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Neutrophil-to-lymphocyte ratio as a bladder cancer biomarker: assessing prognostic and predictive value in SWOG 8710

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Abstract

Background—Risk stratification is a major challenge in bladder cancer (BC), and a biomarker is needed. Multiple studies report the neutrophil-to-lymphocyte ratio (NLR) as a promising candidate; however, these analyses have methodological limitations. Therefore, we performed a category B biomarker study. We tested whether NLR is prognostic for overall survival (OS) after curative treatment or predictive for the benefit from neoadjuvant chemotherapy (NAC).

Methods—We performed a secondary analysis of SWOG 8710—a randomized, phase III trial that assessed cystectomy ± NAC in 317 patients with muscle-invasive BC. We calculated NLR from prospectively collected complete blood counts. We identified 230 patients for the prognostic analysis and 263 for the predictive analysis. We evaluated NLR using proportional hazards models including pre-specified factors (age, gender, T-stage, lymphovascular invasion, treatment arm).

Results—With a median follow-up of 18.6 years, there were 172 and 205 deaths in the prognostic and predictive cohorts, respectively. On multivariable analysis, NLR was not prognostic for OS (HR 1.04, 95%CI [0.98–1.11], *P*=0.24). Furthermore, NLR did not predict for the OS

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benefit from NAC (HR 1.01, 95%CI [0.90 – 1.14], *P*=0.86). Factors associated with worse OS were older age (HR 1.05, 95%CI [1.04–1.07], *P*<0.001) and surgery without NAC (HR 1.39, 95%CI [1.03–1.88], *P*=0.03).

Conclusion—This is the first analysis of NLR in BC to use prospectively collected clinical trial data. In contrast to previous studies, it suggests NLR is neither a prognostic nor predictive biomarker for OS in muscle-invasive BC.

Trial Registration—clinicaltrials.gov Identifier NCT02756637 https://clinicaltrials.gov/show/NCT02756637

Precis

This is the first category B biomarker study testing the neutrophil-to-lymphocyte ratio in bladder cancer. In contrast to previous reports, these data suggest NLR holds neither prognostic nor predictive value for overall survival.

INTRODUCTION

Pre-operative risk stratification is a major challenge in bladder cancer,[1,2] and a robust biomarker is needed.[3–5] One emerging candidate is the neutrophil-to-lymphocyte ratio (NLR). NLR is easily calculated from a complete blood count (CBC) and is felt to reflect the systemic inflammatory state.[6–10] A high ratio may be linked to cancer progression through increased pro-growth and pro-angiogenic factors[9,10] coupled with decreased lymphocyte-mediated tumor surveillance.[11] In clinical studies, elevated NLR correlates with inferior survival in many solid malignancies.[6–8]

Specifically in bladder cancer (BC), previous reports suggest NLR holds prognostic value.[12–24] For example, an elevated pre-treatment NLR has been associated with worse survival after radical cystectomy.[12–16] Other studies link NLR with a higher burden of disease at surgery (e.g., muscle-invasiveness,[17–19] extravesical extension,[13–15,20] and node positivity[14]), raising the possibility that—in addition to being a prognostic biomarker—NLR might also predict which patients will benefit from neoadjuvant chemotherapy.[25] If validated, NLR would be an inexpensive, widely available, and appealing biomarker for BC.

However, results from previous studies are threatened by methodological limitations. These include the use of observational datasets and dichotomization of the NLR variable. Considerably stronger evidence would be generated by rigorously analyzing prospectively collected biomarker specimens from a clinical trial[26–28]—a “category B” study per the biomarker evidence framework of Simon et al.[28] SWOG 8710 is well-suited to such an investigation. This randomized, phase III trial tested radical cystectomy (RC) with or without neoadjuvant chemotherapy (NAC) for muscle-invasive BC.[29] It offers several notable advantages for an analysis of NLR. First, CBCs were prospectively collected at baseline per protocol. Second, the trial’s significant long-term follow-up captures enough events to generate adequate statistical power.[28] Third, in addition to prognostic value, NLR’s predictive value can also be assessed because of the randomization to NAC.

We therefore used SWOG 8710 to evaluate NLR in BC. Specifically, we tested two hypotheses: first, that NLR is a prognostic biomarker for overall survival (OS) after curative treatment; second, that NLR is a predictive biomarker for the OS benefit from NAC.

METHODS

We conducted this work according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines.[27,30] This study's REMARK profile is shown in Table 1.

Patients

We performed a secondary analysis of patients enrolled in SWOG 8710, a multi-institutional, randomized, phase III trial. Full protocol details have been previously reported.[29] In brief, the trial accrued 317 patients between 1987–1998 with T2-T4aN0[31] transitional cell BC and SWOG performance status of 0 or 1. Patients were randomly assigned to RC alone or three cycles of NAC with methotrexate, vinblastine, doxorubicin, and cisplatin followed by RC. Patients were followed clinically every 6 months after treatment; the most recent vital status update occurred on May 8, 2013. All trial participants gave written informed consent and all institutions' relevant ethics committees gave study approval. We conducted the present analysis under a data use agreement with SWOG and with approval of the University of Pennsylvania Institutional Review Board.

Specimen characteristics and assay methods

Trial protocol required enrolling institutions to obtain pre-treatment bloodwork—including a CBC with differential—within 14 days of patient registration. For the present study, a single investigator who was blinded to clinical outcomes abstracted the CBC data from trial flowsheets. We calculated NLR by dividing the number of neutrophils by the number of lymphocytes.

Study design

SWOG 8710 was designed to test a therapeutic question and did not contain a planned biomarker endpoint. However, the trial allows for a category B study of NLR.[28] For the present investigation, we developed a pre-specified analysis plan before examining the data.

From all SWOG 8710 patients, we identified two cohorts—a prognostic cohort and a predictive cohort—to test NLR's value as a biomarker (Figure 1). Prognostic biomarkers give information about cancer outcomes regardless of the specific treatment.[32,33] Therefore, the prognostic cohort comprised patients with pre-treatment NLR who successfully completed curative therapy with RC ± NAC. Patients were excluded if they did not complete curative surgery, and we analyzed the cohort according to treatment received. We additionally tested the prognostic value of NLR separately by treatment arm.[34] Predictive biomarkers, on the other hand, portend differential responses to a particular therapy[32]—in this case, NAC. The predictive comparison was between the two trial arms, and we wished to preserve the benefits of randomization. Therefore, the predictive cohort included any

patient with pre-treatment NLR who was assigned to a trial arm (NAC or no NAC); this group was analyzed according to the intent-to-treat principle.

We chose OS as the study endpoint because this is an unambiguous outcome with clear clinical significance. It also avoids the analytic challenges associated with cancer-specific survival[35] or other surrogate endpoints.[36,37] We defined OS from the date of randomization to the date of death from any cause. Patients alive at last follow-up were treated as right-censored, and we calculated median follow-up using the reverse Kaplan-Meier method.[38]

In addition to NLR, we considered *a priori* the following candidate variables for inclusion in our models: age (continuous), gender (male vs. female), clinical tumor T category (T2 vs. T3/T4a), lymphovascular invasion (LVI) on biopsy or transurethral resection of tumor specimen[39,40] (negative vs. positive), and treatment (RC vs. NAC followed by RC). We incorporated only pre-treatment variables (i.e., no surgical pathology information) because our goal was to test NLR as a pre-treatment biomarker.

We also calculated the minimally detectable hazard ratio (HR) based on the number of death events in each cohort. For the prognostic analysis, we calculated 80% power to detect a HR of at least 1.11 for mortality with each unit increase in NLR at a 2-sided α (Type I error) = 0.05. For the predictive analysis, we calculated 80% power to detect a HR of at least 1.09 for the interaction term of NLR and treatment.

Statistical analysis methods

NLR was measured as a continuous variable. We excluded a single extreme outlier (NLR = 31.3; 94% neutrophils, 3% lymphocytes) that was felt to represent an acutely infected patient. We tested the association of NLR with other candidate variables using the Wilcoxon rank-sum or Kruskal-Wallis tests, as appropriate. We examined the association of each variable with OS using univariable Cox regression models. All variables with $P < 0.2$ on univariable analysis were included in a multivariable Cox regression model.[41] We used the two-sided Wald test to determine significance.

There were complete data for all variables except for 19 (7.2%) missing LVI values. We included these patients in the study, and we addressed missing data using multiple imputation under the assumption that data were missing at random. We additionally performed a sensitivity analysis using only complete cases.

In the regression models, we kept NLR as a continuous variable on its original scale. We checked assumptions of linearity in log hazard by categorizing continuous covariables and plotting coefficient estimates, as well as by examining a plot of Martingale residuals. We checked assumptions of proportional hazards using plots of scaled Schoenfeld residuals and likelihood ratio tests on interactions between covariables of interest and log time. All statistical analyses were performed with Stata (version 14.0, StataCorp, College Station, TX).

RESULTS

Baseline clinical and marker characteristics

The flow of patients in the study and reasons for exclusion are shown in Figure 1. From 317 patients enrolled in SWOG 8710, 263 (83%) patients and 230 (73%) patients comprised the predictive and prognostic cohorts, respectively. Patient and disease characteristics are detailed in Table 2. Excluded patients were more likely to have positive or missing LVI values and higher pre-treatment NLR (Supplemental Tables 1 and 2). There were no other significant differences between included and excluded patients.

Pre-treatment NLR was collected a median of 13 days (interquartile range [IQR] 7–21) before first treatment. The distribution of NLR was similar in both the prognostic and predictive cohorts, with a median NLR of 2.66 (IQR 2.01–4.06) and 2.72 (IQR 2.03–4.17), respectively (Supplemental Figure 1). Higher NLR was associated with positive or missing LVI values in the predictive cohort; NLR was not significantly associated with any other patient or disease characteristic in either cohort (Table 2).

Prognostic and predictive analyses

During a median follow-up of 18.6 years, there were 172 deaths in the prognostic cohort. NLR was not significantly associated with OS on either univariable (HR 1.03; 95% CI [0.97 – 1.10]; $P = 0.30$) or multivariable analyses (HR 1.04; 95% CI [0.98 – 1.11]; $P = 0.24$) (Table 3, Supplemental Figure 2). On univariable analysis, age, gender, T category, and treatment showed some association with OS. On multivariable analysis, both older age (HR 1.05; 95% CI [1.04 – 1.07]; $P < 0.001$) and treatment with RC alone (HR 1.39; 95% CI [1.03 – 1.88]; $P = 0.03$) remained significantly associated with worse OS (Table 3). When the prognostic cohort was analyzed separately by treatment arm, NLR was still not significantly associated with OS (Supplemental Tables 3 and 4).[34]

During a median follow-up of 18.6 years, there were 205 deaths in the predictive cohort. On multivariable analysis, NLR did not predict for response to NAC (HR 1.01, 95% CI [0.90 – 1.14]; $P = 0.86$ for the interaction term) (Table 4, Supplemental Figure 3).

Model assumptions and sensitivity analysis

There were no significant deviations from model assumptions of proportional hazards or linearity. Specifically, the Schoenfeld residuals-based score test did not reject its null hypothesis (prognostic analysis: $P = 0.27$; predictive analysis: $P = 0.19$) and plots of the Schoenfeld residuals did not deviate significantly from zero slope. Likelihood ratio tests further supported the validity of the proportional hazards assumptions (prognostic analysis: $P = 0.45$; predictive analysis: $P = 0.18$). Plots of coefficient estimates for a categorized NLR covariate did not depart significantly from a line of slope zero, and plots of Martingale residuals for each continuous covariate additionally showed no significant nonlinear behavior. A sensitivity analysis on the complete case (i.e., excluding 19 patients with missing LVI values) did not yield significantly different results.

DISCUSSION

In contrast to previous studies, this analysis found that NLR was not a prognostic biomarker for OS in muscle-invasive BC. Furthermore, NLR was not predictive for the OS benefit from NAC.

There are two broad interpretations of this study's findings. First, NLR might truly be a prognostic or predictive biomarker, but this study failed to detect the association. Most randomized clinical trials are designed and powered to test a therapeutic question; secondary biomarker analyses may then be underpowered.[28] However, the long-term follow-up and significant mortality in SWOG 8710 generated a meaningful number of OS events. Therefore, our power analyses suggest that any undetected true association between NLR and OS would likely have a limited effect size (HR less than approximately 1.1 for each unit increase in NLR). The value of such a modest HR would be further reduced by the relatively narrow range of NLR. In summary, it is possible that a small association exists that was not detected in this study—but we would question the practical meaning of such an association. A biomarker must achieve significant differentiation between patients to be clinically useful.

The second interpretation of our findings is that NLR is truly *not* a prognostic or predictive biomarker in BC. Why, then, do previous studies suggest otherwise? Perhaps pitfalls in analysis, reporting, and publication have contributed to a literature that is overly enthusiastic about NLR. This would not be unusual: biomarker studies are almost universally positive. For example, an analysis of over 1,900 publications on cancer prognostic markers found that nearly 95% reported positive results.[42]

A particular analytic concern in biomarker studies is the use of observational datasets. These are susceptible to biases from a lack of standardized inclusion criteria, treatment schemes, and follow-up schedules.[43] Use of observational datasets can significantly inflate prognostic effect sizes compared to data from clinical trials.[44] Therefore, studies of observational data are classified as category C or D and placed at the bottom of the biomarker level of evidence framework proposed by Simon et al.[28] Notably, all previous studies of NLR in BC analyzed observational data. The present report used prospectively collected clinical trial data and is the first such category B study of NLR in BC. Considerably stronger evidence is generated with this approach.[26–28]

A second analytic issue involves handling of the continuous NLR variable. Dichotomizing this variable is strongly discouraged due to information loss and bias.[27,45–47] In fact, certain methods of selecting a cutpoint can raise the false-positive rate to nearly 40%.[48] Yet most previous studies dichotomized NLR[13,17–19,21,23,24] or did not report effect sizes for the continuous variable.[15] This concern is not limited to studies in BC: a recent global review of NLR across various primary cancers found that 96% of publications dichotomized the variable.[6]

In addition to these analytic issues, biases in reporting and publication may also contribute to a surfeit of positive results.[49,50] Reporting bias includes both selective reporting and poor reporting.[26] An example of selective reporting is a study that analyzes multiple endpoints or fits several multivariable models but reports only those with significant *P* values.[26,27] Poor

reporting leads to publications with vague or incomplete details, making it difficult for readers to fully appraise the study. These biases can significantly skew the biomarker literature.[51] The REMARK guidelines offer an opportunity to reduce both types of reporting biases; unfortunately, adherence is not optimal in previous NLR studies (Supplemental Table 5).

Finally, publication bias occurs when authors do not submit negative studies or editors do not accept them.[49,52] The former practice, sometimes termed the "file-drawer problem,"[53] may be a substantial issue in the biomarker literature.[26,27] This concern is heightened for NLR studies because CBCs are a ubiquitous laboratory test. How many investigators queried existing databases for an association of NLR with OS and—finding a null result—decided to avoid the trouble of generating a full manuscript?[54] Indeed, meta-analyses suggest that publication bias affects the NLR literature.[6,55]

Together, these various pitfalls may explain why NLR appeared promising in the previous literature but was negative in the present study. Although these pitfalls were minimized in the current analysis, our work has other important limitations that should be emphasized. First, SWOG 8710 did not contain a planned biomarker endpoint; although prospectively collected data were used, the current analysis was retrospective. This also placed limits on the study's power, as discussed previously. Second, the time period during which SWOG 8710 was conducted colors the interpretation of our study. For example, some of the T3 tumors[31] in the trial would now be considered T2 disease, and current guidelines recommend different chemotherapy regimens than the one used in the trial.[56] Finally, we analyzed CBC values from the trial flowsheets instead of raw laboratory data—raising the possibility of transcription errors. However, we attempted to address this issue by excluding patients with CBC differentials that did not sum to 100%.

In conclusion, this is the first category B analysis of NLR in BC. In contrast to previous studies, these results suggest that NLR is neither a prognostic nor predictive biomarker for OS in muscle-invasive BC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Svatek RS, Shariat SF, Novara G, et al. Discrepancy between clinical and pathological stage: external validation of the impact on prognosis in an international radical cystectomy cohort. BJU Int. 2011; 107(6):898–904. [PubMed: 21244604]
2. Goldsmith B, Baumann BC, He J, et al. Occult pelvic lymph node involvement in bladder cancer: implications for definitive radiation. Int J Radiat Oncol Biol Phys. 2014; 88(3):603–610. [PubMed: 24411628]
3. Kluth LA, Black PC, Bochner BH, et al. Prognostic and Prediction Tools in Bladder Cancer: A Comprehensive Review of the Literature. Eur Urol. 2015; 68(2):238–253. [PubMed: 25709027]
4. Egawa S, Kuruma H. Search for biomarkers of aggressiveness in bladder cancer. Eur Urol. 2006; 50(1):20–22. [PubMed: 16530926]
5. Choudhury A. Molecular Biomarkers in Muscle-Invasive Bladder Cancer. Int J Radiat Oncol Biol Phys. 2015; 92(4):705–706. [PubMed: 26104922]
6. Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014; 106(6):dju124. [PubMed: 24875653]
7. Guthrie GJ, Charles KA, Roxburgh CS, et al. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol. 2013; 88(1):218–230. [PubMed: 23602134]
8. Kumar R, Geuna E, Michalarea V, et al. The neutrophil-lymphocyte ratio and its utilisation for the management of cancer patients in early clinical trials. Br J Cancer. 2015; 112(7):1157–1165. [PubMed: 25719834]
9. Chen ZY, Raghav K, Lieu CH, et al. Cytokine profile and prognostic significance of high neutrophil-lymphocyte ratio in colorectal cancer. Br J Cancer. 2015; 112(6):1088–1097. [PubMed: 25688736]
10. Motomura T, Shirabe K, Mano Y, et al. Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. J Hepatol. 2013; 58(1):58–64. [PubMed: 22925812]
11. Gooden MJ, de Bock GH, Leffers N, et al. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer. 2011; 105(1):93–103. [PubMed: 21629244]
12. Gondo T, Nakashima J, Ohno Y, et al. Prognostic value of neutrophil-to-lymphocyte ratio and establishment of novel preoperative risk stratification model in bladder cancer patients treated with radical cystectomy. Urology. 2012; 79(5):1085–1091. [PubMed: 22446338]
13. Krane LS, Richards KA, Kader AK, et al. Preoperative neutrophil/lymphocyte ratio predicts overall survival and extravesical disease in patients undergoing radical cystectomy. J Endourol. 2013; 27(8):1046–1050. [PubMed: 23517015]
14. Viers BR, Boorjian SA, Frank I, et al. Pretreatment neutrophil-to-lymphocyte ratio is associated with advanced pathologic tumor stage and increased cancer-specific mortality among patients with urothelial carcinoma of the bladder undergoing radical cystectomy. Eur Urol. 2014; 66(6):1157–1164. [PubMed: 24630414]
15. Hermanns T, Bhindi B, Wei Y, et al. Pre-treatment neutrophil-to-lymphocyte ratio as predictor of adverse outcomes in patients undergoing radical cystectomy for urothelial carcinoma of the bladder. Br J Cancer. 2014; 111(3):444–451. [PubMed: 24918819]
16. Kang M, Jeong CW, Kwak C, et al. The Prognostic Significance of the Early Postoperative Neutrophil-to-Lymphocyte Ratio in Patients with Urothelial Carcinoma of the Bladder Undergoing Radical Cystectomy. Ann Surg Oncol. 2016; 23(1):335–342. [PubMed: 26152275]
17. Can C, Baseskioglu B, Yılmaz M, et al. Pretreatment parameters obtained from peripheral blood sample predicts invasiveness of bladder carcinoma. Urol Int. 2012; 89(4):468–472. [PubMed: 23128367]
18. Kaynar M, Yıldırım ME, Badem H, et al. Bladder cancer invasion predictability based on preoperative neutrophil-lymphocyte ratio. Tumour Biol. 2014; 35(7):6601–6605. [PubMed: 24696263]

19. Ceylan C, Doluoglu OG, Keles I, et al. Importance of the neutrophil-to-lymphocyte ratio in muscle-invasive and non-muscle invasive bladder tumors. Urologia. 2014; 81(2):120–124. [PubMed: 24474540]
20. Potretzke A, Hillman L, Wong K, et al. NLR is predictive of upstaging at the time of radical cystectomy for patients with urothelial carcinoma of the bladder. Urol Oncol. 2014; 32(5):631–636. [PubMed: 24629498]
21. Ozyalvacli ME, Ozyalvacli G, Kocaaslan R, et al. Neutrophil-lymphocyte ratio as a predictor of recurrence and progression in patients with high-grade pT1 bladder cancer. Can Urol Assoc J. 2015; 9(3–4):E126–E131. [PubMed: 25844098]
22. Bambury RM, Benjamin DJ, Chaim JL, et al. The safety and efficacy of single-agent pemetrexed in platinum-resistant advanced urothelial carcinoma: a large single-institution experience. Oncologist. 2015; 20(5):508–515. [PubMed: 25845990]
23. Taguchi S, Nakagawa T1, Matsumoto A, et al. Pretreatment neutrophil-to-lymphocyte ratio as an independent predictor of survival in patients with metastatic urothelial carcinoma: A multi-institutional study. Int J Urol. 2015; 22(7):638–643. [PubMed: 25903328]
24. Mano R, Baniel J, Shoshany O, et al. Neutrophil-to-lymphocyte ratio predicts progression and recurrence of non-muscle-invasive bladder cancer. Urol Oncol. 2015; 33(2):67.e1–77.e1.
25. Advanced Bladder Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. Eur Urol. 2005; 48(2):202–205. [PubMed: 15939524]
26. McShane LM, Hayes DF. Publication of tumor marker research results: the necessity for complete and transparent reporting. J Clin Oncol. 2012; 30(34):4223–4232. [PubMed: 23071235]
27. Altman DG, McShane LM, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. BMC Med. 2012; 10:51. [PubMed: 22642691]
28. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst. 2009; 101(21):1446–1452. [PubMed: 19815849]
29. Grossman HB, Natale RB, Tangen CM, et al. Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. N Engl J Med. 2003; 349(9):859–866. [PubMed: 12944571]
30. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies. J Natl Cancer Inst. 2005; 97(16):1180–1184. [PubMed: 16106022]
31. Beahrs, OH., Henson, DE., Hutter, RVP., et al. AJCC Manual for Staging of Cancer. 4th. Philadelphia, PA: Lippincott; 1992.
32. Ballman KV. Biomarker: Predictive or Prognostic? J Clin Oncol. 2015; 33(33):3968–3971. [PubMed: 26392104]
33. Ballman KV. Reply to M. Minden. J Clin Oncol. 2016; 34(14):1703–1704. [PubMed: 26976408]
34. Minden M. Is it time to redefine prognostic and predictive in oncology? J Clin Oncol. 2016; 34(14):1702–1703. [PubMed: 26976412]
35. Barry MJ, Andriole GL, Culkin DJ, et al. Ascertaining cause of death among men in the prostate cancer intervention versus observation trial. Clin Trials. 2013; 10(6):907–914. [PubMed: 23988464]
36. Booth CM, Eisenhauer EA. Progression-free survival: meaningful or simply measurable? J Clin Oncol. 2012; 30(10):1030–1033. [PubMed: 22370321]
37. Gutman, SI., Piper, M., Grant, MD., et al. Methods research report. Rockville, MD: Agency for Healthcare Research and Quality; Progression-free survival: what does it mean for psychological well-being or quality of life?. AHQR Publication No. 13-EHC074-EF. Available from http://effectivehealthcare.ahrq.gov/ehc/products/463/1461/Progression-free-survival-QOL-methods-130410.pdf [accessed February, 19 2016]
38. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996; 17(4):343–346. [PubMed: 8889347]

39. Streeper NM, Simons CM, Konety BR, et al. The significance of lymphovascular invasion in transurethral resection of bladder tumour and cystectomy specimens on the survival of patients with urothelial bladder cancer. BJU Int. 2009; 103(4):475–479. [PubMed: 18990174]
40. Resnick MJ, Bergey M, Magerfleisch L, et al. Longitudinal evaluation of the concordance and prognostic value of lymphovascular invasion in transurethral resection and radical cystectomy specimens. BJU Int. 2011; 107(1):46–52. [PubMed: 20880163]
41. Ioannidis JP. Why most discovered true associations are inflated. Epidemiology. 2008; 19(5):640–648. [PubMed: 18633328]
42. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Almost all articles on cancer prognostic markers report statistically significant results. Eur J Cancer. 2007; 43(17):2559–2579. [PubMed: 17981458]
43. Altman DG, Riley RD. Primer: an evidence-based approach to prognostic markers. Nat Clin Pract Oncol. 2005; 2(9):466–472. [PubMed: 16265015]
44. Tzoulaki I, Siontis KC, Ioannidis JP. Prognostic effect size of cardiovascular biomarkers in datasets from observational studies versus randomised trials: meta-epidemiology study. BMJ. 2011; 343:d6829. [PubMed: 22065657]
45. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med. 2006; 25(1):127–141. [PubMed: 16217841]
46. van Walraven C, Hart RG. Leave 'em alone - why continuous variables should be analyzed as such. Neuroepidemiology. 30(3):138–139.
47. Dawson NV, Weiss R. Dichotomizing continuous variables in statistical analysis: a practice to avoid. Med Decis Making. 2012; 32(2):225–226. [PubMed: 22457338]
48. Altman DG, Lausen B, Sauerbrei W, et al. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. J Natl Cancer Inst. 1994; 86(11):829–835. [PubMed: 8182763]
49. Dwan K, Gamble C, Williamson PR, et al. Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. PLoS One. 2013; 8(7):e66844. [PubMed: 23861749]
50. Kyzas PA, Denaxa-Kyza D, Ioannidis JP. Quality of reporting of cancer prognostic marker studies: association with reported prognostic effect. J Natl Cancer Inst. 2007; 99(3):236–243. [PubMed: 17284718]
51. Kyzas PA, Loizou KT, Ioannidis JP. Selective reporting biases in cancer prognostic factor studies. J Natl Cancer Inst. 2005; 97(14):1043–1055. [PubMed: 16030302]
52. Dickersin K. The existence of publication bias and risk factors for its occurrence. JAMA. 1990; 263(10):1385–1389. [PubMed: 2406472]
53. Young SS, Bang H. The file-drawer problem, revisited. Science. 2004; 306(5699):1133–1134.
54. Ioannidis JP. The importance of potential studies that have not existed and registration of observational data sets. JAMA. 2012; 308(6):575–576. [PubMed: 22871867]
55. Wei Y, Jiang YZ, Qian WH. Prognostic role of NLR in urinary cancers: a meta-analysis. PLoS One. 2014; 9(3):e92079. [PubMed: 24642859]
56. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Bladder Cancer 2.2015. [accessed February, 19 2016] Available from: http://www.nccn.org/professionals/physician_gls/PDF/bladder.pdf.

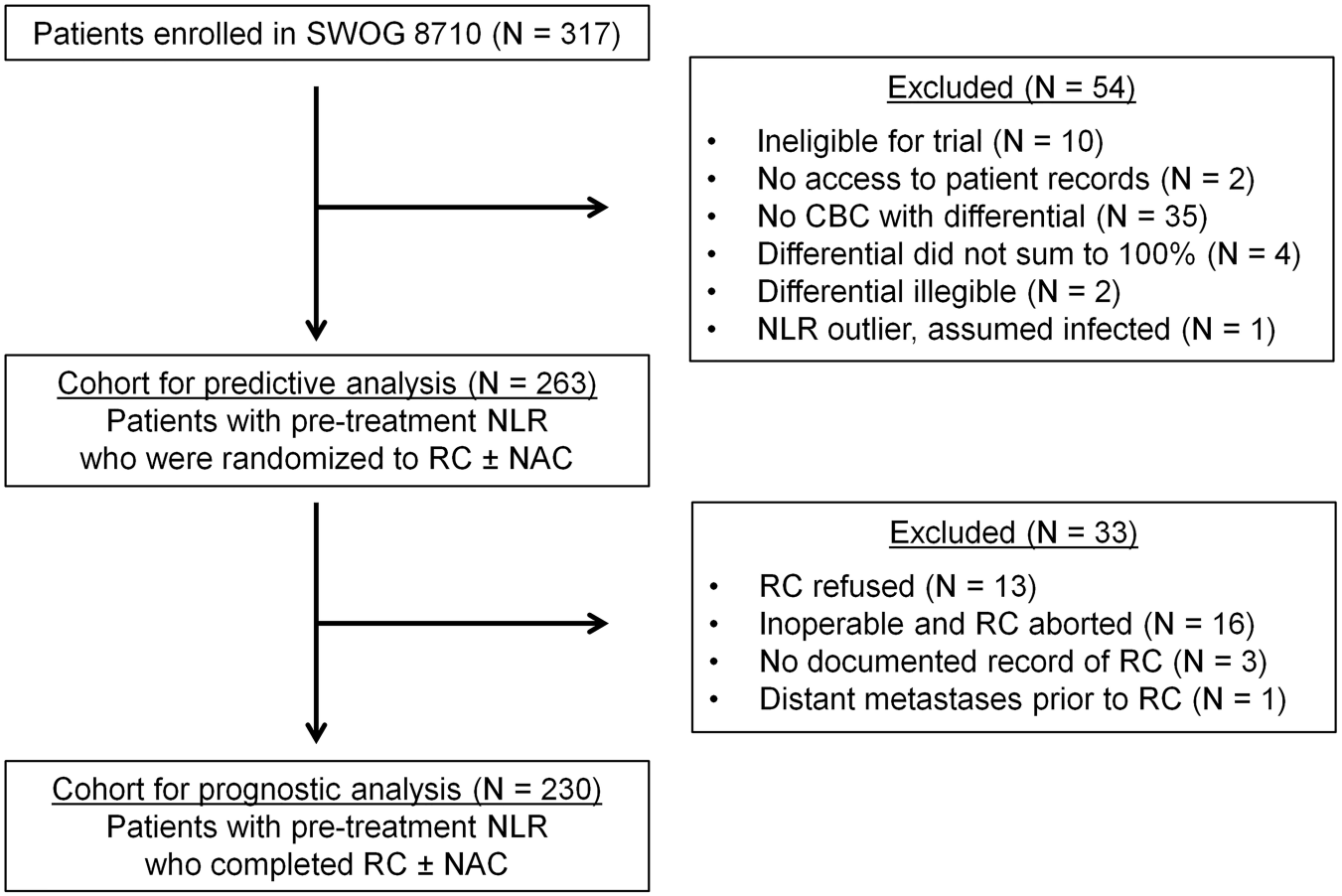

Figure 1.

Patient flow diagram.

Abbreviations: CBC, complete blood count; RC, radical cystectomy; NLR, neutrophil-to-lymphocyte ratio; NAC, neoadjuvant chemotherapy

Table 1

REMARK profile

Marker and variables	Remarks
Marker:	M = pre-treatment NLR (continuous)
Further variables:	v1 = age (continuous), v2 = gender (male vs. female), v3 = clinical T category (T2 vs. T3/T4a)[a] v4 = lymphovascular invasion (yes vs. no)[b] v5 = treatment (RC vs. NAC + RC)[c]
Outcome:	Overall survival (OS)

Patients	N	Remarks
Assessed for eligibility	317	*Disease* bladder cancer, clinical T2-T4aN0 *Patient source* multi-institutional phase III trial *Marker source:* trial flow sheets
Excluded	54	See Figure 1 for details
Predictive cohort	263	Patients with pre-treatment NLR and randomized to RC with or without NAC
Excluded	33	See Figure 1 for details
Prognostic cohort	230	Patients with pre-treatment NLR and completed RC with or without NAC

Analysis	Patients	Events	Variables	Results/remarks
A1: univariable	230	OS: 172	M, v1-v5	Prognostic analysis Table 3
A2: multivariable	230	OS: 172	M, v1-v3, v5	Prognostic analysis Table 3
A3: univariable	263	OS: 205	M, v1-v5	Predictive analysis Table 4
A5: multivariable including interaction of M and v5	263	OS: 205	M, v1-v3, v5, M*v5	Predictive analysis Table 4

[a] per American Joint Committee on Cancer staging manual, 4th edition

[b] as determined on pre-treatment biopsy or transurethral resection of bladder tumor

[c] received treatment for prognostic cohort, assigned treatment for predictive cohort

Abbreviations: NAC, neoadjuvant chemotherapy; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; RC, radical cystectomy; REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies[27,30]

Table 2

Association of neutrophil-to-lymphocyte ratio with patient and disease characteristics

	Prognostic cohort (N = 230)			Predictive cohort (N = 263)		
Characteristic	Total No. (%)	NLR Median (IQR)	*p*[a]	Total No. (%)	NLR Median (IQR)	*p*[a]
Age			0.43			0.23
< 65	129 (56.1%)	2.6 (2.0–3.7)		148 (56.3%)	2.6 (2.0–4.0)	
≥ 65	101 (43.9%)	2.8 (2.0–4.6)		115 (43.7%)	2.8 (2.1–4.8)	
Gender			0.83			0.65
Male	185 (80.4%)	2.7 (2.0–4.1)		213 (81.0%)	2.8 (2.0–4.1)	
Female	45 (19.6%)	2.6 (2.1–4.1)		50 (19.0%)	2.6 (2.0–4.2)	
T category			0.97			0.97
T2	95 (41.3%)	2.7 (2.0–4.1)		105 (39.9%)	2.8 (2.0–4.2)	
T3/T4a	135 (58.7%)	2.6 (2.0–4.1)		158 (60.1%)	2.7 (2.1–4.1)	
LVI			0.07			0.03
Negative	180 (78.3%)	2.6 (2.0–4.0)		202 (76.8%)	2.6 (2.0–4.0)	
Positive	35 (15.2%)	3.0 (2.3–4.5)		42 (16.0%)	3.1 (2.4–4.6)	
Missing	15 (6.5%)	3.3 (2.2–4.8)		19 (7.2%)	3.3 (2.2–4.9)	
Treatment[b]			0.55			0.52
RC	117 (50.9%)	2.8 (2.1–4.1)		133 (50.6%)	3.0 (2.1–4.1)	
NAC + RC	113 (49.1%)	2.6 (2.0–4.1)		130 (49.4%)	2.6 (2.0–4.2)	

[a] Kruskal-Wallis test for LVI, Wilcoxon rank-sum test for all others

[b] Received treatment for prognostic cohort, assigned treatment for predictive cohort

Abbreviations: IQR interquartile range; LVI, lymphovascular invasion; NAC, neoadjuvant chemotherapy; NLR, neutrophil-to-lymphocyte ratio; RC, radical cystectomy

Table 3

Analysis of neutrophil-to-lymphocyte ratio as a prognostic factor for overall survival

	Prognostic cohort (N = 230)					
	Univariable			Multivariable		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age (continuous)	1.06	1.04 to 1.08	<0.001	1.05	1.04 to 1.07	<0.001
Gender (male vs. female)	1.37	0.93 to 2.04	0.12	1.41	0.94 to 2.11	0.09
T category (T3/T4a vs. T2)	1.32	0.97 to 1.79	0.08	1.32	0.96 to 1.81	0.08
LVI (positive vs. negative)	1.00	0.67 to 1.51	0.99	-	-	-
Treatment (RC vs. NAC+RC)	1.32	0.98 to 1.79	0.07	1.39	1.03 to 1.88	0.03
NLR (continuous)	1.03	0.97 to 1.10	0.30	1.04	0.98 to 1.11	0.24

Abbreviations: CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; NAC, neoadjuvant chemotherapy; NLR, neutrophil-to-lymphocyte ratio; RC, radical cystectomy

Table 4

Analysis of neutrophil-to-lymphocyte ratio as a predictive factor for treatment response

	Predictive cohort (N = 263)					
	Univariable			Multivariable with interaction term		
Variable	**HR**	**95% CI**	***P***	**HR**	**CI**	***P***
Age (continuous)	1.04	1.03 to 1.06	<0.001	1.04	1.03 to 1.06	<0.001
Gender (male vs. female)	1.37	0.95 to 1.97	0.09	1.44	0.97 to 2.12	0.07
T category (T3/T4a vs. T2)	1.33	1.00 to 1.77	0.05	1.41	1.06 to 1.88	0.02
LVI (positive vs. negative)	1.10	0.76 to 1.60	0.62	-	-	-
Treatment (RC vs. NAC+RC)	1.20	0.92 to 1.59	0.18	1.35	0.83 to 2.22	0.22
NLR (continuous)	1.05	0.99 to 1.10	0.10	1.05	0.96 to 1.14	0.27
Interaction term (Treatment*NLR)	-	-	-	1.01	0.90 to 1.14	0.86

Abbreviations: CI, confidence interval; HR, hazard ratio; NAC, neoadjuvant chemotherapy; NLR, neutrophil-to-lymphocyte ratio; RC, radical cystectomy