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Preoperative Neutrophil:Lymphocyte and Platelet:Lymphocyte Ratios Predict Endometrial Cancer Survival

Running Title: NLR and PLR predict endometrial cancer survival

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ABSTRACT

Background

Variations in systemic inflammatory response biomarker levels have been associated with adverse clinical outcome in various malignancies. This study determined the prognostic significance of preoperative neutrophil:lymphocyte (NLR), platelet:lymphocyte (PLR) and monocyte:lymphocyte (MLR) ratios in endometrial cancer.

Methods

Clinicopathological and five-year follow-up data were obtained for a retrospective series of surgically-treated endometrial cancer patients (n=605). Prognostic significance was determined for overall (OS) and cancer-specific survival (CSS) using Cox proportional hazards models and Kaplan-Meier analysis. Receiver-operator characteristic and log-rank functions were used to optimise cut-offs. NLR, PLR and MLR associations with clinicopathological variables were determined using non-parametric tests.

Results

Applying cut-offs of ≥2.4 (NLR), ≥240 (PLR) and ≥0.19 (MLR), NLR and PLR (but not MLR) had independent prognostic significance. Combining NLR and PLR scores stratified patients into low (NLR-low and PLR-low), intermediate (NLR-high or PLR-high) and high risk (NLR-high and PLR-high) groups: multivariable hazard ratio (HR) 2.51; P<0.001 (OS); HR 2.26; P<0.01 (CSS) for high *vs*. low risk patients. Increased NLR and PLR were most strongly associated with advanced stage (P<0.001), whereas increased MLR was strongly associated with older age (P<0.001).

Conclusion

NLR and PLR are independent prognostic indicators for endometrial cancer, which can be combined to provide additional patient stratification.

KEYWORDS:

Endometrial cancer, neutrophil:lymphocyte ratio, platelet:lymphocyte ratio, monocyte:lymphocyte ratio, lymphocyte:monocyte ratio, survival.

INTRODUCTION

Endometrial cancer is the commonest gynaecological malignancy in the Western world, accounting for 6.1% of all cancers in European women (Ferlay *et al*, 2013). Almost 8,500 cases are diagnosed annually in the UK, leading to over 2,000 deaths (Cancer Research UK Statistics, 2014). Moreover, the incidence of endometrial cancer in the UK has increased by 43% in 15 years since 1993-5, attributable in part to factors resulting in unopposed oestrogen exposure such as obesity, certain hormone replacement therapies and the use of tamoxifen to treat breast cancer. This rise has also been accompanied by a 14% increase in the number of deaths due to endometrial cancer over the same time period (National Cancer Intelligence Network, 2013).

Endometrial cancers are primarily adenocarcinomas and have been traditionally categorised into types I and II on the basis of aetiology, molecular characteristics and clinical behaviour (Bohkman, 1983). Type I endometrial cancer (endometrioid endometrial cancer, EEC) is the commonest, accounting for approximately 80% of sporadic cases. It typically develops in peri-menopausal women in a background of premalignant hyperplasia and is usually oestrogen/progesterone receptor positive. By contrast, type II endometrial cancers (serous and clear cell carcinomas) usually arise in a background of atrophic (postmenopausal) endometrium, are high-grade, hormone receptor negative and typically follow an aggressive clinical course (Dedes *et al*, 2011). While early stage, low grade EEC is classically managed by curative hysterectomy, late stage EEC/type II endometrial cancers are associated with significant mortality due to their metastatic spread outwith the uterine corpus (Dedes *et al*, 2011; DeLeon *et al*, 2014). Difficulties still exist with the type I/II classification system in terms of prognosis: there is uncertainty as to whether grade 3 EECs should be classified as

type I or type II carcinomas (Boruta *et al*, 2004; Hamilton *et al*, 2006; Soslow *et al*, 2007; Voss *et al*, 2012) while the prognostic significance of tumours with mixed type I/II histology remains the subject of debate (Patsavas *et al*, 2011; Roelofson *et al*, 2012). The existence of grade 1 EECs arising in a background of atrophic endometrium also presents difficulties for this dualistic model (Geels *et al*, 2012). Finally, endometrial carcinosarcomas (formerly named malignant mixed Müllerian tumours) are now considered to be metaplastic carcinomas (McCluggage, 2002) which carry an exceptionally poor prognosis (Amant *et al*, 2005), although their clinical behaviour is to some extent dictated by the histology of their epithelial component (de Jong *et al*, 2011). Thus, there is an ongoing need to identify objective biomarkers, both to improve risk stratification and to guide therapeutic management.

The host response to malignant tumours is characterised by systemic inflammation, resulting in a relative thrombocytosis, neutrophilia and lymphocytopenia. Biomarkers of systemic inflammation such as elevated neutrophil:lymphocyte ratio (NLR), platelet:lymphocyte ratio (PLR) and absolute monocyte counts have shown potential for guiding the clinical management of cancer patients across a range of malignancies (Clarke *et al*, 2011). High preoperative NLR and PLR and, more recently, monocyte:lymphocyte ratio (MLR) have been shown to associate with adverse outcomes in a range of solid tumours (Templeton *et al* 2014a; Templeton *et al*, 2014b; Li *et al*, 2013), although a paucity of data exist on their prognostic significance in the context of endometrial cancer. The aim of this study was therefore to investigate the prognostic significance of preoperative NLR, PLR and MLR in a large retrospective series of surgically treated endometrial cancer patients with five-year follow-up data.

MATERIALS AND METHODS

Patients and data collection

This retrospective study examined the records of a sequential series of 733 patients with a new diagnosis of primary endometrial cancer between January 2005 and December 2007 within the North and West Yorkshire Deanery, UK. Ethical approval was obtained from Leeds East Research Ethics Committee (ref: 05/Q1107/41). Data were obtained from Patient Pathway Manager (Newsham *et al*, 2011) and case notes from Yorkshire Cancer Network units. Follow-up was 3 monthly for first 18 months, 6 monthly for next 18 months, then annually until 5 years when patients were offered to be discharged back to their GP, with the option of continuing annual follow-up visits thereafter. Mobility within the region was limited as judged by the numbers seen in follow-up clinics. Patients who moved from the centre during the follow-up period were reviewed in their local regional hospital by accessing their local electronic records by one of the investigators (MG). All deaths were cross-checked against death certificates and patients were censored at end of follow-up, if death had not occurred. Patients were managed according to regional guidelines, taking into account patient performance status: The extent of surgical staging was based on preoperative histological findings and imaging, where lymphadenectomy (+/- omental sampling and peritoneal washings) was only performed on early stage patients with Type II histology. Following post-operative staging, adjuvant combination chemotherapy (Paclitaxel + Carboplatin) was administered to patients with stage III/IV disease, followed by consolidation external beam radiotherapy (EBRT). Brachytherapy was reserved for those patients with tumour involving the cervical epithelium (if Type II) or stroma (if Type I). Type II stage I cancers were sometimes given EBRT at the discretion of the physician. A total of 128 patients were excluded; these comprised patients who were lost to follow-up (n=7), those with no preoperative blood parameter data available (n=54), patients that did not have a hysterectomy

(*n*=54) and patients with a diagnosis of uterine sarcoma/unknown uterine tumour (*n*=13). Individual Charlson scores were calculated from recorded co-morbidities (Charlson *et al*, 1987). Staging data were converted from the International Federation of Gynaecology and Obstetrics (FIGO) 1988 to the FIGO 2009 staging system (Creasman, 2009) according to pathology reports. Patients' full blood count data (including absolute leukocyte, neutrophil, eosinophil, basophil, monocyte, lymphocyte and platelet counts) were collected from a time frame of less than two weeks prior to hysterectomy and used to calculate NLR, PLR and MLR. We chose to calculate MLR, the reciprocal of the more frequently used lymphocyte:monocyte ratio (LMR), in order to standardise by dividing myeloid lineage counts by lymphoid lineage cell counts for all relevant variables.

Statistical analysis

Data normality was assessed using Kolmogorov-Smirnov tests and associations of NLR, MLR and PLR with other categorised clinicopathological prognostic variables were determined using either Mann-Whitney-*U* tests or Kruskal-Wallis tests followed by *post hoc* pairwise Mann-Whitney-*U* tests. Bonferroni's correction was applied for multiple comparisons, as appropriate. NLR, MLR and PLR correlations were performed using Spearman's rho test.

Overall survival (OS) and cancer specific survival (CSS) were defined as time from diagnosis to death (all causes) and death due to endometrial cancer (where endometrial cancer was listed as a cause of death in the death certificate), respectively. In cases where endometrial cancer was not listed as a cause, deaths were censored in CSS analysis. Survival analyses on

categorical variables were performed using the Kaplan–Meier method and significant differences between groups were identified using the log-rank test. Univariable and multivariable survival analyses were performed using Cox proportional hazards models. NLR, PLR and MLR cut-off optimisation was performed using the software package Cutoff Finder (Budczies *et al*, 2012). Two approaches were used for cut-off determination: a) standard ROC curve analysis based on binary outcome, using Manhattan distance to calculate optimal cut-offs, and b) fitting the Cox proportional hazards models to dichotomised NLR, PLR and MLR variables and the time-dependent survival variable, whereby the optimal cut-off point gave the lowest log-rank *P* value.

Statistical analysis was performed using *R* (R Core Team, 2014) and IBM SPSS (Statistical Package for the Social Sciences; Version 21). Missing data were handled by pairwise exclusion. All statistical tests used in this study were two-sided and *P* values of <0.05 were considered significant.

RESULTS

Patient characteristics

Patient demographics have been summarised in Supplementary Table 1. Median age at diagnosis was 65 years (range 28-95) and all selected patients underwent a total hysterectomy with bilateral salpingo-oophorectomy. Lymphadenectomy (pelvic/para-aortal) was performed in 71% of patients, 33% of patients received adjuvant radiotherapy and 13% of patients received adjuvant chemotherapy. The majority of patients (78%) were diagnosed at early stage (I/II) and EEC was the predominant (77%) histopathological subtype. Median follow-up time (reverse Kaplan-Meier method) was 81.5 months (range 58-103). Throughout the follow-up period there were 166 deaths, 96 of which were attributable to endometrial cancer (see Materials and Methods). The estimated cumulative five-year survival for this patient population was 76±1.7% for overall survival (OS) and 84±1.5% for endometrial cancer-specific survival (CSS).

Prognostic significance of preoperative blood parameters and cut-off determination

To investigate the potential prognostic significance of preoperative blood parameters, univariable Cox proportional hazards analyses were performed on continuous data, whereby each parameter was scaled to its own median value to enable cross-comparison of hazard ratios (Supplementary Table 2). These exploratory analyses revealed leukocyte, neutrophil, lymphocyte and platelet counts to be significantly associated with survival, where increased leukocytes and neutrophils associated with worse OS and CSS, increased lymphocytes associated with better OS and CSS and increased platelets associated with worse CSS (but not OS). No significant relationships between monocyte, eosinophil or basophil counts and

181 survival were identified. These analyses also revealed neutrophil:lymphocyte (NLR),
182 platelet:lymphocyte (PLR) and monocyte:lymphocyte (MLR) ratios to be highly significantly
183 associated with adverse outcome for both OS and CSS (all $P<0.001$), and to have a superior
184 prognostic significance compared to any blood parameters not expressed as a ratio. On this
185 basis, all three ratios were selected for further analysis.

186

187 Although an NLR cut-off of ≥5 is commonly applied in the prognostic setting (particularly in
188 colorectal carcinoma), its use is not universal (Templeton et al, 2014a). Cut-off PLR values
189 used for prognostication in cancers range from 160-300 (Templeton et al, 2014b), and
190 similarly, cut-off LMR values applied to non-haematological malignancies range from 2.9
191 (Stotz *et al*, 2014a) to 5.3 (Li *et al*, 2012). We therefore chose to perform cut-off optimisation
192 for NLR, PLR and MLR on our study cohort. Optimised cut-offs were determined for each
193 parameter using standard ROC curve analysis and time-dependent survival (see Materials and
194 Methods). In ROC curve analyses, the areas under the curve (AUC) for OS were 0.616
195 ($P<0.001$), 0.583 ($P=0.002$) and 0.592 ($P=0.001$) for NLR, PLR and MLR, respectively. For
196 CSS, respective AUC values for NLR, PLR and MLR were 0.620 ($P<0.001$), 0.611
197 ($P=0.001$) and 0.589 ($P=0.006$). For NLR, a cut-off of 2.4 was found to be optimal for OS
198 and CSS using ROC curve determination and this value also gave the lowest log-rank *P* value
199 ($P<0.0001$) for time-dependent survival analysis. For PLR, cut-offs were similar using both
200 approaches (240 and 250 for ROC and time-dependent survival, respectively; both
201 $P<0.0001$), and a cut-off of 240 was selected to maximise the number of patients in the PLR-
202 high group (14%). For MLR, there was a large discrepancy between cut-offs determined by
203 ROC curve (0.19; $P<0.001$) and time-dependent survival analysis (0.66; $P<0.0001$). Since the
204 latter approach defined only 2% of the patient cohort as MLR-high, patients were

dichotomised according to the ROC curve cut-off. This value (0.19) corresponds to an LMR cut-off of 5.3.

Univariable survival analysis of patients stratified according to NLR, PLR and MLR cut-offs and other prognostic parameters

Prognostic parameters for univariable analysis included age, Charlson co-morbidity index, FIGO 2009 stage, grade, histopathological subtype and the presence of lymphovascular space invasion, a known independent prognostic indicator for endometrial cancer (Briët *et al*, 2005). Depth of myometrial invasion, cervical involvement and lymph node status form part of the FIGO staging system and, as such, were not included as independent variables in the analysis. Patients were stratified into four age groups including two 10-year groups around the median age (65 years). All prognostic parameters except the Charlson co-morbidity index were significantly associated with OS and CSS in univariable analysis (Tables 1 and 2, respectively) and were therefore included in subsequent multivariable models.

Kaplan-Meier analysis for OS (Figure 1 A-C) and CSS (Figure 2 A-C) revealed that patients with high preoperative NLR, PLR or MLR (corresponding to a low LMR) had significantly worse OS and CSS. PLR dichotomisation showed the greatest survival difference with a cumulative five-year OS survival rate of 54% (PLR-high) *vs*. 80% (PLR-low), followed by NLR (68% high *vs.* 86% low) and then MLR (72% high *vs.* 83% low). These results were echoed by CSS with estimated cumulative five-year survival rates of 67% (PLR-high) *vs.* 87% (PLR-low), NLR (78% high *vs.* 91% low), and MLR (81% high *vs.* 90% low). However, the enhanced survival difference identified by PLR dichotomisation was offset by the fact

that it only defined a relatively small subset of patients (14%) as high risk (Figures 1C and 2C).

NLR and PLR have independent prognostic significance

Since NLR, PLR and MLR were strongly correlated with each other (Spearman's rho coefficients of 0.728 (NLR *vs.* PLR), 0.682 (NLR *vs.* MLR) and 0.583 (PLR *vs*. MLR; all $P<0.001$), all three factors were adjusted separately in multivariable Cox proportional hazards models which included age, stage, grade, histopathological subtype and lymphovascular space invasion (Tables 1 and 2 for OS and CSS, respectively). Both NLR and PLR were independent prognostic factors for OS and CSS, albeit more highly significant for OS. By contrast, MLR had no independent prognostic value for either OS or CSS.

Combining NLR and PLR provides additional patient stratification

Methods to combine NLR and PLR scores to improve patient stratification in relation to clinical outcome were then investigated. Approaches such as combining the NLR and PLR values as geometric means did not identify cut-offs that performed well in multivariable analysis (data not shown). Indeed, the simplest and most effective approach was to stratify patients into three groups: a) PLR-low and NLR-low, b) PLR-high or NLR-high, and c) PLR-high and NLR-high (Figures 1D and 2D). These corresponded to low, intermediate and high risk groups, with estimated cumulative five-year OS rates of 85%, 76% and 54%, respectively, and estimated cumulative five-year CSS rates of 91%, 83% and 67%. When adjusted for other prognostic parameters (Table 1), both the high and intermediate risk group had significantly worse OS than the low risk group. For CSS (Table 2), the difference

between high and low risk groups was accentuated (compared to when simple NLR and PLR cut-offs were used to dichotomise patients), although the low and intermediate risk groups did not differ significantly from each other in the multivariable model. It is worth noting that only one patient was PLR-high and NLR-low, which might be expected given the strong positive correlation of PLR with NLR. One might, therefore, hypothesise that simply raising the NLR cut-off threshold would also identify this high risk group. We did indeed apply a cut-off of 5.0 - which is the most widely used value in the literature, particularly in colorectal cancer (Guthrie *et al*, 2013a) - to our patient population but this did not perform as well as when applying our optimised cut-offs for either OS (multivariable HR for NLR ≥5: 1.81; 95%CI: 1.17-2.79; *P*=0.008) or CSS (multivariable HR: 1.61; 95%CI: 0.89–2.88; *P*=0.111).

Other prognostic factors in the multivariable model

The multivariable model confirmed the independent prognostic significance of lymphovascular space invasion for both OS (Table 1) and CSS (Table 2). Age, stage and grade were also significant independent prognostic indicators for both OS and CSS but, as expected, the effect of age was magnified for OS compared to CSS, and *vice versa* for grade and stage. When different endometrial cancer histopathological subtypes were compared to EEC as the reference group, only a diagnosis of carcinosarcoma associated with worse OS and CSS, confirming the particularly poor outlook associated with this subtype (Amant *et al*, 2005). A diagnosis of serous or clear cell carcinoma (both *de facto* grade 3 carcinomas) was not independently predictive, perhaps partly due to the relatively small population sizes of these subgroups and the fact that grade was also included in the model. Similarly, a diagnosis of mixed carcinoma (serous or clear cell combined with endometrioid histology) was not independently predictive, although univariable analysis suggested that the risk of endometrial

cancer-related death was lower in this population compared with that of patients diagnosed with pure serous or clear cell tumours (Table 2).

Subgroup analysis of combined NLR and PLR in early and late stage patients

The prognostic value of the combined NLR- and PLR-based stratification system was next investigated in early (I/II) and late stage (III/IV) endometrial cancer subgroups (Figure 3). In multivariable analysis, when adjusting for age, grade and lymphovascular space invasion, combined high NLR and high PLR was significantly associated with worse OS and CSS in both early and late stage subgroups. In the early stage subgroup, the intermediate risk group (with NLR-high or PLR-high status) was significantly associated with worse OS but not CSS (Figure 3A and 3B). By contrast, there was a trend for intermediate risk group to associate with both worse OS and CSS in late stage patients (Figure 3C and 3D), although the numbers in the late stage subgroup were relatively small.

Association of NLR, PLR and MLR with other clinicopathological variables

Potential relationships between NLR, PLR, MLR and other clinicopathological factors were then explored (Table 3). Both NLR and PLR were associated with features of high tumour burden/metastatic potential, including stage (where the association was highly significant; $P<0.001$), lymphovascular space invasion and lymph node positivity. NLR was significantly higher in patients diagnosed with stage IV cancers and PLR was significantly higher in patients with IV cancer compared to those with stages I and II. NLR and PLR were both significantly higher in patients diagnosed with lymphovascular space invasion and with lymph node positivity, although the strength of these associations was greater for

lymphovascular space invasion. Interestingly, NLR was significantly higher in patients with a diagnosis of carcinosarcoma compared to the EEC group. Both NLR and PLR were significantly associated with age (P=0.013 and 0.035, respectively). However, neither NLR nor PLR correlated with age as a continuous variable. By contrast, MLR was highly significantly associated with age (P<0.001) and significantly higher in patients aged ≥75 years compared to the groups aged 55-64 and 65-74 years. MLR also correlated weakly, but significantly, with age as a continuous variable (Spearman's rho coefficient 0.129; P=0.002). MLR did not associate with any other clinicopathological factor except stage (P=0.001), although this association was less significant than that of NLR or PLR. We also investigated potential associations of NLR, PLR and MLR with Charlson co-morbidity index. However, no significant association was found using either Spearman's rho test on ordinal Charlson index data or by Mann-Whitney-U tests on patient populations dichotomised according to Charlson scores at any cut-off point (data not shown).

DISCUSSION

This is the largest study to investigate the prognostic role of preoperative NLR and PLR in endometrial cancer, and the only such study to investigate the prognostic potential of MLR in this disease. Both NLR and PLR were identified as having independent prognostic value when adjusted for age, stage, grade, lymphovascular space invasion and histopathological subtype. In this regard, previous studies on these systemic inflammatory markers in endometrial carcinoma have explored the potential of NLR and PLR in the diagnostic setting (Mete Ural *et al,* 2014; Acmaz *et al*, 2014), or as predictive markers of nodal metastasis (Suh *et al*, 2012) and cervical stromal invasion in EEC (Wang *et al*, 2013). A recent study (Haruma *et al*, 2015) on a cohort of 320 endometrial cancer patients identified high NLR as having independent adverse prognostic significance for OS. However, no independent prognostic significance was identified for PLR, which was only borderline significant for OS even in univariable analysis. The reasons for the discrepant findings for PLR may be due to the fact that although there was close agreement in optimised NLR cut-offs (2.7 compared to 2.4 defined herein), optimised PLR cut-offs were quite dissimilar (174 as opposed to 240 applied herein). This in turn may reflect the smaller cohort size used by Haruma and colleagues (320 compared to 605 in the present study) leading to sub-optimal PLR cut-off determination. It is worth noting that the PLR cut-off determined in the present study defines a relatively small subset of patients (14%) as high risk, although this subset was associated with particularly poor outcome.

A wealth of research supports the prognostic value of NLR in solid tumours, as illustrated by a recently published meta-analysis of 100 studies (Templeton *et al*, 2014a), where the analysis of pooled data showed that high NLR associated with adverse OS, CSS progression-

free and disease-free survival, although only 10% of the studies specifically addressed CSS. Evidence is also mounting for the value of PLR in predicting OS for solid tumours (Templeton *et al*, 2014b). In this regard, preoperative PLR has been demonstrated to be an independent risk factor for worse OS in pancreatic (Smith *et al*, 2014), colorectal (Kwon *et al*, 2012) and ovarian cancers (Asher *et al*, 2011), and to predict independently both worse OS and CSS in breast cancer (Krenn-Pilko *et al*, 2014). By contrast, few studies conducted to date have investigated the prognostic potential of MLR in non-haematological malignancies. Nonetheless, a number of recent publications across a range of carcinomas have indicated that low preoperative LMR is independently predictive of poor OS in nasopharyngeal, lung and colon cancers (Li *et al*, 2013; Hu *et al*, 2014; Stotz *et al*, 2014a), CSS in renal and pancreatic cancers (Hutterer *et al*, 2014; Stotz *et al*, 2014b) and DFS in breast cancer (Ni *et al*, 2014).

Although ROC-based cut-off optimisation for MLR enabled the stratification of endometrial cancer patients into high (MLR-high) and low (MLR-low) risk groups in univariable analysis in the present study, both NLR- and PLR-based stratification performed better in this regard. Moreover, MLR was not an independent prognostic factor for either OS or CSS. Koh and co-workers (2014) demonstrated a significant negative association between LMR and older age, which mirrors the highly significant positive association of MLR with patients aged ≥75 years identified herein. Indeed, the authors suggested that separately defining LMR thresholds for elderly patients may improve the prognostic accuracy of this marker, albeit at the cost of complicating analyses.

The role of inflammation in carcinogenesis and tumour progression is well established. Existing models purport that the inflammatory tumour microenvironment facilitates the subversion of the host immune response by cancer cells, thereby enabling their escape from immunosurveillance, inhibiting apoptosis, promoting genome instability, angiogenesis, invasion and metastatic spread (Coussens and Werb, 2002). However, the biology underlying the relationships between NLR, PLR and MLR, systemic inflammation and the inflammatory tumour microenvironment remain comparatively poorly understood. Both high PLR and NLR have been found to be associated with advanced stage and aggressive disease (Raungkaewmanee *et al*, 2012; Guthrie *et al*, 2013a, 2013b; Feng *et al*, 2014), in line with their highly significant association with advanced stage and the presence of lymphovascular invasion noted in the present study. An emerging link between circulatory cytokines and increased NLR in cancer patients may reflect increased tumour burden/aggressiveness and consequent systemic pro-inflammatory effects, although it is not possible to establish clear causal relationships in these observational studies. Elevated circulatory concentrations of interleukin (IL)-1 receptor antagonist, IL-6, IL-7, IL-8, IL-12, monocyte chemoattractant protein (MCP)-1 and platelet-derived growth factor (PDGF)-BB were found to be associated with high NLR, while a highly significant association was also found between serum IL-8 and TNM stage in colorectal cancer (Kantola *et al*, 2012). Moreover, Motomura and colleagues (2013) demonstrated an association between elevated serum and peritumoural IL-17, high NLR and increased peritumoural macrophage infiltration in hepatocellular carcinoma patients. In line with this, Guthrie *et al* (2013b) showed elevated serum IL-6 in colorectal cancer patients to be associated with high NLR and the presence of tumour necrosis, which is both a feature of aggressive disease and an inflammatory trigger. Analogously, Stone and colleagues (2012) provided experimental evidence for the role of IL-6 release by ovarian cancer cells in stimulating hepatic thrombopoeitin production and

paraneoplastic thrombocytosis, which itself stimulates tumour growth and angiogenesis and is a feature of advanced disease and poor outlook in ovarian cancer patients. In this regard, however, PLR proved to be a more sensitive prognostic indicator than absolute platelet count in our cohort of endometrial cancer patients. Thus far, no studies have investigated links between circulatory cytokines and PLR or MLR/LMR in cancer patients. As we observed strong correlations between NLR, PLR and MLR, it is likely that these are related phenomena that reflect the complex interactions between the host immune system and the inflammatory tumour microenvironment, together with other patient-specific factors such as age, nutritional status and underlying inflammatory conditions (McMillan, 2009; Bhat *et al*, 2013; Gunay *et al*, 2014), all of which likely combine to influence patient survival.

Despite their inter-relationships, both NLR and PLR proved to be better prognostic indicators than MLR in endometrial cancer. By combining NLR and PLR scores using the cut-offs defined for the present study's cohort, it was possible to stratify patients into low (NLR-low, PLR-low), intermediate (NLR-high or PLR-high) and high risk (NLR-high, PLR-high) groups. This approach was particularly successful for predicting OS in multivariable models and accentuated the survival difference between the low and high risk groups in CSS. Moreover, subgroup analysis revealed the findings from this stratification method (high *vs*. low risk) to hold true for both early and late stage subgroups, although differences in the prognostic significance of the intermediate risk group in early and late stage subgroups were noted. Thus, NLR and PLR are biomarkers of systemic inflammation that only partially overlap in terms of prognostic information, such that they can be combined to provide additional risk stratification for endometrial cancer patients.

Standard therapy for endometrial cancer includes total hysterectomy and bilateral salpingo-oophorectomy. The extent of associated lymph node dissection and adjuvant chemo- or radiotherapy is dependent upon tumour type, stage and grade at diagnosis, along with individual patient factors such as age, functional status and the presence of co-morbidities (Dinkelspeil *et al*, 2013; DeLeon *et al*, 2014). Although extensive lymph node dissection has been shown to improve prognostication, it is also associated with marked morbidity, while a survival benefit for low risk early stage endometrial cancer patients has not been demonstrated. However, it potentially alters or eliminates the need for adjuvant therapy in high/intermediate risk patients (Burke *et al*, 2014). Furthermore, endometrial cancer is a heterogeneous disease presenting diagnostic and prognostic difficulties (Gilks *et al*, 2011; Geels *et al*, 2012; Roelofson *et al*, 2012), and whilst novel genomic classification methods offer much promise in this regard (DeLeon *et al*, 2014; Murali *et al*, 2014), they have yet to be implemented clinically. The present data suggest that NLR and PLR may have potential merit as additional prognostic tools to support clinical decision-making in the surgical and adjuvant therapeutic management of endometrial cancer.

The strengths of the current study are its large patient cohort and comparatively long follow-up period. The limitations are its retrospective design, where it is difficult to control for potential confounding factors. As such, it was beyond the scope of this study to investigate potential interactions between systemic inflammatory markers and response to adjuvant therapies. Independent validation of our cut-offs in prospective studies, including clinical trials, is also warranted prior to their implementation. A further limitation is the heterogeneity of tumour types/stages included in this study. Nevertheless, subgroup analysis revealed combined NLR and PLR to have prognostic value in both early and late-stage endometrial cancers, independent of other prognostic variables. In contrast to many other studies, we

accounted for patient co-morbidities, many of which involve underlying systemic inflammation e.g. coronary heart disease and chronic obstructive pulmonary disease (Bhat *et al*, 2013; Günay *et al*, 2014), by compiling Charlson co-morbidity indices for our patient cohort. However, we found no significant association between Charlson score and NLR, PLR, MLR or survival. In this sense, systemic inflammatory markers may provide a simple and more objective alternative to Charlson scores for predicting survival in endometrial cancer patients.

In conclusion, this study highlights the potential of NLR and PLR as additional prognostic tools. These are simple measures which are essentially cost-neutral and which could aid decision-making in the clinical management of endometrial cancer patients.

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FIGURE LEGENDS

Figure 1. Overall survival of endometrial cancer patients stratified according to MLR, NLR and PLR cut-offs. Kaplan-Meier overall survival (OS) curves plus log-rank *P* values for patients stratified using a monocyte:lymphocyte ratio (MLR) cut-off of 0.19 (A), a neutrophil:lymphocyte ratio (NLR) cut-off of 2.4 (B, D) and a platelet:lymphocyte ratio (PLR) cut-off of 240 (C, D). High and low ratio values are indicated by ↑ and ↓, respectively, applying the cut-offs defined above. The number of patients falling into each category is also indicated.

Figure 2. Cancer specific survival of endometrial cancer patients stratified according to MLR, NLR and PLR cut-offs. Kaplan-Meier cancer specific survival (CSS) curves plus log-rank *P* values for patients stratified using a monocyte:lymphocyte ratio (MLR) cut-off of 0.19 (A), a neutrophil:lymphocyte ratio (NLR) cut-off of 2.4 (B, D) and a platelet:lymphocyte ratio (PLR) cut-off of 240 (C, D). High and low ratio values are indicated by ↑ and ↓, respectively, applying the cut-offs defined above. The number of patients falling into each category is also indicated.

Figure 3. Survival analysis of combined NLR and PLR-based categorisation in early and late stage endometrial cancer subgroups. Kaplan-Meier overall survival (OS; A, C) and cancer specific survival (CSS; B, D) curves plus log-rank *P* values in stage I/II (A, B) and stage III/IV (C, D) endometrial cancer patients. High and low ratio values are indicated by ↑ and ↓, respectively, applying cut-offs of 2.4 for NLR and 240 for PLR. The number of patients falling into each category is also indicated on the Kapan-Meier plots. Corresponding multivariable analysis data of combined NLR and PLR-based categorisation adjusted for age,

650 grade and lymphovascular space invasion are tabulated below each plot. HR = hazard ratio;

651 CI = confidence interval.

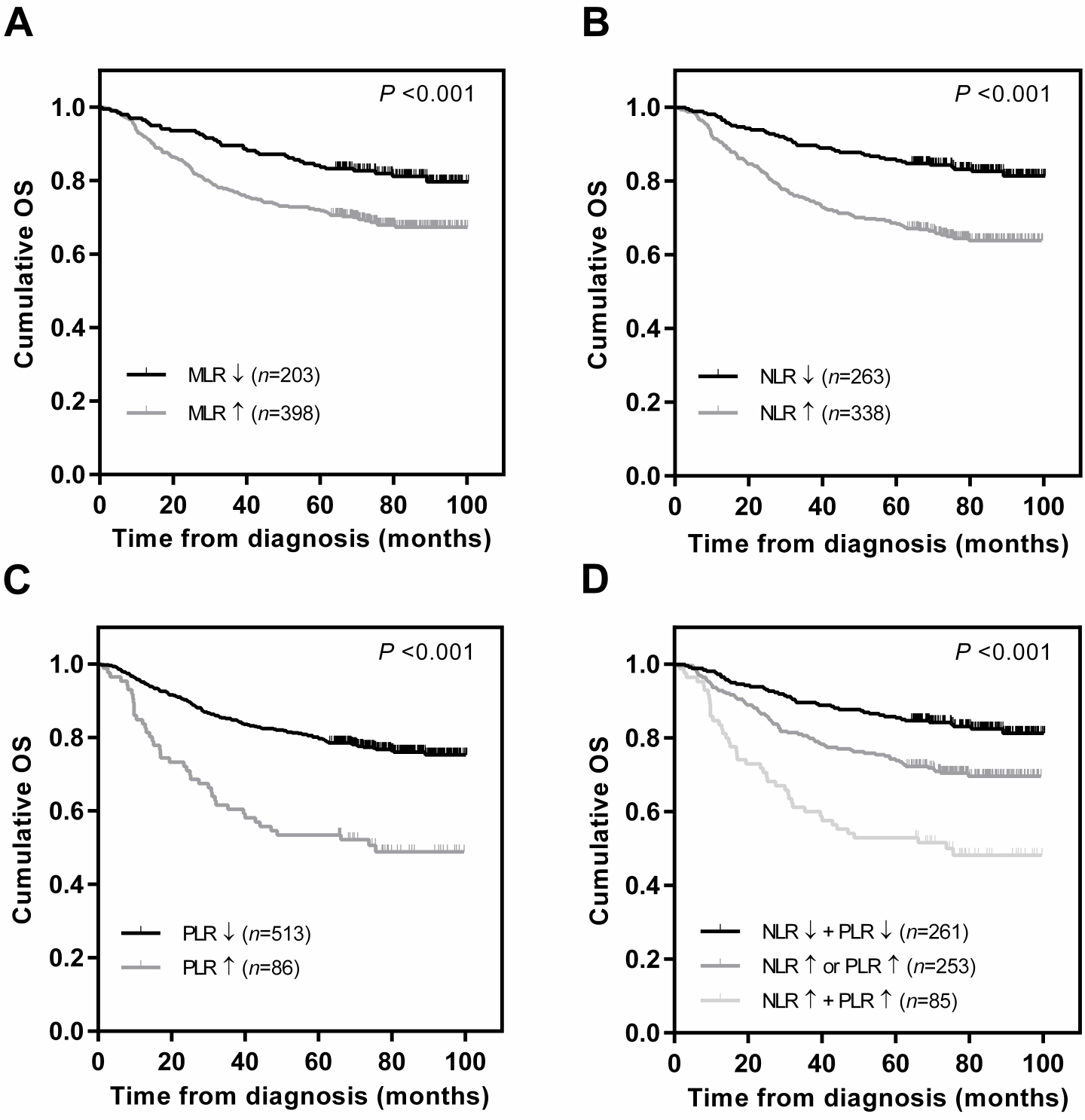

A
P <0.001
Cumulative OS
1.0
0.8
0.6
0.4
0.2
0.0
MLR ↓ (n=203)
MLR ↑ (n=398)
0
20
40
60
80
100
Time from diagnosis (months)
B
P <0.001
Cumulative OS
NLR ↓ (n=263)
NLR ↑ (n=338)
Time from diagnosis (months)
C
P <0.001
Cumulative OS
PLR ↓ (n=513)
PLR ↑ (n=86)
Time from diagnosis (months)
D
P <0.001
Cumulative OS
NLR ↓ + PLR ↓ (n=261)
NLR ↑ or PLR ↑ (n=253)
NLR ↑ + PLR ↑ (n=85)
Time from diagnosis (months)

Figure 1

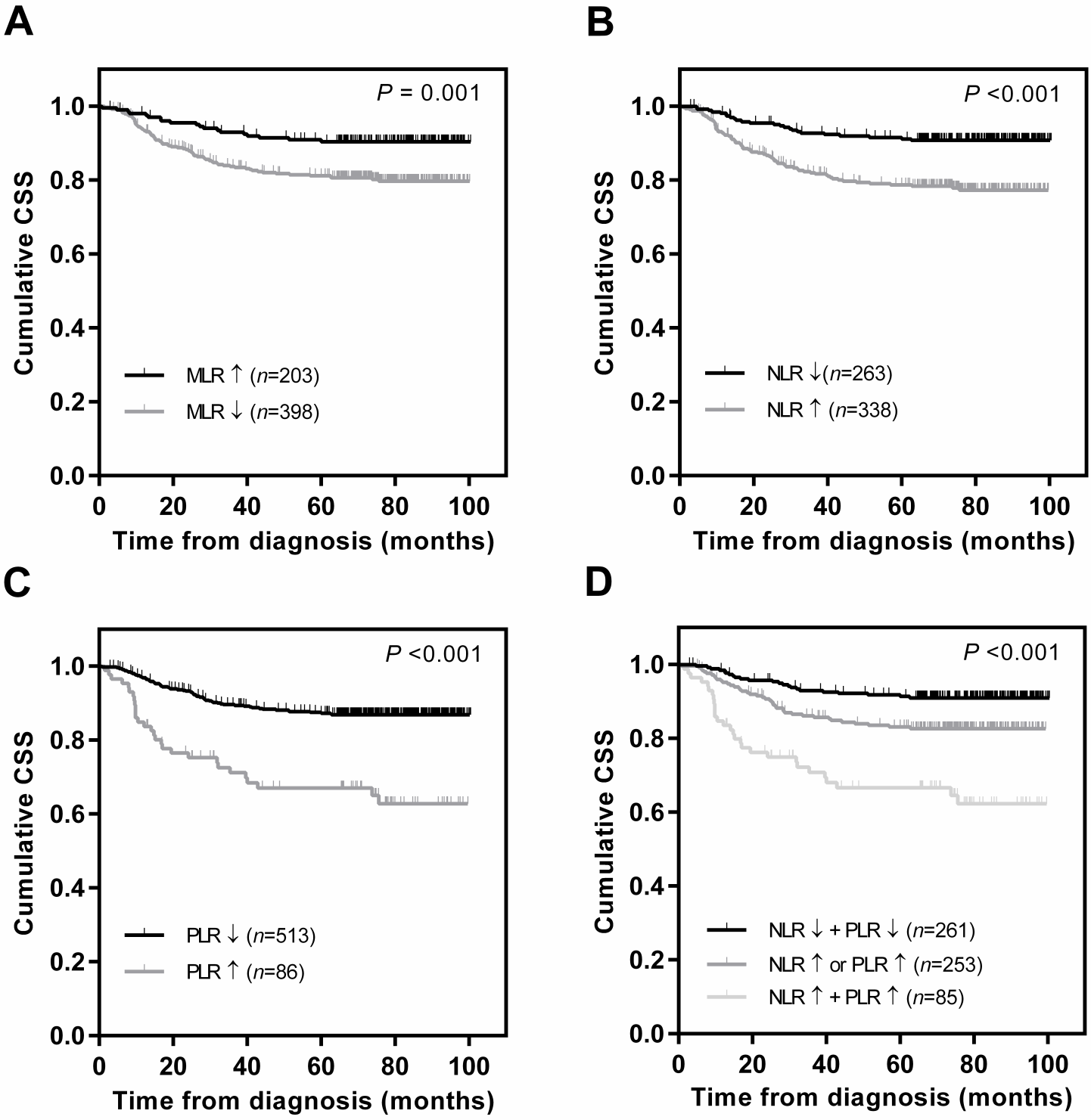
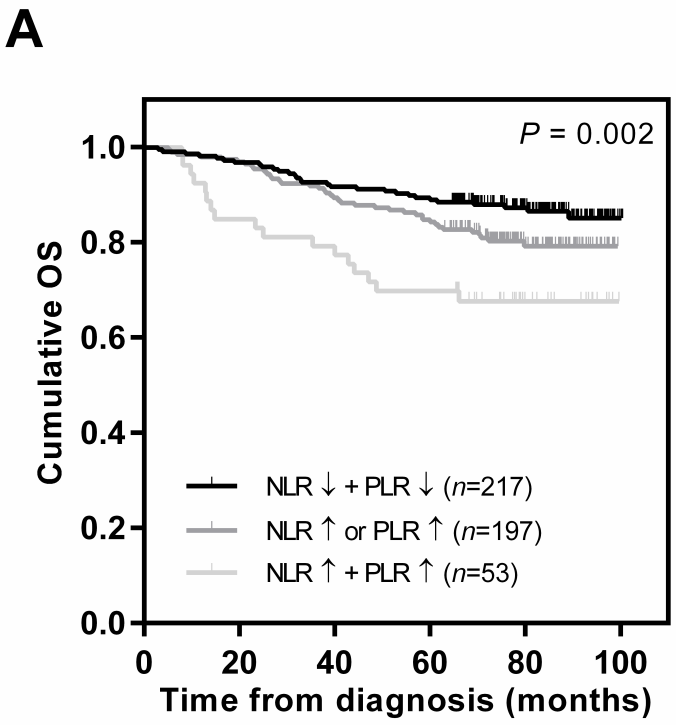
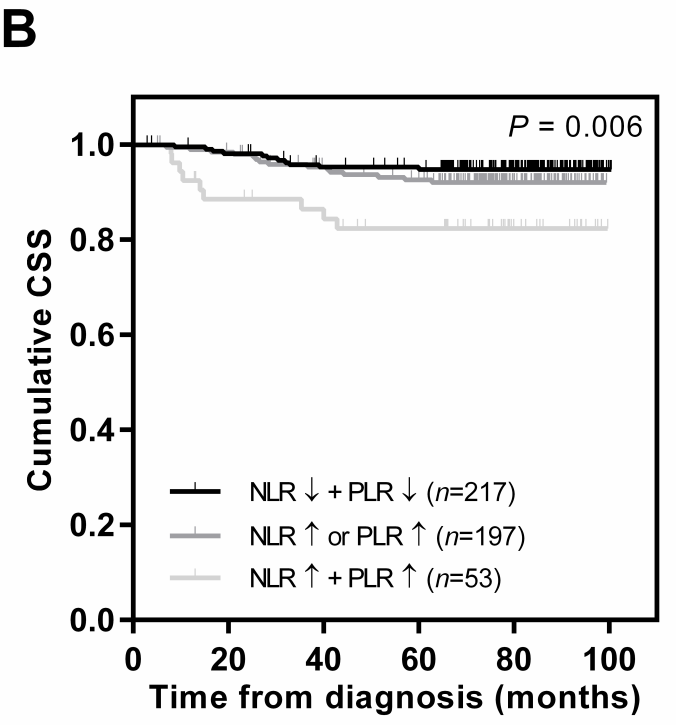

A
P = 0.001
Cumulative CSS
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Time from diagnosis (months)
MLR ↑ (n=203)
MLR ↓ (n=398)
B
P <0.001
Cumulative CSS
Time from diagnosis (months)
NLR ↓(n=263)
NLR ↑ (n=338)
C
P <0.001
Cumulative CSS
Time from diagnosis (months)
PLR ↓ (n=513)
PLR ↑ (n=86)
D
P <0.001
Cumulative CSS
Time from diagnosis (months)
NLR ↓ + PLR ↓ (n=261)
NLR ↑ or PLR ↑ (n=253)
NLR ↑ + PLR ↑ (n=85)

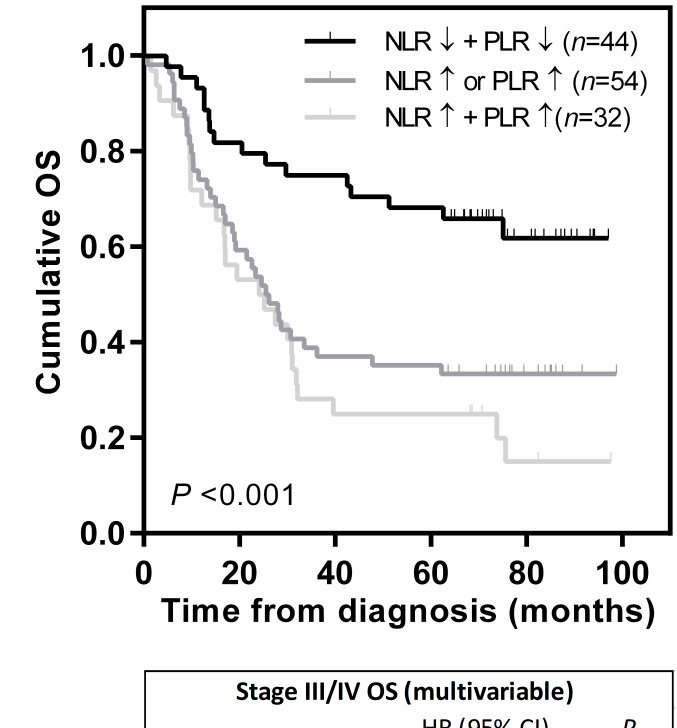
Figure 2



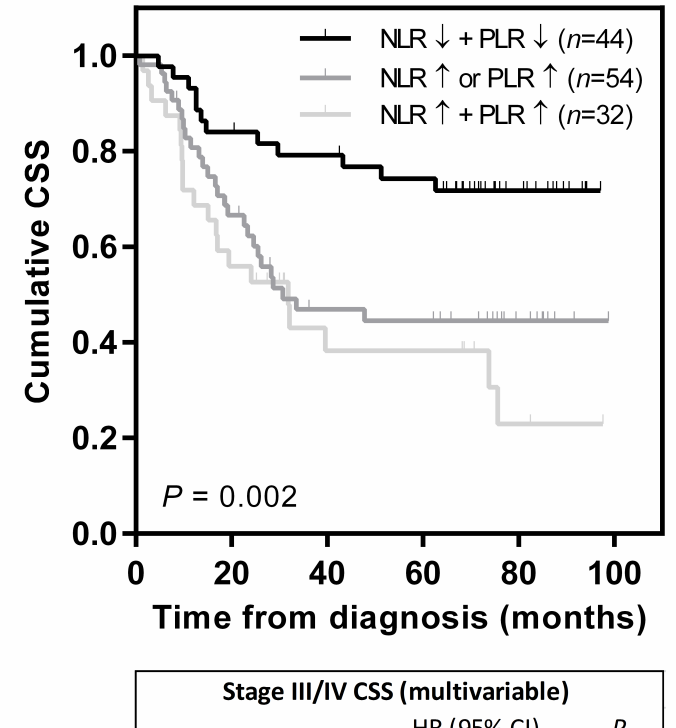
Stage I/II OS (multivariable)	HR (95% CI)	P
NLR low + PLR low	1 (referent)	
NLR high or PLR high	1.70 (1.03 - 2.81)	0.037
NLR high + PLR high	2.78 (1.50 - 5.18)	0.001



Stage I/II CSS (multivariable)	HR (95% CI)	P
NLR low + PLR low	1 (referent)	
NLR high or PLR high	1.52 (0.69 - 3.34)	0.294
NLR high + PLR high	3.51 (1.43 - 8.60)	0.006



Stage III/IV OS (multivariable)	HR (95% CI)	P
NLR low + PLR low	1 (referent)	
NLR high or PLR high	1.87 (1.00 - 3.51)	0.050
NLR high + PLR high	2.48 (1.30 - 4.73)	0.006



Stage III/IV CSS (multivariable)	HR (95% CI)	P
NLR low + PLR low	1 (referent)	
NLR high or PLR high	1.96 (0.95 - 4.07)	0.069
NLR high + PLR high	2.48 (1.30 - 4.73)	0.021

Figure 3

Table 1. Overall survival of patients stratified according to NLR, PLR and MLR cut-offs, together with other prognostic parameters

Parameter	Univariable		Multivariable	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
MLR				
Low (< 0.19)	1 (Referent)		1 (Referent)	
High (≥ 0.19)	1.89 (1.31 - 2.72)	0.001	1.23 (0.84 - 1.82)	0.294
NLR				
Low (< 2.4)	1 (Referent)		1 (Referent)	
High (≥ 2.4)	2.37 (1.68 - 3.34)	<0.001	1.82 (1.27 - 2.62)	0.001
PLR				
Low (< 240)	1 (Referent)		1 (Referent)	
High (≥ 240)	2.72 (1.92 - 3.84)	<0.001	1.89 (1.30 - 2.75)	0.001
Combined NLR + PLR				
NLR low + PLR low	1 (Referent)		**1 (Referent)**	
NLR high or PLR high	1.89 (1.30 - 2.73)	0.001	**1.59 (1.08 - 2.35)**	**0.018**
NLR high + PLR high	3.92 (2.58 - 5.96)	<0.001	**2.54 (1.61 - 4.01)**	**<0.001**
Age (years)				
<55	1 (Referent)		**1 (Referent)**	
55-64	1.84 (0.91 - 7.49)	0.091	**2.72 (1.28 - 5.78)**	**0.009**
65-74	3.44 (1.75 - 6.75)	<0.001	**4.41 (2.17 - 5.78)**	**<0.001**
≥75	7.46 (3.84 - 14.50)	<0.001	**7.64 (3.74 - 15.61)**	**<0.001**
Charlson co-morbidity index				
	0.93 (0.79 - 1.10)	0.419	-	-
Stage (FIGO 2009)				
I	1 (Referent)		**1 (Referent)**	
II	1.49 (0.84 - 2.65)	0.169	**1.16 (0.63 - 2.14)**	**0.633**
III	4.29 (3.01 - 6.12)	<0.001	**2.31 (1.51 - 3.53)**	**<0.001**
IV	12.66 (8.03 - 19.97)	<0.001	**5.57 (3.19 - 9.74)**	**<0.001**
Grade				
1	1 (Referent)		**1 (Referent)**	
2	1.71 (1.06 - 2.75)	0.027	**1.38 (0.83 - 2.29)**	**0.216**
3	5.26 (3.56 - 7.77)	<0.001	**1.98 (1.15 - 3.40)**	**0.014**
Histopathological subtype				
Endometrioid (EEC)	1 (Referent)		**1 (Referent)**	
Serous	5.48 (3.51 - 8.56)	<0.001	**1.66 (0.95 - 2.90)**	**0.076**
Clear Cell	8.31 (4.31 - 16.05)	<0.001	**1.71 (0.70 - 4.16)**	**0.243**
Carcinosarcoma	6.35 (3.90 - 10.35)	<0.001	**2.28 (1.25 - 4.16)**	**0.007**
Mixed (EEC + non-EEC)	2.30 (1.44 - 3.67)	<0.001	**1.06 (0.61 - 1.86)**	**0.838**
Lymphovascular space invasion				
Absent	1 (Referent)		**1 (Referent)**	
Present	3.69 (2.66 - 5.13)	<0.001	**1.66 (1.12 - 2.46)**	**0.012**

Univariable and multivariable analysis using Cox proportional hazards models. NLR, PLR, MLR and combined NLR+PLR were adjusted separately in models that all included age, stage, grade, histopathological subtype and lymphovascular space invasion. Results from the multivariable model which included combined NLR+PLR score are indicated in bold. Abbreviations: NLR = neutrophil:lymphocyte ratio; PLR = platelet:lymphocyte ratio; MLR = monocyte:lymphocyte ratio; FIGO = International Federation of Gynaecology and Obstetrics; EEC = endometrioid endometrial carcinoma;

HR = hazard ratio; CI = confidence interval.

Table 2. Cancer specific survival of patients stratified according to NLR, PLR and MLR cut-offs, together with other prognostic parameters

Parameter	Univariable		Multivariable	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
MLR				
Low (< 0.19)	1 (Referent)		1 (Referent)	
High (≥ 0.19)	2.22 (1.35 - 3.68)	0.002	1.26 (0.73 - 2.15)	0.409
NLR				
Low (< 2.4)	1 (Referent)		1 (Referent)	
High (≥ 2.4)	2.72 (1.70 - 4.36)	<0.001	1.68 (1.03 - 2.76)	0.040
PLR				
Low (< 240)	1 (Referent)		1 (Referent)	
High (≥ 240)	3.24 (2.09 - 5.03)	<0.001	1.76 (1.09 - 2.87)	0.022
Combined NLR + PLR				
NLR low + PLR low	1 (Referent)		**1 (Referent)**	
NLR high or PLR high	2.02 (1.22 - 3.36)	0.007	**1.46 (0.87 - 2.47)**	**0.156**
NLR high + PLR high	4.91 (2.84 - 8.49)	<0.001	**2.26 (1.24 - 4.13)**	**0.008**
Age (years)				
<55	1 (Referent)		**1 (Referent)**	
55-64	1.36 (0.63 - 2.95)	0.431	**2.13 (0.92 - 4.97)**	**0.079**
65-74	1.97 (0.93 - 4.16)	0.076	**2.55 (1.12 - 5.79)**	**0.025**
≥75	4.10 (1.97 - 8.55)	<0.001	**3.87 (1.69 - 8.86)**	**0.001**
Charlson co-morbidity index				
	0.84 (0.66 - 1.08)	0.168	-	-
Stage (FIGO 2009)				
I	1 (Referent)		**1 (Referent)**	
II	2.61 (1.22 - 5.58)	0.013	**1.66 (0.74 - 3.72)**	**0.223**
III	7.90 (4.79 - 13.03)	<0.001	**3.34 (1.88 - 5.96)**	**<0.001**
IV	28.03 (15.85 - 49.57)	<0.001	**7.84 (3.90 - 15.76)**	**<0.001**
Grade				
1	1 (Referent)		**1 (Referent)**	
2	2.71 (1.23 - 5.97)	0.013	**1.77 (0.79 - 3.13)**	**0.169**
3	12.19 (6.28 - 23.66)	<0.001	**3.47 (1.56 - 7.71)**	**0.002**
Histopathological subtype				
Endometrioid (EEC)	1 (Referent)		**1 (Referent)**	
Serous	7.21 (4.10 - 12.66)	<0.001	**1.58 (0.79 - 3.13)**	**0.193**
Clear Cell	12.22 (5.72 - 26.11)	<0.001	**1.98 (0.70 - 5.55)**	**0.197**
Carcinosarcoma	10.38 (5.90 - 18.27)	<0.001	**2.40 (1.20 - 4.79)**	**0.013**
Mixed (EEC + non-EEC)	2.32 (1.19 - 4.49)	0.013	**0.77 (0.36 - 1.63)**	**0.493**
Lymphovascular space invasion				
Absent	1 (Referent)		**1 (Referent)**	
Present	6.51 (3.97 - 10.66)	<0.001	**2.02 (1.14 - 3.56)**	**0.016**

Univariable and multivariable analysis using Cox proportional hazards models. NLR, PLR, MLR and combined NLR+PLR were adjusted separately in models that all included age, stage, grade, histopathological subtype and lymphovascular space invasion. Results from the multivariable model which included the combined NLR+PLR score are indicated in bold. Abbreviations: NLR = neutrophil:lymphocyte ratio; PLR = platelet:lymphocyte ratio; MLR = monocyte:lymphocyte ratio; FIGO = International Federation of Gynaecology and Obstetrics; EEC = endometrioid endometrial

carcinoma; HR = hazard ratio; CI = confidence interval.

Table 3. Associations of NLR, PLR and MLR with other clinicopathological factors

Factor	*n* (%)	NLR, Median (IQR)	*P*	PLR, Median (IQR)	*P*
Total	605 (100)	2.56 (1.87 - 3.59)	-	144 (112 - 200)	-
Age (years)					
<55	100 (16.5)	2.66 (1.96 - 3.64)[a,b]	0.013	142 (113 - 210)[a,b]	0.036
55-64	198 (32.7)	2.36 (1.71 - 3.30)[a]		134 (103 - 185)[a]	
65-74	185 (30.6)	2.49 (1.92 - 3.43)[a,b]		147 (112 - 202)[a,b]	
≥75	122 (20.2)	2.91 (2.17 - 3.88)[b]		156 (120 - 210)[b]	
Stage (FIGO 2009)					
I	414 (68.4)	2.47 (1.79 - 3.32)[a]	<0.001	140 (108 - 192)[a]	<0.001
II	57 (9.4)	2.41 (1.88 - 3.73)[a]		145 (86 - 201)[a]	
III	101 (16.7)	2.81 (2.01 - 4.00)[a]		156 (120 - 207)[a,b]	
IV	31 (5.1)	3.68 (2.75 - 5.29)[b]		206 (135 - 285)[b]	
Missing data	2 (0.3)	-		-	
Grade					
1	256 (42.3)	2.46 (1.84 - 3.30)	0.055	136 (106 - 193)	0.131
2	156 (25.8)	2.45 (1.81 - 3.80)		151 (115 - 195)	
3	193 (31.9)	2.77 (2.03 - 3.83)		150 (115 - 214)	
Histopathological subtype					
Endometrioid (EEC)	468 (77.4)	2.48 (1.86 - 3.51)[a]	0.021	143 (111 - 195)	0.206
Serous	38 (6.3)	2.90 (1.83 - 3.40)[a,b]		132 (112 - 199)	
Clear Cell	13 (2.1)	2.88 (2.39 - 3.20)[a,b]		130 (113 - 229)	
Carcinosarcoma	29 (4.8)	3.15 (2.60 - 5.58)[b]		179 (131 - 258)	
Mixed (EEC + non-EEC)	57 (9.4)	2.47 (1.78 - 3.55)[a,b]		158 (112 - 208)	
Lymph nodes					
Negative	356 (58.8)	2.48 (1.80 - 3.38)	0.024[ł]	143 (109 - 197)	0.045[ł]
Positive	70 (11.6)	2.85 (2.18 - 4.00)		165 (121 - 206)	
No lymphadenectomy	168 (28.6)	-		-	
Missing data	6 (1.0)	-		-	
Lymphovascular space invasion					
Absent	346 (57.2)	2.46 (1.80 - 3.26)	0.002[ł]	138 (108 - 192)	0.006[ł]
Present	248 (41.0)	2.77 (2.05 - 4.05)		154 (116 - 213)	
Missing data	11 (1.8)	-		-	

ł = Mann-Whitney-*U* test *P* values (all other *P* values are from Kruskall-Wallis tests); a,b depict significant differen
post hoc Mann-Whitney-*U* tests with Bonferroni corrections for multiple comparisons. Abbreviations: FIGO = Inter
and Obstetrics; EEC = endometrioid endometrial carcinoma; IQR = interquartile range.

MLR, Median (IQR)	*P*
0.216 (0.170 - 0.293)	-
0.214 (0.163 - 0.296)[a,b] 0.204 (0.158 - 0.272)[a] 0.220 (0.175 - 0.275)[a] 0.249 (0.194 - 0.354)[b]	<0.001
0.214 (0.163 - 0.279)[a] 0.209 (0.179 - 0.298)[a] 0.223 (0.170 - 0.304)[a] 0.300 (0.219 - 0.391)[b] -	0.001
0.214 (0.167 - 0.285) 0.214 (0.167 - 0.274) 0.221 (0.178 - 0.314)	0.272
0.213 (0.167 - 0.281) 0.221 (0.168 - 0.360) 0.227 (0.215 - 0.278) 0.248 (0.216 - 0.339) 0.220 (0.170 - 0.305)	0.109
0.212 (0.163 - 0.279) 0.223 (0.178 - 0.329) - -	0.050[†]
0.212 (0.165 - 0.285) 0.224 (0.178 - 0.304) -	0.104[†]

ıces between categories following
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