recent version of this article, please click the DOI URL in the line above.

Received 14 September 2020
Revised 30 November 2020
Accepted 02 December 2020

CA125 test result, test-to-diagnosis interval and stage in ovarian cancer: a cohort study using electronic health records

Garth Funston BSc MB BChir[1*], Luke T.A. Mounce BSc MSc PhD[2], Sarah Price PhD[2], Brian Rous MA MB BChir PhD FRCPath[3], Emma J Crosbie BSc MBChB PhD FRCOG[4,5], Willie Hamilton CBE MD FRCP FRCGP[6], Fiona M Walter MA MD FRCGP[7]

[1] Clinical Research Fellow, The Primary Care Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK

[2] Research Fellow, University of Exeter, Exeter, UK

[3] Consultant Histopathologist, National Cancer Registration and Analysis Service, Public Health England, Cambridge, UK

[4] Professor of Gynaecological Oncology, Gynaecological Oncology Research Group, Division of Cancer Sciences, University of Manchester, Manchester, UK

[5] Consultant in Gynaecological Oncology, Department of Obstetrics and Gynaecology, Manchester University NHS Foundation Trust, Manchester Academic Health Sciences Centre, Oxford Road, Manchester, UK

[6] Professor of Primary Care Diagnostics, University of Exeter, Exeter, UK

[7] Principal Researcher in Primary Care Cancer Research, The Primary Care Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK

*gf272@medschl.cam.ac.uk

Abstract

Background
Cancer-Antigen-125 (CA125) is recommended as a first-line investigation in women in UK General Practice with symptoms of possible ovarian cancer.

Aim
To compare time between initial primary care CA125 test and diagnosis (test-to-diagnosis interval), tumour morphology and stage, in women with normal (<35U/ml) and abnormal (≥35U/ml) CA125 prior to ovarian cancer diagnosis.

Design
Retrospective cohort study using routinely collected primary care and cancer registry data.

Methods
Associations between CA125 result with test-to-diagnosis interval, stage and ovarian cancer morphology were examined.

Results
351 (77%) women had an abnormal and 105 (23%) a normal initial CA125 result prior to ovarian cancer diagnosis. Median test-to-diagnosis interval was 35 days (Interquartile range [IQR]:21-53) in those with abnormal, and 64 days (IQR:42-127) in those with normal CA125s. Tumour morphology differed by CA125 result, with indolent borderline tumours being less common in those with abnormal (n=47, 13%) than normal (n=51, 49%) CA125s (p<0.001). Staging data was available for 381 women. 106 of 304 (35%) women with abnormal CA125s and 66 of 77 (86%) women with normal CA125s were diagnosed at an early stage: normal CA125s were associated with early-stage diagnosis (Odds Ratio:12.1, 95% CI:5.8-25.1, p<0.001).

Conclusion
Despite experiencing longer intervals between testing and diagnosis, women with normal CA125s more frequently had indolent tumours and were more commonly diagnosed at an early stage, compared to those with abnormal CA125s. Whilst more sensitive testing approaches might expedite diagnosis for some women, it is not known whether this would translate to earlier stage diagnosis and improved survival.

Keywords
Ovarian cancer, Cancer antigen 125, CA125, early diagnosis, diagnostic intervals, general practice

How this fits in
CA125 is widely used as an initial test for ovarian cancer in symptomatic women in primary care, but a recent study reported that CA125 levels were normal in 23% of women prior to diagnosis. In this study, we found that although women with normal CA125 results take longer to receive a diagnosis following testing, they are more likely to be diagnosed with less aggressive more curable forms of disease and at an earlier stage than women with abnormal CA125 results. This provides some reassurance for those using and being tested

for CA125, but improving the sensitivity of primary care testing approaches for ovarian cancer could still be of benefit to patients.

Introduction

Ovarian cancer is the 6th most common cancer to affect UK women, with over 7,000 women diagnosed each year.[1] It has the worst prognosis of all gynaecological cancers, accounting for over 4,000 UK deaths annually.[2] Whilst overall ovarian cancer prognosis is relatively poor, this varies markedly based on tumour type, with 5-year relative survivals of 47% for invasive epithelial cancer (the most common type) compared to 93% for ovarian germ cell tumours and 97% for borderline tumours.[3,4]

Most women with ovarian cancer are diagnosed after presenting with symptoms in primary care, but ovarian cancer symptoms, such as bloating and abdominal pain, are non-specific and therefore individual symptoms have relatively low positive predictive values (PPVs) for the disease.[5,6] In 2011 the guideline-producing body for England, Wales and Northern Ireland, the National Institute for Health and Care Excellence (NICE), advocated testing for the serum biomarker CA125 in women with symptoms of possible ovarian cancer in primary care.[7] NICE recommended that women with an elevated CA125 (≥35U/ml) should then undergo ultrasound testing; however, they did not provide guidance on the follow-up or investigation of women with 'normal' (<35 U/ml) CA125 results. Many other countries including Ireland, Australia, Canada and the USA also recommend CA125 as a primary care test for ovarian cancer.[8]

CA125 is a glycoprotein found within healthy ovaries, but blood levels commonly increase in ovarian cancer. Around 80% of women with ovarian cancer have raised CA125 levels pre-surgery.[9] CA125 is more frequently elevated in advanced than early stage disease and in some tumour types than others.[10] Some clinicians and researchers have expressed concerns that using CA125 as a single first line investigation might delay diagnosis and lead to worse outcomes in women whose ovarian cancer is not associated with CA125 levels ≥35 U/ml.[11] Yet, there is little research exploring the relationship between CA125, time to diagnosis and outcomes.

In this study, we examined the association of initial primary care pre-diagnostic CA125 result with time between testing and diagnosis (test-to-diagnosis interval), tumour morphology and disease stage in women with ovarian cancer.

Methods

Study design, setting and data sources

This retrospective cohort study utilised data from the Clinical Practice Research Datalink (CPRD) GOLD database, a dataset containing postcode linked deprivation measures (provided by CPRD), and data from the National Cancer Registration and Analysis Service (NCRAS). CPRD GOLD consists of anonymised, coded primary care data, including laboratory results and diagnoses, for around 7% of the UK population.[12] The deprivation dataset consists of a five-level Townsend score - an area-level deprivation metric in which higher

scores indicate greater material deprivation. NCRAS data (the English cancer registry) consists of detailed information on cancers diagnosed in England, including stage and morphology.[13] CPRD-NCRAS linkage was performed at the patient level by NHS Digital.[3] To match NCRAS coverage, this study was restricted to England.

Study period and cohort

We utilised a data sample obtained for a related study.[14] The sample consisted of women with a CA125 test recorded in CPRD between 1st May 2011 and 31st December 2014. From this sample, we excluded those <18 years old, those registered at a GP practice not deemed 'up-to-standard' on data quality by CPRD,[12] those with a record of ovarian cancer on or before the CA125 test date and those with a CA125 test in the 12 months prior to the first CA125 test during the study period. To maximise data quality, only CA125 entries recorded in standard CA125 units (U/ml, IU/ml, KU/L, KIU/L) and with a laboratory upper reference limit were accepted. Similarly, we excluded CA125 values associated with clearly erroneous upper reference limits (245, 420 and 455 U/ml) as these could also indicate issues with the recording or coding of CA125 values.[14] We then identified women diagnosed with ovarian cancer, as recorded in the cancer registry, within 12 months of CA125 testing. This group formed the study cohort.

We defined ovarian cancer, on the basis of International Classification of Diseases (ICD)-10 codes, as an ovarian malignancy (C56), a fallopian tube malignancy (C57.0), a peritoneal malignancy (C48.1, C48.2), or a neoplasm of uncertain behaviour of the ovary (D39.1).[14] Fallopian and peritoneal cancers arise from the same tissue type, and are diagnosed, staged and treated in the same way, as cancer arising from the surface of the ovary. Borderline tumours are non-invasive, usually diagnosed at an early stage and have a good prognosis but may recur and generally require surgery. Borderline tumours are included in NICE guidance on ovarian cancer detection.[7]

CA125 category

NICE recommends using a CA125 cut-off of 35 U/ml. Therefore, we classified women on the basis of the initial CA125 test into two groups: CA125-abnormal (≥35U/ml) and CA125-normal (<35U/ml).

Co-variates

We used a code list to identify symptoms of possible ovarian cancer included in current NICE guidelines (abdominal/pelvic pain, abdominal distension/bloating, change in bowel habit, fatigue, weight loss, urinary frequency/urgency, loss of appetite, pelvic mass or ascites), which were recorded in CPRD in the 30 days prior to CA125 testing.[15] Level of deprivation was determined using the five-level Townsend score within the deprivation measures dataset.

Test-diagnosis interval

The date of cancer diagnosis is recorded for all tumours within NCRAS. We calculated the test-to-diagnosis interval (days from first CA125 test, in the year before diagnosis, to diagnosis date as recorded in NCRAS) for all women.

Cancer stage, and morphology

Tumour behaviour, morphology and stage were identified from the cancer registry. Tumours were classified on the basis of ICD10 codes as: “Borderline Epithelial”, “Invasive Epithelial”, “Invasive Non-epithelial” and “Invasive Not Otherwise Specified (NOS)”. Stage was categorised as early (stage I-II) or late (stage III-IV).

Statistical analysis

Accelerated failure time (AFT) models were used to examine the association between CA125 result and test-to-diagnosis interval. AFT models are a parametric time-to-event analysis previously utilised in CPRD studies.[16] AFT models can be used to calculate Time Ratios. A Time Ratio >1 indicates that a variable prolongs the time to an event (e.g. diagnosis) while a ratio <1 indicates that the variable is associated with an earlier event. A univariate model was constructed to examine the relationship between CA125 result and test-to-diagnosis interval. A multivariable model was constructed incorporating age, a binary variable denoting the presence/absence of relevant symptoms prior to CA125 testing, and Townsend score. The presence or absence of a symptom was included as there is evidence that symptoms are more likely to be coded, rather than recorded in free text (which is unavailable for research), when they are more severe/persistent - which could result in expedited referral and diagnosis.[17] Weibull, generalised gamma, log-normal and log-logistic distributions were examined. Log-logistic distribution was the best-fit parameterisation, according to the Akaike information criterion. We report Time Ratios with associated p-values and 95% confidence intervals.

Fisher’s exact test was used to assess whether women with abnormal and normal CA125 tests differed significantly in tumour morphology. We then performed pairwise analyses to assess whether there was a significant difference for each morphology category. We corrected for multiple comparisons, setting our significance level at p=0.01.[18]

In a subgroup for whom stage data was recorded, logistic regression was used to examine the association between CA125 result and stage at diagnosis (classified as early stage: I-II and late stage: III-IV). We adjusted for age, the presence/absence of a recorded symptom and Townsend score. Given their favourable prognosis, we performed a sub-analysis excluding borderline tumours. We explored the relationship between explanatory variables with missing stage using logistic regression. We report crude and adjusted odds ratios (OR) with 95% confidence intervals and associated p-values.

All analysis was performed in Stata version 15.1.

Results

CPRD provided data on 55,519 women who were eligible for NCRAS linkage and who had had a CA125 test between 1st May 2011 and 31st December 2014. After exclusions, 456 women diagnosed with ovarian cancer in the year following CA125 testing were included in the study (**Figure 1**). 105 women (23%) had a normal initial CA125 and 351 (77%) an abnormal CA125. 41 (9%) women had a repeat CA125 test performed prior to diagnosis. 30 women with an abnormal initial CA125 had a repeat test of which 29 (97%) remained abnormal. 11 women with a normal initial CA125 test had a repeat test and 8 (73%) had an

increase in their CA125 level, but in only 3 cases (27%) was this increase sufficient to reach the ≥35 U/ml threshold.

Mean age was higher in those with abnormal CA125 results and a greater proportion of women with abnormal CA125 results had a coded symptom of possible ovarian cancer (**Table 1**).

Test-to-diagnosis interval

Overall median test-to-diagnosis interval in the cohort was 42 days (Interquartile range [IQR]: 25-62 days); 35 days (IQR: 21-53 days) in those with abnormal CA125 results and 64 days (IQR: 42-127 days) in those with normal CA125 results (**Table 2)**. AFT models demonstrated a significant association between CA125 result and test-to-diagnosis interval. A Time Ratio of 2.0 (95% CI:1.7-2.4, p<0.001) indicated that the test-to-diagnosis interval in those with normal CA125s was twice as long as in those with abnormal CA125s. The Time Ratio remained unaltered when adjusting for age, the presence/absence of a recorded symptom and Townsend score.

Tumour morphology

Tumour morphology differed significantly by CA125 result (p=<0.001) (**Table 3**). Invasive epithelial cancers were the most common type in women with abnormal CA125 results (81%) while borderline tumours were the most common type in women with normal CA125 results (49%). Serous tumours accounted for 52% of invasive tumours in those with an abnormal CA125 compared to 30% in those with a normal CA125 result.

Stage at diagnosis

Staging information was missing for 75 women – 47 (13%) women with an abnormal CA125 and 28 (27%) women with a normal CA125. In women for whom stage was recorded (n=381), 106 (35%) of those with an abnormal CA125 were diagnosed with early stage disease compared to 66 (86%) of those with a normal CA125.

Logistic regression, performed in patients with recorded stage and adjusted for age, the presence or absence of a recorded symptom and Townsend score, demonstrated that the odds of being diagnosed with early stage disease were 12.1 times higher in women with normal than abnormal CA125s (**Table 5**). A sub-analysis conducted after excluding borderline tumours (**Supplementary Table 2**) demonstrated a significant association between having a normal CA125 and being diagnosed at an early stage (OR:9.0, 95% CI:4.0-19.8).

There was strong evidence to support an association between having a normal CA125 result and missing stage in a logistic regression model. No such association was identified when borderline tumours were excluded from analysis.

Discussion

Summary

Women with normal CA125 results in primary care prior to ovarian cancer diagnosis took twice as long to be diagnosed following testing than those with abnormal results. Despite

this, in women for whom staging data was available, 86% of those with normal CA125 results were diagnosed at an early stage compared to only 35% of those with abnormal CA125 results. In addition, indolent borderline ovarian tumours were more common, and aggressive invasive epithelial cancers less common, in women with normal than abnormal CA125 results.

Strengths and limitations

A major strength of this study is its large size - our sample is equivalent to over 6% of all ovarian cancers diagnosed in the UK each year.[1] Our results should be generalisable to women tested for CA125 in primary care prior to ovarian cancer diagnosis, as we used a primary care database which is generally representative of the UK population.[12] Ovarian cancer diagnoses were identified from NCRAS, which reports a near 100% case ascertainment.[13]

This study has limitations. When defining our cohort, we assumed that cancer diagnosed within 12 months of the initial CA125 test was present at the time of testing. A period of one year, which has been used in similar studies,[14,19,20] and which was specified prior to data analysis,[14] was chosen as a compromise between minimising the inclusion of incidental cancers and maximising the inclusion of relevant cancers. Examining a longer follow-up was not possible as NCRAS data was only available till the end of 2015, but given that only one woman out of 456 was diagnosed in month 12, extending follow-up is unlikely to significantly alter the results. We did not examine a shorter follow-up period e.g. 6 months, as this would have preferentially excluded patients from the CA125 normal group (who have longer test-diagnosis intervals) and would therefore produce biased results.

Patients with severe disease, who often have more severe symptoms, frequently experience expedited diagnoses when compared to those with less severe disease, an observation sometimes referred to as the 'sick quick' phenomenon.[21] As CA125 is also more likely to be elevated in women with more severe disease, this may act as a confounder. We have adjusted our analyses for the presence/absence of relevant coded symptoms, as symptoms may be more likely to be coded (rather than mentioned in free text) if they are more severe,[17] but we are unlikely to have been able to adjust fully for severity of symptoms and disease.

We considered adjusting for ethnicity in our analyses, but it was not included as not all patients have an ethnicity recorded within CPRD.[22] We have not identified any evidence within the literature indicating that ethnicity is associated with either diagnostic interval or stage at diagnosis for ovarian cancer and would not expect it to significantly alter our results.

A normal CA125 result was significantly associated with missing stage. This is to be expected, as stage is less frequently recorded in the cancer registry for borderline tumours, which are more common in women with normal CA125s. It is reassuring that when borderline tumours were excluded no significant association between CA125 result and missing stage was identified and a normal CA125 result was still strongly associated with early stage diagnosis. While we have no reason to suspect that study findings would differ if staging data were available for all patients, the magnitude of the association between CA125 result and stage should be interpreted with caution.

Comparison with existing literature

Previous research has identified an association between false negative results and longer healthcare intervals. In one study, patients with a negative chest X-ray prior to lung cancer diagnosis experienced longer primary care intervals than those with an abnormal chest X-ray, while in another study patients with a false negative rheumatoid factor in primary care, prior to a rheumatoid arthritis diagnosis, took longer to be referred to a specialist.[23,24] Other researchers have postulated that negative results could provide false reassurance to patients thereby delaying their re-presentation in the presence of persistent symptoms. Similarly, false-reassurance could affect GPs, prompting them to seek alternative diagnoses and delaying referral.[23,25]

Few studies have investigated the relationship between false negative results and outcomes in symptomatic patients, although one study found that patients with false negative fine-needle aspiration before thyroid cancer diagnosis had worse outcomes.[26] In our study, for the majority of women with normal CA125 results, cancer was detected at an early stage, in contrast to women with abnormal results, despite longer test-to-diagnosis intervals. This finding could be due to differences in tumour type. Borderline tumours were four times as common in women with normal than abnormal CA125 levels. Borderline tumours less frequently cause elevations in CA125 than their invasive counterparts, tend to grow slowly and 80% are diagnosed at an early stage.[27] In contrast, invasive epithelial tumours, which typically have an insidious onset and poor survival, were twice as common in women with abnormal than normal CA125s. Further, aggressive invasive serous tumours, which are more frequently diagnosed at a later stage and more frequently elevate CA125 levels than other invasive tumour types,[10] accounted for half of invasive tumours in women with abnormal CA125 results and only a third of invasive cancers in women with normal CA125 results.

Implications for research and practice

CA125 detected 77% of ovarian cancer and 88% of the invasive epithelial subtype, which is responsible for the majority of ovarian cancer mortality.[28] An abnormal CA125 test is therefore helpful in identifying women with possible ovarian cancer, especially the most lethal type, but a normal CA125 does not exclude disease. It is reassuring that most women with normal CA125 results were diagnosed at an early stage, despite taking longer to be diagnosed. However, given the observational nature of this study we could not determine to what extent women with normal CA125 results experienced disease progression or worse survival as a result of their prolonged test-to-diagnosis intervals. Diagnostic strategies which use novel serum biomarkers or imaging modalities in combination with CA125, may detect additional ovarian cancer cases,[8,29] which could expedite diagnosis in some women. However, large prospective studies would be needed to determine whether implementing more sensitive testing strategies, with the aim of reducing test-to-diagnosis intervals by a median of a month for a small proportion of women, would lead to earlier stage diagnosis and improved survival.

Regardless of its impact on survival, reducing unnecessary delay in ovarian cancer diagnosis is likely to be beneficial for women with normal CA125 results. Delay in cancer diagnosis is associated with psychological distress, particularly among women, and perceived delays can damage doctor-patient relationships.[30,31] Earlier diagnosis of ovarian cancer could reduce morbidity, even if a stage shift is not achieved, by detecting lower volume disease.[29]

Possible strategies to reduce diagnostic delay could include appropriate safety netting with reassessment, re-testing or alternative investigations e.g. ultrasound, if symptoms persist or worsen. In our study, only a small proportion (9%) of women with normal initial CA125 results had a repeat test. There was an increase in levels in 75% of cases but in only 23% was this increase sufficient to reach the 35 U/ml threshold, supporting the idea that rising levels below the 35 U/ml threshold could be used to prompt further investigation.[29] The nature, duration and severity of presentation should also be considered when deciding upon a follow-up strategy. For example, if the patient develops a pelvic mass (which has a high positive predictive value for ovarian cancer) an urgent referral is warranted,[15,32] whereas alternative follow-up strategies may be more appropriate for less highly predictive presentations.

Although we have employed the NICE advocated threshold of 35 U/ml in this study to categorise results as 'normal' or 'abnormal', this is an oversimplification. The probability of cancer is much higher in women with a value of 34 U/ml compared to those with a value of 1 U/ml,[14] yet these women are all classified as 'normal'. In addition, the probability of cancer also varies markedly with age. Newly developed primary care prediction models which estimate of the probability of ovarian cancer in women of any age at any CA125 level (1-1000 U/ml) could help select women for further investigation, but further evaluation is needed to determine whether this would improve ovarian cancer outcomes.[14]

Funding
This research arises from the CanTest Collaborative, which is funded by Cancer Research UK [C8640/A23385], of which GF is Clinical Research Fellow and WH and FMW are Directors. The study was also funded by the National Institute of Health Research (NIHR) School of Primary Care Research [FR17 424] (GF, FMW). EJC is supported by the National Institute for Health Research Manchester Biomedical Research Centre [IS-BRC-1215-20007]. The funders of this study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The views expressed are those of the authors and not necessarily those of the NIHR, the Department of Health and Social Care or Cancer Research UK.

Ethical/regulatory approval
Approval for this research was obtained from the Independent Scientific Advisory Committee (ISAC) for the Medicines and Healthcare products Regulatory Agency (protocol number 18_184).

Competing interests
We have no competing interests to declare.

Acknowledge
This work uses data provided by patients and collected by the NHS as part of their care and support.

References

1 Cancer Research UK. Ovarian cancer incidence statistics. www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer/incidence#heading-One (accessed Sept 12, 2019).

2 Cancer research UK. Ovarian cancer mortality statistics. www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer/mortality (accessed June 29, 2020).

3 American Cancer Society. Survival Rates for Ovarian Cancer. 2018. https://www.cancer.org/cancer/ovarian-cancer/detection-diagnosis-staging/survival-rates.html#references (accessed July 15, 2020).

4 Kalapotharakos G, Högberg T, Bergfeldt K, Borgfeldt C. Long-term survival in women with borderline ovarian tumors: A population-based survey of borderline ovarian tumors in Sweden 1960-2007. *Acta Obstet Gynecol Scand* 2016; **95**: 473–9.

5 National Cancer Intellegence Network. Routes to Diagnosis 2006-2016 by year, V2.1a. 2019. http://www.ncin.org.uk/publications/routes_to_diagnosis (accessed May 21, 2020).

6 Funston G, O'Flynn H, Ryan NAJ, Hamilton W, Crosbie EJ. Recognizing Gynecological Cancer in Primary Care: Risk Factors, Red Flags, and Referrals. *Adv Ther* 2018; **35**: 577–589.

7 National Collaborating Centre for Cancer. Ovarian Cancer: The recognition and initial management of ovarian cancer (NICE Clinical Guidelines No. 122). Cardiff (UK), 2011.

8 Funston G, Melle VM, Ladegaard Baun M-L, *et al.* Variation in the initial assessment and investigation for ovarian cancer in symptomatic women: a systematic review of international guidelines. *BMC Cancer* 2019; **19**: 1028.

9 Medeiros LR, Rosa DD, da Rosa MI, Bozzetti MC. Accuracy of CA 125 in the diagnosis of ovarian tumors: A quantitative systematic review. *Eur J Obstet Gynecol Reprod Biol* 2009; **142**: 99–105.

10 Jacobs I, Bast RC. The CA 125 tumour-associated antigen: a review of the literature. *Hum Reprod* 1989; **4**: 1–12.

11 Olaitan A. Recognition and initial management of ovarian cancer: summary of NICE guidance (Rapid response). *BMJ* 2011; **342**: d2073.

12 Herrett E, Gallagher AM, Bhaskaran K, *et al.* Data Resource Profile: Clinical Practice Research Datalink (CPRD). *Int J Epidemiol* 2015; **44**: 827–36.

13 Henson KE, Elliss-Brookes L, Coupland VH, *et al.* Data Resource Profile: National Cancer Registration Dataset in England. *Int J Epidemiol* 2019; published online April 23.

14 Funston G, Hamilton W, Abel G, Crosbie EJ, Rous B, Walter FM. The diagnostic performance of CA125 for the detection of ovarian and non-ovarian cancer in primary care: a population-based cohort study. *PLoS Med* 2020; **17**: e1003295.

15 National Institute for Health and Care Excellence. Suspected cancer: recognition and referral (NG12). 2015. www.nice.org.uk/guidance/ng12 (accessed July 18, 2019).

16 Ridd MJ, Santos Ferreira DL, Montgomery AA, Salisbury C, Hamilton W. Patient-doctor continuity and diagnosis of cancer: Electronic medical records study in general practice. *Br J Gen Pract* 2015; **65**: e305–e311.

17 Price SJ, Stapley SA, Shephard E, Barraclough K, Hamilton WT. Is omission of free text records a possible source of data loss and bias in Clinical Practice Research Datalink

studies? A case-control study. *BMJ Open* 2016; **6**: e011664.
18 McDonald J. Handbook of Biological Statistics (3rd Edition). Baltimore: Sparky House Publishing, 2014.
19 Watson J, Salisbury C, Banks J, Whiting P, Hamilton W. Predictive value of inflammatory markers for cancer diagnosis in primary care: a prospective cohort study using electronic health records. *Br J Cancer* 2019; **120**: 1045–51.
20 Neal RD, Din NU, Hamilton W, *et al.* Comparison of cancer diagnostic intervals before and after implementation of NICE guidelines: analysis of data from the UK General Practice Research Database. *Br J Cancer* 2014; **110**: 584–92.
21 Tørring ML, Frydenberg M, Hansen RP, Olesen F, Vedsted P. Evidence of increasing mortality with longer diagnostic intervals for five common cancers: A cohort study in primary care. *Eur J Cancer* 2013; **49**: 2187–98.
22 Mathur R, Bhaskaran K, Chaturvedi N, *et al.* Completeness and usability of ethnicity data in UK-based primary care and hospital databases. *J Public Health (Oxf)* 2014; **36**: 684–92.
23 Bjerager M, Palshof T, Dahl R, Vedsted P, Olesen F. Delay in diagnosis of lung cancer in general practice. *Br J Gen Pract* 2006; **56**: 863–8.
24 Miller A, Nightingale AL, Sammon CJ, *et al.* Negative Rheumatoid Factor in Primary Care Delays Referral of Patients with Rheumatoid Arthritis. *Rheumatology* 2014; **53**: i29.
25 Petticrew MP, Sowden AJ, Lister-Sharp D, Wright K. False-negative results in screening programmes: Systematic review of impact and implications. *Health Technol Assess (Rockv)* 2000; **4**: 1–120.
26 Yeh MW, Demircan O, Ituarte P, Clark OH. False-negative fine-needle aspiration cytology results delay treatment and adversely affect outcome in patients with thyroid carcinoma. *Thyroid* 2004.
27 Fischerova D, Zikan M, Dundr P, Cibula D. Diagnosis, treatment, and follow-up of borderline ovarian tumors. *Oncologist* 2012; **17**: 1515–33.
28 Elias KM, Guo J, Bast RC. Early Detection of Ovarian Cancer. *Hematol Oncol Clin North Am* 2018; **32**: 903–14.
29 Gilbert L, Basso O, Sampalis J, *et al.* Assessment of symptomatic women for early diagnosis of ovarian cancer: results from the prospective DOvE pilot project. *Lancet Oncol* 2012; **13**: 285–91.
30 Risberg T, Sørbye SW, Norum J, Wist EA. Diagnostic delay causes more psychological distress in female than in male cancer patients. *Anticancer Res* 1996; **16**: 995–9.
31 Norman A, Sisler J, Hack T, Harlos M. Family physicians and cancer care. Palliative care patients' perspective. *Can Fam Physician* 2001; **47**: 2006–16.
32 Hamilton W, Peters TJ, Bankhead C, Sharp D. Risk of ovarian cancer in women with symptoms in primary care: population based case-control study. *BMJ* 2009; **339**: b2998.

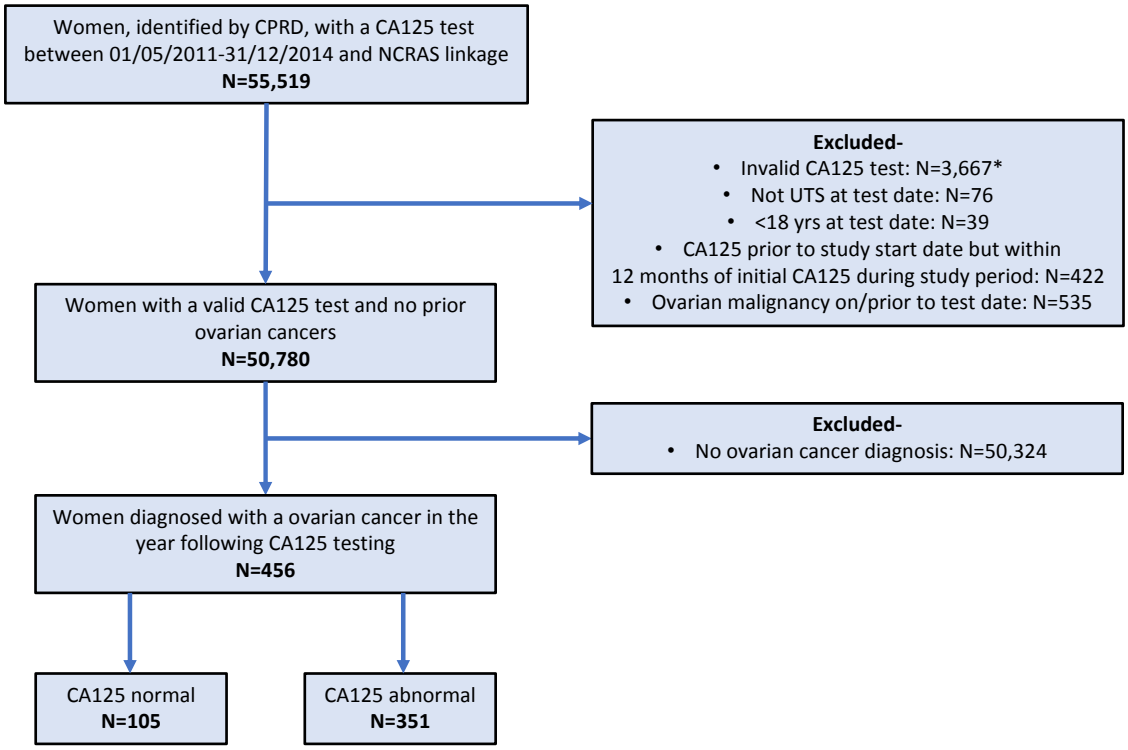

Figure 1. Application of selection criteria.
*No CA125 value, no or incorrect units, no upper threshold or spurious upper threshold recorded.

Table 1. Patient groups and baseline characteristics.

	n	Mean age at diagnosis [range]	Patients with a symptom of possible ovarian cancer recorded pre-testing, n (%)*	Townsend score, n (%)*
Abnormal CA125	351	65 [22-93]	212 (60.4)	Level 1: 80 (22.8) Level 2: 100 (28.5) Level 3: 78 (22.2) Level 4: 61 (17.4) Level 5: 32 (9.1)
Normal CA125	105	57 [18-87]	59 (56.2)	Level 1: 24 (22.9) Level 2: 31 (29.5) Level 3: 25 (23.8) Level 4: 14 (13.3) Level 5: 11 (10.5)
Overall cohort	456	63 [18-93]	271 (59.4)	Level 1: 104 (22.8) Level 2: 131 (28.7) Level 3: 103 (22.6) Level 4: 75 (16.5) Level 5: 43 (9.4)

*Percentage of each group with symptoms and Townsend score

Table 2. Median intervals by CA125 result and crude and adjusted associations between CA125 result and Test-to-diagnosis interval.

	n	Median Test-to-diagnosis interval in days [IQR]	Unadjusted		Adjusted*	
			Time Ratio (95% CI)	p value	Time Ratio (95% CI)	p value
Abnormal CA125	351	35 [21-53]	Reference		Reference	
Normal CA125	105	64 [42-127]	2.0 (1.7-2.4)	<0.001	2.0 (1.6-2.4)	<0.001

*Adjusted for age, presence/absence of a recorded symptom and Townsend score.
Individual associations for all variables are displayed in **Supplementary Table 1.**
IQR = Interquartile range

Table 3. Tumour morphology by CA125 result.

	n	Borderline, n (%)	Invasive			Overall analysis, p-value
			Epithelial, n (%)	Non-epithelial, n (%)	NOS, n (%)	
Abnormal CA125	351	47 (13)	284* (81)	4 (1)	16 (5)	<0.001
Normal CA125	105	51 (49)	39^ (37)	9 (9)	6 (6)	
Pairwise analysis, p-value	-	<0.001	<0.001	<0.001	0.6	-

NOS= Not otherwise specified i.e. could not be classified as epithelial or non-epithelial based on the information within the cancer registry. P-values are derived from Fisher's exact test for independence. Row percentages are shown.

* 158 serous, 16 endometrioid, 14 mucinous, 14 clear cell, 13 other epithelial and 69 epithelial cancers of unknown morphology.

^16 serous, 4 endometrioid, 8 mucinous, 3 clear cell, 4 other epithelial and 4 epithelial cancers of unknown morphology.

Table 4. The association between CA125 test result, age and presence/absence of a recorded symptom with early stage (I-II) diagnosis.

		Unadjusted		Adjusted*	
	n	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Abnormal CA125	304	Reference	-	Reference	-
Normal CA125	77	11.2 (5.7-22.1)	<0.001	12.2 (5.8-25.5)	<0.001
Age (years)	-	0.95 (0.93-0.96)	<0.001	0.94 (0.92-0.96)	<0.001
No symptom record	148	Reference	-	-	-
Symptom record	233	0.51 (0.33-0.77)	0.001	0.35 (0.21-0.59)	<0.001

*Model also adjusted for Townsend score. Data not shown as Townsend score was insignificant (p=0.9).