Neutrophil counts and cancer prognosis: an umbrella review of systematic reviews and meta-analyses of observational studies

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ABSTRACT

OBJECTIVE

To evaluate the strength and validity of evidence on the association between the neutrophil to lymphocyte ratio (NLR) or tumour-associated neutrophils (TAN) and cancer prognosis.

DESIGN

Umbrella review of systematic reviews and meta-analyses of observational studies.

DATA SOURCES

Medline, EMBASE, and Cochrane Database of Systematic Reviews.

ELIGIBILITY CRITERIA

Systematic reviews or meta-analyses of observational studies evaluating the association between NLR or TAN and specific cancer outcomes related to disease progression or survival.

DATA SYNTHESIS

The available evidence was graded as strong, highly suggestive, suggestive, or weak through the application of pre-set grading criteria. For each included meta-analysis, the grading criteria considered the significance of the random effects estimate, the significance of the largest included study, the number of studies and individuals included, the heterogeneity between included studies, the 95% prediction intervals, presence of small study effects, excess significance and credibility ceilings.

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RESULTS

239 meta-analyses investigating the association between NLR or TAN and cancer outcomes were identified from 57 published studies meeting the eligibility criteria, with 81 meta-analyses from 36 studies meeting the criteria for inclusion. No meta-analyses found a hazard ratio (HR) in the opposite direction of effect (HR<1). When assessed for significance and bias related to heterogeneity and small study effects, only three (4%) associations between NLR and outcomes in gastrointestinal and nasopharyngeal cancers were supported by strong evidence.

CONCLUSION

Despite many publications exploring the association between NLR and cancer prognosis, the evidence is limited by significant heterogeneity and small study effects. There is a lack of evidence on the association between TAN and cancer prognosis, with all nine associations identified arising from the same study. Further research is required to provide strong evidence for associations between both TAN and NLR and poor cancer prognosis.

REGISTRATION

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KEYWORDS

Cancer, Neutrophils, White Blood Cells, Neutrophil to lymphocyte ratio, Tumour-associated neutrophils, Prognosis, Umbrella review

SHORT TITLE

Neutrophils and cancer prognosis: an umbrella review

KEY RESULT

When assessed for significance and bias related to heterogeneity and small study effects, only three (4%) associations between NLR and overall survival and progression-free survival in gastrointestinal and nasopharyngeal cancers were supported by strong evidence.

WHAT THIS PAPER ADDS

WHAT IS ALREADY KNOWN ON THE TOPIC

- Neutrophil counts have been linked to the progression of cancer due to their tumourigenic role in the cancer microenvironment.

- Numerous meta-analyses and individual studies have explored the association between neutrophil counts and cancer outcomes for a variety of cancer sites, leading to a large body of evidence with variable strength and validity.

- Uncertainty exists around the association between neutrophils and cancer outcomes, depending on the site, outcome and treatments considered.

WHAT THIS STUDY ADDS

- All meta-analyses included in this review indicated an association between high neutrophil counts and poor cancer prognosis.

- There is strong evidence supporting the association between the neutrophil to lymphocyte ratio and poor cancer prognosis in some respiratory and gastrointestinal cancers.

- Further research is required to strengthen the existing body of evidence, particularly for the association between tumour-associated neutrophils and cancer outcomes.

INTRODUCTION

Cancer is the second leading cause of death worldwide(1), contributing to over 8.7 million deaths globally(2). Cancer incidence is increasing(2), due in part to the epidemiological transition of increasing mortality and morbidity from chronic diseases(3). This increase highlights the importance of identifying prognostic indicators associated with cancer progression(4). C-reactive protein (CRP)(5), serum albumin(6), fibrinogen and differential leukocyte counts(7–9) are all indicators of inflammation that have been linked to cancer prognosis. In recent years, the role of neutrophils in the tumour microenvironment has been explored due to their paradoxical role in both the prevention and facilitation of tumour progression(10).

Neutrophils are the most abundant white blood cells (WBCs)(11), making up 50-70% of the body's circulating leukocytes(12). Neutrophil counts, particularly the neutrophil to lymphocyte ratio (NLR), have emerged as indicators of cancer prognosis and several systematic reviews and meta-analyses have explored their potential as a prognostic indicator in cancer(13). The NLR was first recognised for its association with systemic inflammation in the critically ill(14) and meta-analyses on the association between elevated NLR and poor prognosis have reported a wide range of effect sizes depending on the site of cancer(13). It is currently unclear how the association between NLR and poor prognosis varies depending on the site of cancer or the treatment considered. The close association between inflammation and cancer progression indicates that elevated tumour-associated neutrophils (TAN), also known as neutrophils which infiltrate tumours(15), are a potential prognostic indicator(10,16,17). Many systematic reviews and meta-analyses explore the association between neutrophils and cancer prognosis. However, the myriad of different cancer sites, stages, treatments and survival outcomes measured complicates the interpretation of this body of evidence.

Umbrella reviews allow for the analysis of broad subject areas to examine the strength and credibility of associations using the results of published systematic reviews and meta-analyses(18,19). Through umbrella review methods, the strength and consistency of the literature is assessed to evaluate bias and identify which associations are supported by strong evidence(18). Here we carried out an umbrella review of systematic reviews and meta-analyses with the aim of comprehensively evaluating the validity and strength of reported associations between NLR or TAN and cancer prognosis and identify potential biases in relevant literature.

METHODS

Literature search

Searches were conducted in Medline, Embase and the Cochrane Database (*Appendix 1*) and aimed to include all systematic reviews and meta-analyses published in English from inception up to 23 June 2017. Indicators of neutrophil counts included NLR and TAN (intratumoural neutrophils, peritumoural neutrophils and stromal neutrophils). Overall survival (OS), cancer-specific survival (CSS), progression-free survival (PFS), disease-free survival (DFS) and reoccurrence-free survival (RFS) were considered as cancer outcomes. Articles were initially screened by title and abstract to determine eligibility for full text screening and inclusion using RefWorks web-based bibliography and database manager(20).

Inclusion and exclusion criteria

Included studies were systematic reviews and meta-analyses of individual observational studies in humans with any cancer diagnosis and NLR or TAN measurements taken around the time of diagnosis. Systematic reviews which did not include a meta-analysis were excluded. Meta-analyses were excluded if they did not assess a cancer outcome in our inclusion criteria, included more than one outcome in a single analysis, or either did not specify the cancer site studied or included all sites in a single analysis (e.g. analyses of cancers grouped as "other" were excluded). Meta-analyses were also excluded if they did not provide sufficient detail for replication, such as the individual hazard ratio (HR), 95% confidence interval and total sample size of each included study. If a single study included multiple meta-analyses, all meta-analyses were individually assessed for eligibility.

When more than one meta-analysis was identified for a single association at a specific site they were assessed for concordance in the direction, magnitude and significance of their effect estimates. If the duplicate meta-analyses agreed in significance, magnitude and direction of effect, the meta-analysis with the greatest number of studies was included. If the duplicates had any disagreement, all meta-analyses were excluded for the association.

Data extraction

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Data extraction forms were generated to record information from each meta-analysis and the included individual studies. From each meta-analysis the study's first author, year of publication, outcome measure, indicator and cancer diagnosis were extracted. For each included individual study, the first author, year of publication, total population, epidemiological design, HR and 95% confidence interval were extracted. Each meta-analysis was allocated to one of six categories according to cancer site as follows: all cancers, gastrointestinal, gynaecological, hepatocellular, respiratory and urinary cancers.

Data analysis

Estimation of summary effects – The weighted inverse variance method was used to reproduce all included meta-analyses in R(21) with the "meta" package(22) and "metagen" command. For each cancer site specific indicator and outcome pair, the summary effect size and 95% confidence interval were calculated using fixed and random effects methods. The random effects model was used to compute summary effect size estimates taking into account the observed heterogeneity, since cancer is a highly heterogeneous disease(23,24). Estimates from the fixed effects model are also presented.

Assessment of reproducibility – Each included meta-analysis was reproduced to yield both fixed and random effects estimates. Reproduced random or fixed effect estimates which did not match the results reported in the original study were assessed for absolute and percent difference. Meta-analyses with a difference in HR of only 0.01 were attributed to rounding errors. Studies with larger discrepancies were investigated to determine the source of disagreement. Where there were issues with reproducibility, the calculated values of the random effects model were used to assess the evidence for the association.

Assessment of heterogeneity – Heterogeneity within each meta-analysis was assessed with Cochrane's Q test and quantified using the I^2 statistic(25). Cochrane's Q test detects a departure from homogeny in the effect sizes of individual studies when $p<0.10$(25). The I^2 statistic was also used to quantify the percentage of variation which can be attributed to heterogeneity due to common limitations associated with Cochrane's Q test(25). Values exceeding 50% or 75% are considered to show large or very large heterogeneity respectively. The 95% confidence intervals around each I^2 value were also included to evaluate the

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uncertainty around estimates of heterogeneity(26). Large measures of heterogeneity, representing true heterogeneity or inconsistency due to bias(27), were further assessed through prediction intervals and Egger's test for funnel plot asymmetry.

Estimation of prediction intervals – In order to assess the impact of heterogeneity, 95% prediction intervals were calculated for the summary random effect estimates(28). Prediction intervals account for the uncertainty caused by heterogeneity when estimating the distribution of true effect sizes in an association and yield an interval which predicts the effect size of future studies investigating the same association(28). In studies with large amounts of heterogeneity the prediction interval may be wide enough to include the null value (HR<1), suggesting that the true effect size may also include the null value.

Assessment of small study effects – Small study effects and funnel plot asymmetry were quantified through Egger's test using the command "metabias" from the R package "meta"(22) to determine if heterogeneity occurred due to chance(29). The presence of small study effects was confirmed by a low significance value in Egger's test (p<0.10) indicating bias or true heterogeneity(30). Since the Egger's test is underpowered in meta-analyses including less than ten individual studies(31), further assessment was carried out in these meta-analyses to determine if the summary effect size estimate was greater than the point estimate of the largest included study, indicating potential small study effects(32).

Evaluation of excess significance - The test for excess significance (TES)(33) was used to determine if the number of observed positive results differed significantly from the expected number of significant results. TES results can reveal reporting bias if the number of observed studies with significant results in each meta-analysis is significantly larger than the expected number using a two-tailed binomial probability test (p<0.10)(34). The expected number of significant results in each meta-analysis was calculated as the sum of the statistical power estimate, or the probability that each study will find a positive result(33,34). The estimated power for each individual study was calculated in Stata 14(35), using the "power cox" command to calculate the power of each test given its sample size, effect size and significance level(36). The estimation of power for each individual study also requires an estimation of the true effect size, so the effect size of the largest

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study was used to give the most conservative estimation of true effect. Estimates from both fixed and random effects models were included for sensitivity analysis. The "binom.test" command in R was used to assess the significance of differences in the number of observed versus expected significant studies through an exact binomial test(37).

Credibility ceilings - Credibility ceilings were utilised for sensitivity analysis and to test the methodological limitations of using observational studies to calculate combined effect estimates(38,39). Credibility ceiling calculations inflate the variance of each study included in a meta-analysis to account for the probability *c* that the true effect size is in the opposite direction of effect of the observed point estimate(39). Inflated variances were calculated in Stata 14(35,38). The summary effect size and heterogeneity of each meta-analysis was assessed with ceiling values ranging from 5 to 20%.

Grading the evidence - Associations between neutrophil counts and cancer prognosis were categorised into strong, highly suggestive, suggestive, or weak through assessment of the strength and validity of the evidence for each meta-analysis, according to pre-defined criteria outlined in *Supplementary Figure 1*(40,41). In order for an association to be considered strong, the meta-analysis must yield a p-value of less than 10^{-6} in the random effects model(42), include more than 1,000 individuals, show significance at p<0.05 in the largest included study, find no heterogeneity (p>0.10) through the Q test, detect less than 50% variance due to heterogeneity through the I^2 statistic, yield a prediction interval excluding the null value (HR=1), display no evidence of small study effects or excess significance, and the association must maintain significance at p<0.05 with the application of a credibility ceiling of 10%. The number of studies in each meta-analysis was also included as eligibility criterion for strong evidence since a sample size greater than three is required for reliable assessment of heterogeneity and small study effects(25,31,43).

Quality assessment - Studies with meta-analyses categorised as providing either highly suggestive or strong evidence underwent quality assessment through AMSTAR 2, a tool for assessing the methodological quality of systematic reviews (44). Studies were assessed by two reviewers (MAC and MC) and consensus reached on any disagreements in quality.

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Statistical analyses were carried out R(21), including the packages "meta"(22) version 4.8-4 and "ggplot2"(45) version 2.2.1, and Stata 14(35).

Patient involvement

No patients were involved in development of the study design nor were they asked to advise on interpretation. No ethical approval was required for this review since it relied entirely on anonymised, published data.

RESULTS

Characteristics of included meta-analyses

The 57 published articles meeting the criteria for inclusion contained 239 meta-analyses (*Appendix 2*). The 81 meta-analyses meeting the eligibility criteria arose from 36 of these articles, published between 2014 and 2017 (*Figure 1*)(46–81). These meta-analyses included individual studies which presented NLR or TAN categorically as either high or low and investigated 40 associations for 27 different cancer diagnoses, including nine subtypes for treatment and four for cancer stage (*Supplementary Table 1*). The meta-analyses were grouped as all cancers (n=8), gynaecological (n=6), gastrointestinal (n=24), hepatocellular (n=11), respiratory (n=10) and urinary cancers (n=22) (*Figures 2A and 2B*). Included meta-analyses summarised effect size estimates from 693 individual studies, with OS as the most frequently assessed outcome (n=41). In 51 meta-analyses (63%) total sample size exceeded 1,000 individuals and each meta-analysis had a median of five studies. However, 57 meta-analyses (70%) included less than ten studies and 17 (21%) included only two studies. The characteristics of included meta-analyses are summarised in *Supplementary Table 2*.

A total of 74 duplicate meta-analyses were excluded. Nineteen meta-analyses assessing six associations were excluded due to disagreement in significance between duplicates. A further 55 duplicate meta-analyses that agreed in significance, magnitude and direction of effect were excluded for 31 associations and only the meta-analysis with the largest number of studies was included for each association (*Appendix 3*).
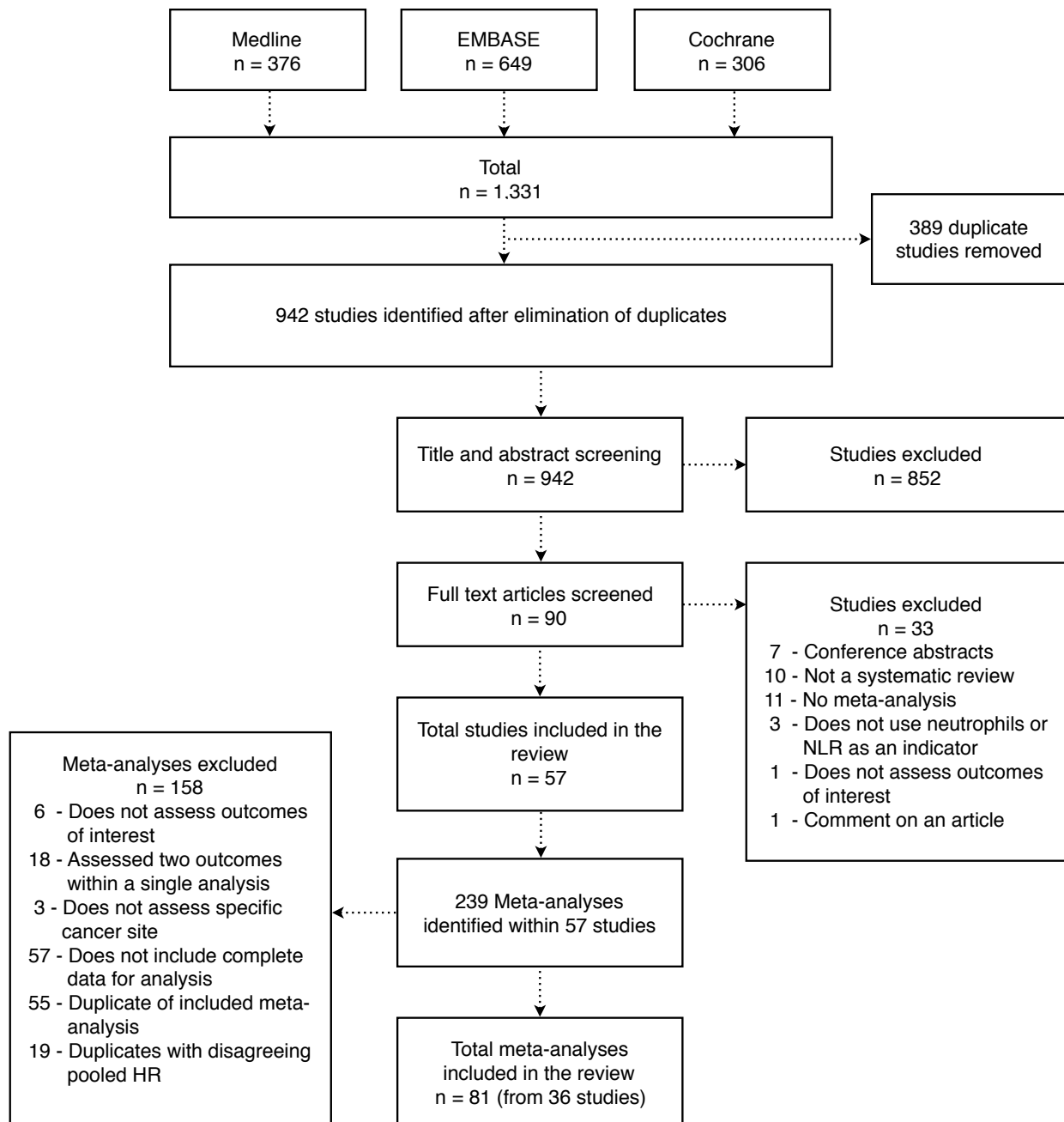
**Figure 1** - Flowchart of study and meta-analysis selection.

Summary effect size

All estimated summary effect sizes for both fixed and random effects estimates are shown in *Supplementary Figures 2-82*.

Using a threshold of p<0.05 for statistical significance, 72 of the 81 meta-analyses (89%) were significant with random effects. At a more stringent threshold of $p<10^{-6}$, the number of statistically significant meta-analyses for random effects dropped to 38 (47%) (*Supplementary Table 2*). The 38 meta-analyses with significance at $p<10^{-6}$ assessed both NLR and intratumoural neutrophils as indicators of poor prognosis in 19 cancer sites. Thirty-five of these 38 meta-analyses assessed NLR as an indicator of poor prognosis in gynaecologic, gastrointestinal, hepatocellular, respiratory, urinary, and all cancers. Intratumoural neutrophils were assessed as an indicator of poor prognosis in three of the 38 meta-analyses (8%), including urinary and all cancers.

In 20 meta-analyses (25%), the largest study included was not statistically significant at p<0.05. However, 16 of these meta-analyses still had a statistically significant summary random effects estimate. In two meta-analyses, the largest study included had an effect size in the opposite direction to the random effects estimate (HR<1). The largest study effect sizes tended to be more conservative estimates of effect than the random effect estimates, with 58 meta-analyses (72%) yielding a HR which was greater than the point estimate of the largest included study. However, there was correlation between the log(HR) of the summary random effects and the largest study for each meta-analysis, indicating consistency in the results (*Figure 2C*).

In order to determine the impact of study size on the magnitude of the summary effect size, random effects estimates were plotted against inverse variance for each meta-analysis. When compared to meta-analyses with large variances those with smaller variances studies produced more conservative estimates, displaying a smaller range of HR estimates and a slight tendency toward a null value (HR=1). Meta-analyses with large variance displayed a wide range of random effects HR and included an increased number of HR estimates greater than two (*Figure 2D*).
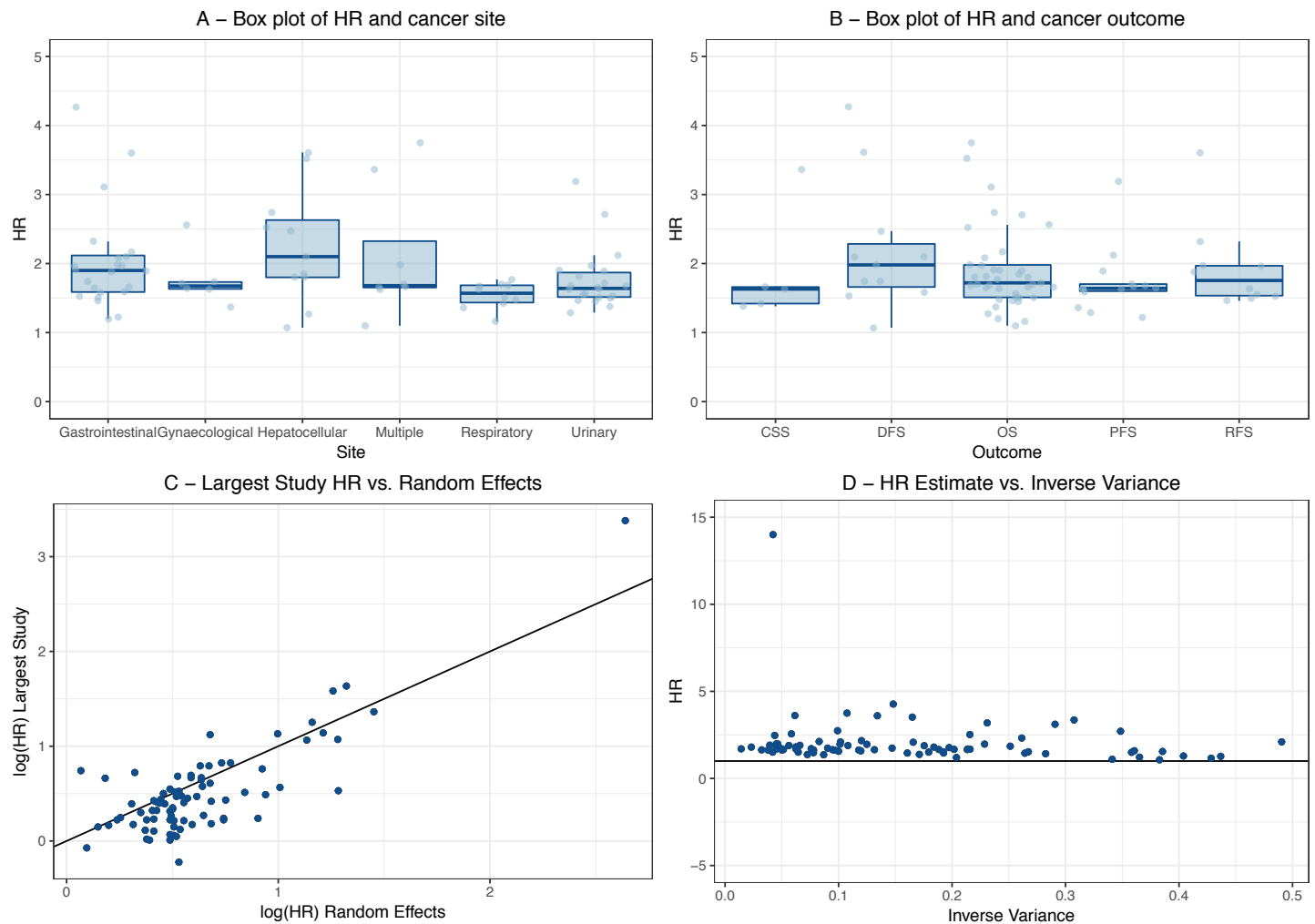
Figure 2 - Assessment of consistency in meta-analyses. **A, B** - Box plots of random effects HR estimates for each meta-analysis by cancer site and outcome. The Y-axis labelled "HR" details the effect size for each meta-analysis describing an association between NLR or TAN and cancer prognosis for each site grouping. The X-axis labelled "Site" in Figure 2 A represents each site group meta-analyses have been sorted into. The multiple subgroup contains all cancers, defined as a grouping of cancer diagnosis unrelated to site, stage or treatment. The X-axis labelled "Outcome" in Figure 2 B represents the prognostic outcome assessed in each meta-analysis. The outlier of HR=14 for NLR and OS in rectal cancer has been excluded from these figures. **C** - Log(HR) of largest study versus log(HR) of random effects estimates for each meta-analysis. The Y-axis labelled "log(HR) Largest Study" represents the log of the HR of the largest study included in each analysis. The X-axis labelled "log(HR) Random Effects" represents the log of the HR of the random effects estimate calculated in each meta-analysis. **D** - Random effects estimates versus inverse variance. The Y-axis labelled "Random Effects Estimates Hazard Ratio" represents the HR of random effects estimate for each meta-analysis. The X-axis labelled "Inverse Variance" represents the inverse of the variance ($\frac{1}{Variance}$) for each meta-analysis.

Reproducibility

In 21 of the 81 included meta-analyses, the HR was reproduced imperfectly. Nine of the 21 imperfectly reproduced meta-analyses were within 0.01 of the reported HR, and the differences were attributed to rounding errors. The remaining 12 meta-analyses were within 10% of the reported HR, with six meta-analyses (50%) reporting an HR with less than a 2% difference from the calculated HR (*Appendix 4*).

Heterogeneity between studies

Cochrane's Q test was significant at p<0.10 in 40 of the 81 included meta-analyses (50%). In 20 meta-analyses (25%), the I^2 statistic was greater than 75%, indicating a high level of variability between studies due to heterogeneity. An additional 19 meta-analysis yielded I^2 values between 50% and 75% *(Appendix 5)*. In 30 meta-analyses (37%), the I^2 statistic was less than 50%, but only six (9%) yielded a I^2 statistic with a 95% CI that did not include 50%. The confidence interval of the I^2 statistic was not used as criteria for grading evidence, since large fluctuations in I^2 occur when meta-analyses include less than 15 studies(43).

Prediction intervals were not calculated for 17 (21%) meta-analyses which had included only two individual studies. The prediction intervals of 46 meta-analyses (57%) included the null value of HR=1. Of 64 meta-analyses (79%) including at least three individual studies, 18 had prediction intervals which excluded the null value (HR=1). The 12 meta-analyses (15%) including exactly three individual studies yielded very wide prediction intervals, all of which included the null value of HR=1.

Small study effects

Sixty-five (80%) of the 81 included meta-analyses were judged to have evidence of small study effects *(Appendix 5).* Sixty-four meta-analyses included three or more studies and were eligible for further assessment through Egger's test for asymmetry(22). Thirty-seven (58%) of these 64 meta-analyses yielded significant p-values (p<0.10). However only 24 (30%) meta-analyses included ten or more individual studies, a cut off required to give Egger's sufficient power(31,32). Twenty-one (88%) of the 24 meta-analyses including 10 of more individual studies yielded a significant Egger's test (79%), indicating funnel plot asymmetry(30).

Forty meta-analyses analysed between three and nine studies and Egger's test was significant (p<0.1) in 16 of these (40%). In 11 of the remaining 24 meta-analyses (46%), the summary effects estimate from the random effects model was larger than the point estimate of the largest study and they were considered to have evidence of small study effects.

Excess significance

Seventeen meta-analyses (21%) showed evidence of excess significance bias according to the TES when the effect size of the largest included study was utilised as an estimate of true effect size (*Appendix 6*). When the fixed summary effect sizes were utilised as an estimation of true effect size, fifteen meta-analyses (16%) showed evidence of excess significance. No meta-analyses showed evidence when the random summary effect sizes were used.

Credibility ceilings

The summary effect size estimates and significance of each meta-analysis matched that of the random effects model at a credibility ceiling of 0%, with 72 of the 81 meta-analyses being significant at p<0.05 (89%) (*Table 1*). At a ceiling of 5%, 65 maintained significance (71%) and 50 (61%), 37 (46%), and 22 (27%) maintained significance at ceilings of 10%, 15%, and 20%, respectively. All of the meta-analyses remained consistent in direction of effect (HR>1) up to a ceiling of 15% and 2 (2%) yielded an effect estimate in the opposite direction (HR<1) with a ceiling of 20%. The I^2 value of each meta-analysis decreased with each increase in ceiling value.

Table 1 – Credibility ceiling results

All meta-analyses	Ceiling 0%	Ceiling 5%	Ceiling 10%	Ceiling 15%	Ceiling 20%
X = 1, n (%)					
Number of meta-analyses with effect size > 1.0	81 (100)	81 (100)	81 (100)	81 (100)	79 (98)
Number of meta-analyses with nominal statistical significance	72 (89)	65 (71)	50 (62)	37 (46)	22 (27)

Grading the evidence

Each included meta-analysis was evaluated to determine if the associations they explored was supported by strong, highly suggestive, suggestive or weak. In nine meta-analyses (11%), no significance was detected at a threshold of p<0.05. The remaining seventy-two meta-analyses (89%) provided at least weak evidence of an association (p<0.05) (*Table 2*).

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Table 2 - Grading of evidence

Evidence	Criteria	Increased risk of poor prognosis
Strong (n=4)	p<10-6* with random effects; >1000 individuals included; >3 studies included; largest study significant at p<0.05; Q test significant at p<0.10; I2 less than 50%, prediction interval does not include null value (HR=1); small study effects not detected; excess significance not detected	CLM-OS (NLR), CLM SR-OS (NLR), Nasopharyngeal-PFS (NLR)
Highly Suggestive (n=22)	p<10-6* with random effects; >1000 individuals included; largest study significant at p<0.05	All cancers-DFS (NLR), All cancers-PFS (NLR), Breast-DFS (NLR), Breast-OS (NLR), Cervical-OS (NLR), Gynaecologic-OS (NLR), Gastric-OS (NLR), Pancreatic-OS (NLR), Colorectal-OS (NLR), CLM-RFS (NLR), HCC-OS (NLR), HCC-DFS (NLR), HCC transplant-OS (NLR), HCC transplant-DFS (NLR), Biliary Tract-OS (NLR), Lung-OS (NLR), Nasopharyngeal-OS (NLR), NSCLC-OS (NLR), Upper urinary and bladder-OS (NLR), Bladder-OS (NLR), CR prostate-OS (NLR), Renal-OS (NLR), Advanced renal-OS (NLR)
Suggestive (n=17)	p<10-4* with random effects; >1000 individuals included	All cancers-OS (NLR), All cancers-OS (IN), Cervical-PFS (NLR), Ovarian-PFS (NLR), Gastric SR-OS (NLR), Colorectal-DFS (NLR), Colorectal-PFS (NLR), CLM SR-RFS (NLR), Colorectal SBR-DFS (NLR), HCC RFA-DFS (NLR), Nasopharyngeal-CSS (NLR), NSCLC-PFS (NLR), Urinary-OS (NLR), Upper urinary and bladder-CSS (NLR), Upper urinary and bladder-PFS (NLR), CR prostate-PFS (NLR), Renal-PFS (NLR),
Weak (n=29)	p<0.05* with random effects	All cancers-CSS (IN), Soft Tissue Sarcoma-OS (NLR), Gastric-DFS (NLR), Gastric-PFS (NLR), Biliary Tract-RFS (NLR), Pancreatic-CSS (NLR), Rectal-OS (NLR), Rectal-DFS (NLR), Rectal-RFS (NLR), CLM NS-OS (NLR), CLM NS-RFS (NLR), Colorectal PC-DFS (NLR), HCC and ICC-OS (IN), HCC mixed Tx-OS (NLR), HCC SR-OS (NLR), HCC RFA-OS (NLR), HCC TACE-OS (NLR), Lung-PFS (NLR), MPM-OS (NLR), NHC-OS (IN), Upper urinary-RFS (NLR), Bladder-RFS (NLR), Prostate-PFS (NLR), Urothelial carcinoma-OS (NLR), Urothelial carcinoma-RFS (NLR), Renal-OS (IN), Renal-RFS (NLR), Advanced renal-PFS (NLR), Localised renal-RFS (NLR)
Not Significant (n=9)	Not significant at p<0.05* with random effects	All cancers-OS (PN), All cancers-OS (SN), Gastric-OS (IN), Oesophageal-PFS (NLR), HCC SR-DFS (NLR), NSCLC-OS (IN), Localised prostate-OS (NLR), Renal-CSS (NLR), Localised renal-OS (NLR)

(NLR) Neutrophil to lymphocyte ratio, (IN) Intratumoural neutrophils, (PN) Peritumoural neutrophils, (SN) Stromal neutrophils, (OS) Overall survival, (DFS) Disease-free survival, (PFS) Progression-free survival, (RFS) Reoccurrence-free survival, (CSS) Cancer specific survival, (PC) Palliative chemotherapy, (SR) Surgical resection, (CLM) Colorectal liver metastasis, (NS) Non-surgical, (HCC) Hepatocellular carcinoma, (ICC) Intrahepatic cholangiocarcinoma, (RFA) Radiofrequency ablation, (TACE) Trans-arterial chemoembolization, (MPM) Malignant pleural mesothelioma, (NSCLC) Non-small cell lung cancer, (NHC), Neck and head cancer, (CR) Castration resistant. *p-values of random effects model. All cancers are defined as a grouping of cancer diagnosis unrelated to site, stage or treatment. No meta-analyses presented evidence of elevated neutrophils and improved cancer prognosis (HR<1).

Strong evidence was evident in three meta-analyses (4%) for associations between NLR and poor cancer prognosis. These associations included colorectal liver metastasis (CLM) (n=2) and nasopharyngeal cancer (n=1), with OS being the most frequently assessed outcome (n=2, 67%). Increased NLR was associated with reduced PFS in nasopharyngeal cancer and reduced OS in CLM, with and without surgical resection.

Twenty-three meta-analyses (28%) presented associations supported by highly suggestive evidence, including associations between increased NLR and poor prognosis in all cancers, breast, cervical, gynaecologic, gastric, biliary, pancreatic, colorectal, CLM, hepatocellular, lung, nasopharyngeal, non-small cell lung cancer (NSCLC), bladder, upper urinary and bladder, castration resistant prostate, renal and advanced renal cancer. The most commonly assessed outcome for highly suggestive associations was OS (n=17), followed by DFS (n=4), PFS (n=1) and RFS (n=1).

Seventeen meta-analyses provided suggestive evidence (21%) for an association between high NLR (n=16) or intratumoural neutrophils (n=1) and poor cancer prognosis, and twenty-nine meta-analyses provided weak evidence (36%) for an association between high NLR or intratumoural neutrophils and poor cancer prognosis. The association between intratumoural neutrophils and overall survival in all cancers was classified as suggestive, but there was weak evidence supporting associations with peritumoural neutrophils or stromal neutrophils. Details of the grading for each meta-analysis are included in *Appendix 7*.

Quality assessment

The 26 meta-analyses categorised as providing either high suggestive (n=23) or strong evidence (n=3) arose from 20 individually published studies. Of these studies, three were ranked as critically low quality (15%), four as low quality (20%) and thirteen as moderate quality (65%). None of the assessed studies were ranked as high quality. The two studies which yielded the three meta-analyses categorised as providing strong evidence were both ranked as moderate quality (*Appendix 8*).

DISCUSSION

A total of 81 associations between elevated NLR or TAN and cancer outcomes in 27 cancer sites were reviewed to assess the strength of the evidence supporting them. Three associations were supported by strong evidence and the evidence supporting associations including NLR was stronger than those including TAN. Although the studies included showed consistency in direction of effect, we detected poor reproducibility of findings and evidence of heterogeneity.

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Risk of elevated neutrophil to lymphocyte ratio

Previous studies have documented the prognostic role of neutrophils, particularly the NLR, and their link with poor outcomes for many cancer sites(13). The findings of this umbrella review support the association between elevated NLR and poor cancer prognosis, with 89% of the included meta-analyses providing a significant HR through random effects estimates (p<0.05) and no meta-analyses indicating a HR in the opposite direction of effect (HR<1).

All associations supported by strong evidence assessed elevated NLR in gastrointestinal and nasopharyngeal cancers. Nasopharyngeal cancers have also been linked to inflammation caused by smoking(82), presenting a potential confounder for the association. CLM represents a unique case where metastasis has already occurred and may present a link between elevated NLR and poor prognosis in metastasised cancers. Future research should assess the association between the NLR and prognosis in gastrointestinal cancers, with and without metastasis, and in respiratory cancers, by smoking status, to ensure these do not confound associations.

Risk of intratumoural neutrophils

Although previous studies suggest a link between intratumoural neutrophils and the progression of cancer in the tumour microenvironment(15,17,83), the evidence for the association between intratumoural neutrophils and OS in all cancers was only classified as suggestive, with all other associations including TAN classified as weak or not significant. The significance of these associations may have been limited by small sample size and it is important to note that all meta-analyses considering TAN arose from a single study and may be to the same limitations.

It is important to note that all meta-analyses on associations concerning TAN arose from the same publication by Shen at al. 2014(48) and may be subject to the same limitations. The association between intratumoural neutrophils and cancer outcomes holds potential for a strong association due to the large effect size observed in this study and the plausibility of the biological mechanism behind the relationship(10). However, a recently

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published individual study on this association found that high levels of TAN had a protective effect in cancer(84), indicating that additional research is required to clarify the association.

Risk of peritumoural and stromal neutrophils

Peritumoural and stromal neutrophils are hypothesised to play a role in creating a tumourigenic microenvironment through promotion of cellular proliferation and metastasis(17,85,86). However, the association for both peritumoural and stromal neutrophils and OS in all cancers was not supported by statistically significant evidence (p>0.05). Additional research is required to determine if any statistically significant association exists.

Strengths

A key strength of this review comes from the use of umbrella review methodology, which only includes meta-analyses as evidence for quantitative data analyses(18). The use of meta-analyses ensures that effect size estimates are a balanced representation of the available evidence, as demonstrated by the sensitivity analysis of the association between elevated NLR and OS in rectal cancer from Dong et al. 2016(59) (*Appendix 9*). When an extreme outlier detected in this meta-analysis was removed from the analysis, the random effects estimate was not considerably altered due to the small weighting given to studies with large variances.

The TES did not detect a greater number of positive studies than expected in any meta-analyses, indicating that individual studies were not selectively included based on their significance(33). However, it is important to note that the TES is limited in power since biases that increase reporting of positive results will also increase the reported effect size, leading to a greater number of expected positive studies(33).

Although there are considerable differences between the included meta-analyses in terms of cancer site, stage and treatment, all the HR estimates reported in these meta-analyses were in the same direction of effect. This finding suggests consistency in the relationship between neutrophil indicators and poor outcomes across a wide spectrum of cancer diagnoses.

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Limitations

Only 34% of the identified meta-analyses were eligible for inclusion and 24% of the identified meta-analyses were excluded because they did not include sufficient data to be reproduced. Furthermore, we were unable to perfectly reproduce 26% of the included studies, highlighting issues with transparency and reproducibility of findings in epidemiologic research(87). Umbrella reviews also fail to include evidence published in individual studies after the last published meta-analyses. However, all the included meta-analyses in our study were recently published, with the oldest published in 2014, so the exclusion of individual studies in our case may be minimal. This exclusion of individual studies is of greatest concern in the association between TAN and cancer outcomes, due to a single meta-analysis being available.

The findings of this review are reliant on the quality of the included meta-analyses. This methodological limitation is of concern since 35% of the studies which yielded meta-analyses with highly suggestive or strong evidence were ranked as low or critically low quality by AMSTAR 2. There is also some concern over consistency, since meta-analyses aggregated the results of individual studies which categorised patients' NLR or TAN levels as high or low using different cut off values (*Appendix 10*). Due to heterogeneity in these values, it is not possible to establish a dose-response relationship between neutrophil counts and cancer prognosis. Assessment of the individual studies included in each meta-analysis was outside of the scope of this review.

The assessment of heterogeneity using Cochrane's Q test and the I^2 statistic is problematic with varying study size. Cochrane's Q test has weak power when there are few studies and excess power in detecting heterogeneity when studies are numerous, both of which are complications in this review(25).

Causal association

This umbrella review does not address causality directly and cannot determine whether the association between neutrophils and poor prognosis in cancer is causal or due to confounding or reverse causality(88,89).

Although previous studies have highlighted the paradoxical role of neutrophils in both tumour progression and suppression(17), this review suggests that the overall effect of high neutrophil counts is tumourigenic.

This review supports the relationship between elevated NLR and poor outcomes in cancer in terms of effect size and consistency of findings. We cannot address temporality as the studies included measured biomarkers before the initiation of treatment but generally after diagnosis. However, the biological mechanisms behind inflammation and cancer progression suggest temporality, as elevated NLR and TAN are proposed to promote increased cell proliferation(17), angiogenesis(90) and risk of metastasis(17,91) as contributors to poor prognosis.

Clinical significance and future research

Future research should focus on strengthening the current evidence base for specific cancers which displayed suggestive and highly suggestive associations, addressing heterogeneity and, importantly, reverse causality. Unveiling a causal association between neutrophils and cancer outcomes could lead to cancer treatments which involve neutrophils as a therapeutic target and validate the NLR as a prognostic indicator in cancer. A casual association between neutrophils and poor prognosis could give further insight into experimental therapy which lowers neutrophils counts in the body to improve outcomes in cancer(85,92,93).

CONCLUSION

The quantitative evidence presented suggests an association between elevated NLR and poor outcomes in cancer patients across a wide spectrum of diagnoses, stages of disease and courses of treatment. The evidence is strongest for associations between NLR and OS in CLM and nasopharyngeal cancer. The association between TAN and poor prognosis in cancer patients is limited by significant heterogeneity, small study effects and the existence of a single meta-analysis on the association. Further research is required to remove the limitations of the existing evidence.

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Acknowledgements

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Availability of data and materials

Data and computational code used for processing and analysis are available at https://github.com/megcupp/Neutrophils-and-Cancer-Prognosis.

All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Authors' contributions

AJBT conceived the study. MAC, AJBT and IT designed the study. MAC collected data and performed the analysis with input from MC, IT, ABJT and EE. MAC and AJBT wrote the manuscript with contributions from all authors. All authors critically revised and approved the manuscript. AJBT and MAC are the guarantors.

Transparency declaration

The guarantors (AJBT and MAC) affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval and consent to participate

Not required.

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