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### EPAC-lung

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# EPAC-lung: European pooled analysis of the prognostic value of circulating tumour cells in small cell lung cancer

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**Background:** Circulating tumour cell (CTC) number is an independent prognostic factor in patients with small cell lung cancer (SCLC) but there is no consensus on the CTC threshold for prognostic significance. We undertook a pooled analysis of individual patient data to clinically validate CTC enumeration and threshold for prognostication.

**Methods:** Four European cancer centres, experienced in CellSearch CTC enumeration for SCLC provided pseudo anonymised data for patients who had undergone pre-treatment CTC count. Data was collated, and Cox regression models, stratified by centre, explored the relationship between CTC count and survival. The added value of incorporating CTCs into clinico-pathological models was investigated using likelihood ratio tests.

**Results:** A total of 367 patient records were evaluated. A one-unit increase in log-transformed CTC counts corresponded to an estimated hazard ratio (HR) of 1.24 (95% CI: 1.19–1.29, P<0.0001) for progression free survival (PFS) and 1.23 (95% CI: 1.18–1.28, P<0.0001) for overall survival (OS). CTC count of  $\geq 15$  or  $\geq 50$  was significantly associated with an increased risk of progression (CTC  $\geq 15$ : HR 3.20, 95% CI: 2.50–4.09, P<0.001; CTC  $\geq 50$ : HR 2.56, 95% CI: 2.01–3.25, P<0.001) and an increased risk of death (CTC  $\geq 15$ :

HR 2.90, 95% CI: 2.28–3.70,  $P < 0.001$ ; CTC  $\geq 50$ : HR 2.47, 95% CI: 1.95–3.13,  $P < 0.001$ ). There was no significant inter-centre heterogeneity observed. Addition of CTC count to clinico-pathological models as a continuous log-transformed variable, offers further prognostic value (both likelihood ratio  $P < 0.001$  for OS and PFS).

**Conclusions:** Higher pre-treatment CTC counts are a negative independent prognostic factor in SCLC when considered as a continuous variable or dichotomised counts of  $\geq 15$  or  $\geq 50$ . Incorporating CTC counts, as a continuous variable, improves clinic-pathological prognostic models.

**Keywords:** Small cell lung cancer (SCLC); biomarker; meta-analysis; liquid biopsies; circulating tumour cells (CTCs); prognostic models

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## Introduction

Circulating tumour cells (CTCs) have been identified in a broad range of tumour types including lung cancer but are rarely seen in benign disease or healthy normal volunteers (HNV), thus making them an attractive biomarker (1). The current ‘gold standard’ method of CTC enumeration is the CellSearch<sup>®</sup> platform. CellSearch has been shown to be reliable and reproducible, with the FDA approving CellSearch CTC enumeration to inform on prognosis in metastatic breast, colorectal and prostate cancer (2-4). This efficient and semi-automated platform offers the opportunity for comparable large-scale studies with minimal inter user variation (5,6).

Small cell lung cancer (SCLC) characteristically presents with central rapidly growing tumours in which biopsies frequently harbour extensively necrotic tissue and scant tumour. Liquid biopsies offer an opportunity for systematic tumour interrogation, particularly important in this ‘recalcitrant’ cancer where emergence of chemo resistance is rapid, metastatic disease is early and prognosis is poor (7-9).

Despite its poor prognosis it is clinically evidence that SCLC patient outcomes are heterogeneous. A host of clinical and laboratory factors have been associated with poor outcomes in SCLC including performance status, age, sex, disease stage, LDH, albumin, creatinine, and sodium (10-17). Scoring systems that incorporate these details, such as the Manchester prognostic score, have been found to significantly associate with poorer survival (18). However, these have to some extent become obsolete as the guidelines for staging and care have updated, whilst efforts to upgrade prognostic scores often remain limited by the absence of

pre-treatment variables recorded in large cancer databases (19,20). Identification of novel independent prognostic biomarkers that characterise patient subgroups remain important for prognostication and for stratifying patients in clinical trials.

An abundance of CTCs can be detected in the blood of patients with SCLC compared to other tumour types. Between 70–95% of patients with SCLC have detectable CTCs (21-30). Some relatively small single centre studies have aimed at evaluating the effect of the presence of CTCs on survival with some degree of discordance of prognostic results (22-24,26,29,31,32). This may be due to selection bias in the small patient series or a consequence of the semi-automated method of CTC enumeration, where CellSearch captures and identifies potential CTC candidates but ultimately individual trained users make the final decision on what represents a CTC.

Previous studies have identified thresholds of  $\geq 2$  and  $\geq 50$  CTCs as significant for inferior survival in heterogeneous cohorts of extensive and limited stage patients (22,27,31). The Phase III CONVERT study, which investigated once daily *vs.* twice daily chemoradiation in limited stage SCLC, found a threshold of 15 CTCs to be most significant for survival (32). These studies demonstrate that thresholds will vary according to the series studies and further consensus on the threshold, derived from a range of studies, would be required for clinical implementation.

This European cancer centre collaboration was established with the purpose to pool independent datasets for analysis of clinical associations and prognostic value of CTCs counts in SCLC. The primary outcome was to evaluate the relationship between pre-treatment CTC

count and survival. Secondary analyses investigated inter site heterogeneity in CTC enumeration and the added value of incorporating CTCs into our clinic-pathological model. We present the following article in accordance with the REMARK reporting checklist (available at <http://dx.doi.org/10.21037/tlcr-20-1061>).

## Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of Gustave Roussy (Commission scientifique des Essais thérapeutiques) on July 20 2016. Centres were required to have local ethics committee approval for CTC enumeration and a recorded baseline CTC count prior to treatment for each individual and informed consent was taken from all individual participants.

### *Study design and population*

The study protocol was designed by the study management team and reviewed by all investigators. Invitations to participate in the study were sent to 4 European Cancer Centres; known to treat SCLC patients and with the capabilities to enumerate CTCs with the CellSearch platform between Jan 2003 and March 2017. Eligible patients had a confirmed diagnosis of SCLC with available prospective or retrospective progression free survival (PFS) and overall survival (OS) data. Centres were required to have local ethics committee approval for CTC enumeration and a recorded baseline CTC count prior to treatment for each individual. Cases were excluded if CTC counts influenced clinical decision making by resulting in a treatment switch, thus avoiding confounding bias in the survival analysis.

### *Procedures*

The Gustave Roussy cancer centre and the Cancer Research UK Manchester Institute (CRUK MI) partnered to establish the 'EPAC-lung' (European Pooled Analysis of CTCs in lung cancer) consortium. Other centres known to collect SCLC CTCs were then invited. Pseudo-anonymised patient data was collected, encrypted, and send to the central database by local investigators. The data included anonymised patient ID; centre ID; line of systemic treatment; baseline total CTC count

by CellSearch (per 7.5 mL); CellSearch date; date of tumour progression and/or death; gender; age; ECOG performance status; smoking status; stage of disease (extensive *vs.* limited); planned treatment; and location/number of metastatic sites. Screening of data was performed by the study management team and any queries returned to the relevant centre.

Collection of blood, immuno-magnetic selection and immuno-fluorescent staining of CTCs were performed using the CellSearch<sup>®</sup> system, as previously described (6,33). All studies did not use the automated image analysis software ACCEPT, an open-source programme to identify CTC (<https://github.com/LeonieZ/ACCEPT> and [www.cancer-id.eu](http://www.cancer-id.eu)).

Submitted data included CTCs counts previously published by participating centres (*Figure 1*) (24,26,31,32).

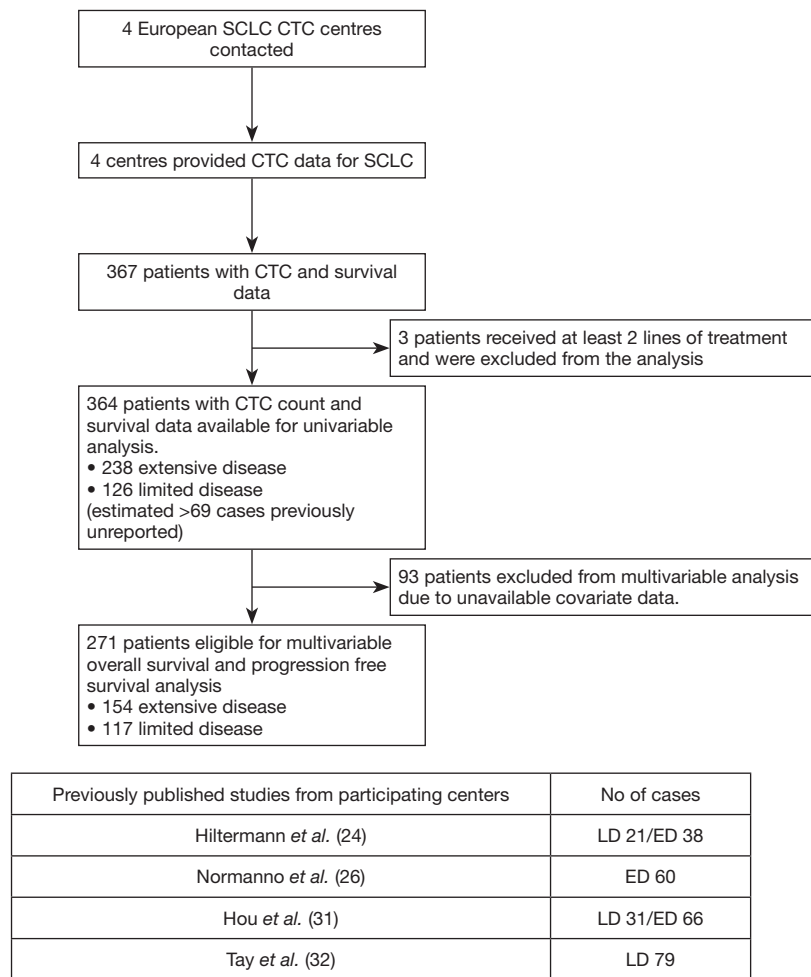
### *Statistical analysis*

Study design and results are in accordance with recommendations for tumour markers (REMARK) criteria (34). Overall survival (OS) was defined as the time from first CTC analysis until death from any cause. Patients still alive were censored at the date of last follow-up. Progression free survival (PFS) was defined as time from first CTC analysis until confirmed tumour progression (as per assessment by RECIST 1.1 criteria) or death, whichever came first. If no event occurred, the record was censored at the date of last follow up.

The primary objective was to evaluate the prognostic effect of the quantitative amount of baseline pre-treatment CTC count (per 7.5 mL) by the CellSearch method in SCLC on OS and PFS. Analysis of CTCs as a continuous variable precluded the need for ROC curve analysis, although additional cut-offs of 15 and 50 were taken from previous single centre studies (31,32) in an effort to facilitate a standardised future approach to CTC adoption.

Associations between CTCs and survival were investigated using the Cox proportional hazard model and stratified by cancer centre. In order to investigate the linear relationship between CTCs and hazard of progression in the Cox regression model, cubic splines were used; a log-transformation was used in order to satisfy the linearity hypothesis. In addition to assessing CTCs as a continuous variable, pre-defined CTC thresholds were also included. Heterogeneity between centres was measured using chi-squared tests in the Cox regression models.

We prespecified a clinicopathological model for



**Figure 1** CONSORT diagram and table of previously published data. LD, limited disease; ED, extensive disease.

the multivariable Cox regression which included age (continuous), gender (male/female), baseline treatment (platinum doublet *vs.* other), smoking status (never smoked or former *vs.* current smoker), number of metastases (up to 1 *vs.* >1), performance status (ECOG score <2 *vs.* ECOG score  $\geq$ 2) and sites of metastasis, then stratified by centre. Due to the low number of never smokers (3 patients) these were merged into the former smoker group for analysis. To assess the added value of CTCs to this clinico-pathological model in a multivariable Cox regression, we used likelihood ratios tests.

Associations between CTC counts and study population characteristics were analysed using Fishers exact test or Wilcoxon test. Kaplan Meier curves were used to estimate survival distributions. A two-sided significance level of 0.05 was considered significant.

## Results

Four European cancer centres participated in the study, submitting pre-treatment CTC counts and survival data for 364 patients, of which 238 (65%) had extensive stage disease. The median pre-treatment CTC count was 19 with a range 0–44,896 CTCs detected. Two or more CTCs were detected in 266 (73%) patients of which 191 (53%) had  $\geq$ 15 CTCs, and 139 (38%) had  $\geq$ 50 CTCs counts of  $\geq$ 15 and  $\geq$ 50 was numerically higher for increased age, poorer performance status, extensive stage disease and increased number of metastasis. *Table 1* displays the patient characteristics for the overall population and *Table 2* patient characteristics divided by CTC cut-offs.

A total of 271 patients had sufficient clinical information available to be included in the multivariable analysis (see flow chart in *Figure 1*). Clinical data from one centre had

**Table 1** Patient characteristics in the overall population (n=364)

Patients characteristics	% [N] or median (IQR)
Centre	
Groningen	18.13 [66]
Manchester	63.74 [232]
Naples	16.48 [60]
Paris	1.65 [6]
Gender	
Male	55.22 [201]
Female	44.78 [163]
Age at baseline (years)	65.9 (59.4 to 71)
Baseline treatment	
Platinum doublet	57.14 [208]
Platinum doublet ± thoracic radiotherapy ± PCI	27.47 [100]
Other (CAV, Topotecan, Vin, Gem, immunotherapy)	15.38 [56]
Line of therapy	
1st line	99.45 [362]
2nd/3rd line	0.05 [2]
Performance status (ECOG)	
0	22.97 [79]
1	47.09 [162]
2	22.09 [76]
3	7.27 [25]
4	0.58 [2]
Missing (N)	20
Smoking status	
Never	1.02 [3]
Former	42.66 [125]
Current	56.31 [165]
Missing (N)	71
Status	
Alive	9.07 [33]
Dead	90.93 [331]

**Table 1** (continued)

**Table 1** (continued)

Patients characteristics	% [N] or median (IQR)
Baseline number of metastases	
≤1	47.78 [172]
>1	52.22 [188]
Missing (N)	4
Baseline CTC count (continuous)	19.0 (1.0 to 228.8)
Baseline CTC count (cut-off =15)	
<15	47.53 [173]
≥15	52.47 [191]
Baseline CTC count (cut-off =50)	
<50	61.81 [225]
≥50	38.19 [139]

to be excluded in the multivariate analysis as the patients smoking status was not recorded which was found to be clinically significant in the clinico-prognostic model.

**Survival**

The median follow-up for the pooled population was 62.4 months (95% CI: 46.3–68.9). The median PFS was 6.24 months (95% CI: 5.72–6.97) and median OS 7.85 months (95% CI: 6.93–8.87) at which time 338 patients had progressed and 331 patients died respectively.

For PFS, there was no significant heterogeneity observed between cancer centres for the prognostic effect of log transformed CTC counts ( $X_3^2=3.12$ ,  $P=0.37$ ) or dichotomised CTC thresholds of  $\geq 15$  ( $X_3^2=3.22$ ,  $P=0.36$ ), or  $\geq 50$  ( $X_3^2=3.85$ ,  $P=0.28$ ) (Figure 2). In the primary analysis, a one-unit increase in log-transformed CTC counts corresponded to an estimated hazard ratio (HR) equal to 1.24 (95% CI: 1.19–1.29,  $P<2e-16$ ). Using the cutoffs of 15 and 50 CTCs, a pre-treatment CTC count of  $\geq 15$  or  $\geq 50$  was significantly associated with an increased risk of progression (CTC  $\geq 15$  HR 3.20, 95% CI: 2.50–4.09,  $P<0.001$ , CTC  $\geq 50$  HR 2.56, 95% CI: 2.01–3.25,  $P<0.001$ ) in univariable analysis (Figure 3A,B). The median PFS was 9.72 months (95% CI: 8.34–11.89) for  $<15$  CTCs vs. 4.67 months (95% CI: 4.14–5.45) for  $\geq 15$  CTCs and median

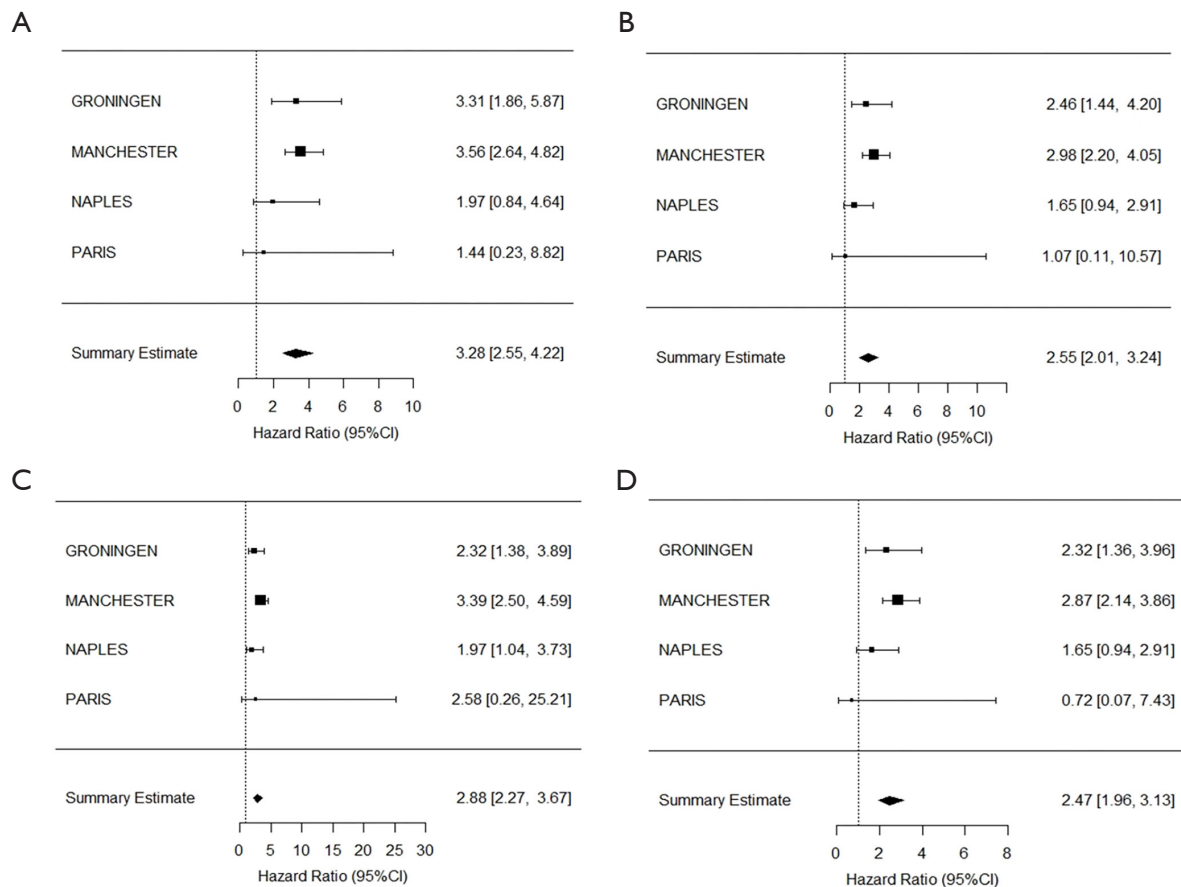
**Table 2** Baseline characteristics of patients with SCLC according to CTC count cut off in the overall population (n=364).

Characteristics	Cut-off =15		Cut-off =50	
	N (%) or median (IQR)		N (%) or median (IQR)	
	CTC <15	CTC ≥15	CTC <50	CTC ≥50
Age at baseline (years)	65 (57.7–71)	66 (60–71.5)	65 (58.6–71)	66 (60–72.0)
Gender				
Male	90 (24.7)	111 (30.5)	114 (31.3)	87 (23.9)
Female	83 (22.8)	80 (22.0)	111 (30.5)	52 (14.3)
Performance status (ECOG)				
≤2	162 (47.1)	155 (45.1)	208 (60.5)	109 (31.7)
>2	3 (0.9)	24 (7.0)	6 (1.7)	21 (6.1)
Missing (N)	8	12	11	9
Smoking status				
Never/former smoker	62 (21.2)	66 (22.5)	82 (28)	46 (15.7)
Current smoker	86 (29.4)	79 (27)	107 (36.5)	58 (19.8)
Missing (N)	25	46	36	35
Baseline treatment				
Platinum doublet	76 (21.1)	131 (36.4)	113 (31.4)	94 (26.1)
Other	94 (26.1)	59 (16.4)	109 (30.3)	44 (12.2)
Missing (N)	3	1	3	1
Treatment line				
1 <sup>st</sup> line extensive	73 (20.1)	166 (45.6)	111 (30.5)	128 (35.2)
Curative/limited	100 (27.5)	25 (6.9)	114 (31.3)	11 (3.0)
Baseline number of metastatic sites				
≤1	117 (32.5)	55 (15.3)	139 (38.6)	33 (9.2)
>1	53 (14.7)	135 (37.5)	83 (23.1)	105 (29.2)
Missing (N)	3	1	3	1
Stage				
Extensive	72 (19.8)	166 (45.6)	110 (30.2)	128 (35.2)
Limited	101 (27.7)	25 (6.9)	115 (31.6)	11 (3.02)

PFS for the higher CTC threshold <50 CTCs 7.75 months (95% CI: 7.03–9.46) vs. 4.57 months (95% CI: 3.75; 5.45) for ≥50 CTCs.

Regarding OS, no significant heterogeneity was observed between centres regarding the prognostic effect of CTCs for log-transformed CTCs ( $X_3^2=2.60$ ,  $P=0.457$ ), nor  $CTC \geq 15$  ( $X_3^2=3.08$ ,  $P=0.380$ ), nor  $CTC \geq 50$  ( $X_3^2=4.18$ ,  $P=0.243$ ) (Figure 2C,D). In the primary analysis, a one-unit

increase in log-transformed CTC counts corresponded to an estimated hazard ratio (HR) equal to 1.23 (95% CI: 1.18–1.28,  $P < 2e-16$ ). Also, pre-treatment CTC counts of  $\geq 15$  was associated with an increased risk of death (OS HR 2.90, 95% CI: 2.28–3.70,  $P < 0.001$ ), as was pre-treatment CTC count  $\geq 50$  (OS HR 2.47, 95% CI: 1.95–3.13,  $P < 0.001$ ) (Figure 3C,D). The median OS for <15 CTCs was 12.30 months (95% CI: 10.50–16.00) vs. 5.65 months (95% CI:



**Figure 2** Forest plots of progression free survival (A,B) and overall survival (C,D) according to dichotomised CTCs counts at 15 (A,C) and 50 CTCs (B,D) per 7.5 mL of blood. The HR and 95% CI are represented by a square box and horizontal line. Box sizes are proportional to the number of events in each centre.

4.76–6.44) for  $\geq 15$  and for  $< 50$  CTCs the median OS was 10.84 months (95% CI: 8.97–12.45) *vs.* 5.29 months (95% CI: 4.40–6.31) for  $\geq 50$  CTCs.

**CTCs as an independent prognostic indicator**

Prespecified clinico-pathological prognostic models were built incorporating identified prognostic factors, including age at baseline, gender, baseline treatment, performance status, smoking status, site of metastasis and number of metastasis.

The addition of log transformed CTC counts to clinico-pathological models resulted in a significant improvement in estimation of PFS (LR of 17.99,  $P=2.23e-05$ ) and OS (LR 20.14,  $P=7.19e-06$ ), confirming that CTC counts are an independent prognostic factor beyond established factors.

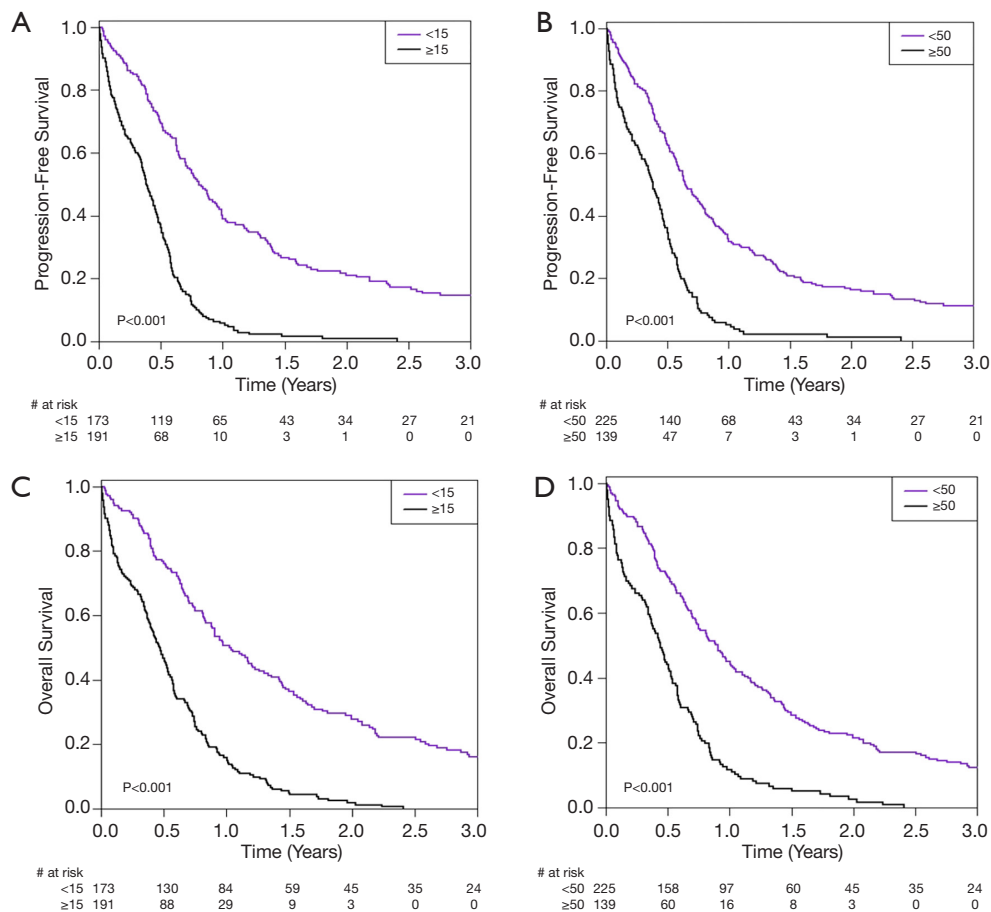
Incorporating dichotomised CTC counts of  $\geq 15$  also yielded a significant LR for PFS (LR 15.36,  $P=8.89e-05$ ) and OS (LR 13.35,  $P<0.001$ ), while the higher threshold of  $> 50$  CTCs improved estimation of OS (LR 4.51,  $P=0.03$ ) but not PFS (LR 2.65,  $P=0.103$ ).

**Discussion**

In this European multicentre collaboration, we have confirmed that pre-treatment CTC count, enumerated by CellSearch, is an independent prognostic factor in SCLC. We observed minimal between-centre variability utilising this semi-automated enumeration platform.

Incorporation of CTC count, especially as a continuous variable, added value to our prespecified prognostic clinical-pathological model.





**Figure 3** Kaplan-Meier curves of progression-free survival for baselines CTC  $\geq 15$  (A) and  $\geq 50$  (B). Kaplan-Meier curves for overall survival stratified by baselines CTC count  $\geq 15$  (C) and  $\geq 50$  (D) CTCs per 7.5 mL.

To our knowledge, this is the largest study to date evaluating the prognostic value of CellSearch CTC count in SCLC, and the only study that has analysed previous published and unpublished results from a number of European centres, thus addressing concerns regarding single centre heterogeneity. These findings support previous single centre reports (<100 patients), which have concluded that the presence of CTCs is associated with poor survival (23–26,29,31,32,35). Previous attempts at meta-analyses of the prognostic implications of CTCs in SCLC have yielded conflicting results, hampered by (I) selection bias through restriction to patients that have already been reported in published literature, (II) variability of CTC isolation platform employed for enumeration, and (III) univariable survival estimates only (30,36).

In this study, we observed only minimal heterogeneity in the association between CTC value and prognosis,

supporting CellSearch as a standardised comparable platform for future studies. This result helps facilitate multi-site collaborations, dispelling any hypothetical concerns regarding the potential for inter-user inconsistency that may derive from image interpretation or lack of automated reporting software (37). This is particularly important as efforts are made to develop standards for CTC reporting across Europe through the CANCER-ID consortium ([www.CANCER-ID.eu](http://www.CANCER-ID.eu)).

The limitations of our study include a residual potential for selection bias, incomplete data collection and the absence of a centralised pathological review. However, attempts to reduce bias have been made by large patient numbers and application of an established protocol in the limited number of centres performing CellSearch CTC quantification. A significant number of patient records were excluded from the study population due to

incomplete data submission, including all data from one centre where smoking status could not be provided. The resulting study population incorporated published and unpublished data, supplemented by stratification according to cancer centre.

Our findings offer a definitive view of CTC prognostication in a cohort of limited and extensive stage SCLC. A previous large multicentre clinical trial, CONVERT, investigated the significance of baseline CTCs in a subset of 79 patients with limited SCLC, identifying a threshold of  $\geq 15$  CTCs as most strongly associated with poor survival (32). Other studies with a mix of limited and/or extensive stage SCLC patients have proposed numerous significant thresholds for prognosis (23,24,29,31). Our study has indicated that when using an appropriate log-transformation the effect of CTCs is pretty linear in a Cox regression model and that it is not a specific cut-off that drives prognosis.

Technology that isolates and/or enriches CTCs has evolved rapidly. Epitope dependent technologies such as CellSearch enrich for EpCAM expressing CTCs (33,38) whereas epitope independent systems e.g., Parsortix (39,40) and RosetteSep (41,42) exploit physical characteristics of CTCs to harvest cells independently of surface markers. RareCyte (43) and HD-SCA (44) can interrogate huge number of individual cells with the potential to identify rare CTC subpopulations. Discrete prognostic threshold for CTC enumeration will vary dependent upon CTC enrichment methodology and case series, favouring analysis of CTCs as a continuous variable.

Future work assessing longitudinal changes in CTC counts, in well powered studies, may also confirm CTCs as a surrogate for response and predictive for outcome, impacting clinical decision making. This study has confirmed the prognostic significance of baseline CTCs and would advocate incorporation of CTC counts into prognostic models and clinical trials, improving stratification of patients and trial design.

CTCs are already proving a hugely valuable resource in translation medicine. With established SCLC CTC derived xenografts (CDXs) (45) and the potential for SCLC CTC culture. Molecular characterisation of CTCs, employing a CNV classifier, has already proven to predict sensitivity to chemotherapy in extensive stage patients (46). As research into these clinically informative biomarkers increases, we have demonstrated the benefits of increased power and reduced bias from a collaborative approach of pooling multi-centre data.

## Conclusions

In summary, this European collaboration has demonstrated that CTCs are an independent prognostic factor in SCLC. There was minimal inter site variability between European centres when utilising standardised CTC enumeration platforms, permitting pooled analysis of previously published and unpublished data. The continued pursuit of circulating biomarker research may soon yield more clinically applicable results which will establish their routine baseline and longitudinal use at critical junctures in patient care.

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