Prognostic role of neutrophil-lymphocyte ratio in localized and metastatic renal cell carcinoma. A population-based cohort study.

Research year rapport

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Preface

This research year report is based on a study carried out during my one year of research at the Department of Clinical Epidemiology, Aarhus University Hospital.

It has indeed been such a lovely year! First of all, I would like to express my sincerest gratitude to my supervisors Mette Nørgaard and Frede Donskov, for being enthusiastic about the project and for guidance throughout the year. Mette, thank you for always being optimistic, guiding me on the right track and for sharing your epidemiologic knowledge and Frede, thank you for inviting me to MDT at Skejby and RCC meetings in CPH and for guiding me in a clinical perspective. I must also express my thanks to Lars Pedersen and Buket Öztürk at KEA for their help with data management and statistics, and a special thanks to professor Zuo-Feng Zang at UCLA Fielding School of Public Health for having me for a two-month research stay at Department of Epidemiology, UCLA Fielding School of Public Health, Los Angeles, USA.

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Abbreviations

NLR	Neutrophil-lymphocyte ratio
RCC	Renal cell carcinoma
mRCC	Metastatic renal cell carcinoma
CPR	Civil personal registration number
CRS	Danish Civil Registration System
DNPR	Danish National Patient Registry
DPR	Danish National Pathology Registry
DCR	Danish Cancer Registry
LABKA	Clinical Laboratory Information System Research Database
HR	Hazard Ratio
CI	Confidence interval
CCI	Charlson Comorbidity Index
LLN	Lower limit of normal (values)
UPN	Upper limit of normal (values)

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Abstract

Background

Inflammation has an impact in tumor development. Using data from Danish registries, we examined the prognostic role of neutrophil-lymphocyte ratio (NLR) among renal cell carcinoma (RCC) patients.

Methods

From 1999-2015, we included patients from the North and Central Denmark regions with a RCC diagnosis and a NLR measurement within 30 days prior to diagnosis. Patients were categorized according to NLR levels (≤3.0 and >3.0) and were followed until death, emigration or up to 5 years. We estimated survival probabilities using Kaplan-Meier curves and hazard ratios (HR) with 95% confidence intervals (95%CI) using Cox regression (adjusted for predefined, potential confounders).

Results

We included 979 patients. The 5-year survival rate was 35.2% in patients with NLR >3.0 and 69.4% in patients with NLR ≤3.0. Unadjusted HR was 3.1 (95%CI, 2.5;3.9) and when adjusted for all covariates, HR was 1.8 (95%CI, 1.4;2.2).

Among 76 patients with RCC recurrence, 59 had NLR measured at time of recurrence. Elevated NLR was associated with poorer prognosis (adjusted HR=2.1 (95%CI, 0.8;5.7)).

Conclusion

Elevated NLR at time of RCC diagnosis and at RCC recurrence is associated with a poorer prognosis.

Dansk resume

Baggrund

Inflammation har en betydning i udviklingen af kræft. Ved brug af danske registre undersøgte vi den prognostiske betydning af neutrofil-lymfocyt ratio (NLR) hos patienter med renalcellekarcinom (RCC).

Metode

Fra 1999-2015 inkluderede vi patienter fra region Nord- og Midtjylland med en RCC diagnose og NLR-måling indenfor 30 dage før diagnosedatoen. Patienter blev kategoriseret ift. NLR-niveau (≤3,0 og >3,0) og fulgt indtil død, emigration eller op til 5 år. Vi lavede overlevelsesanalyser vha. Kaplan-Meier kurver og udregnede hazard ratioer (HR) med 95%-konfidensinterval (95% CI) vha. Cox regression (justeret for prædefineret, potentielle confounders).

Resultater

Vi inkluderede 979 patienter. Patienter med NLR>3,0 havde en 5-års overlevelse på 35,2%, sammenlignet med 69,4% hos patienter med NLR<3,0. Ujusteret HR var 3,1 (95%CI, 2,5-3,9) og når justeret for alle kovarianter, var HR på 1,8 (95%CI, 1,4-2,2).
Blandt 76 patienter med RCC recidiv havde 59 en NLR-måling på recidivtidspunktet. Forhøjet NLR var associeret med dårligere prognose (justeret HR=2,1 (95%CI, 0,8-5,7)).

Konklusion

Forhøjet NLR på tidspunktet for RCC-diagnose og RCC-recidiv er associeret med en dårligere prognose.

MANUSCRIPT

Introduction

Kidney cancer is among the 10 most common cancers in both men and women worldwide with renal cell carcinoma (RCC) being the most common histologic subtype accounting for approximately 90% of primary kidney tumors[1]. In Denmark, about 900 new cases are diagnosed per year with increasing incidences over the last 10 years and despite progress in survival rates, kidney cancer has relatively high mortality with a five-year survival rate of 62% for men and women[2,3]. Several tumor and patient-related factors affect RCC prognosis. The extent of disease, usually measured using the TNM staging system, Fuhrman grade and patients' performance status are combined in the UCLA integrated prognostic model for localized disease[4] and T stage, N stage, tumor size, Fuhrman grade and presence of tumor necrosis are combined in Leibovich prognostic index, also used in localized RCC[5]. Concerning metastatic RCC (mRCC), the most widely used prognostic model is the MSKCC/Motzer score from 1999[6]. It includes five indicators: performance status (Karnofsky score <80% (yes/no)), serum calcium level >2.5 mmol/L (yes/no), serum LDH level >1.5xULN (yes/no), hemoglobin level <LLN (yes/no), and time from diagnosis to start of systemic treatment <1 year (yes/no). Other well-established prognostic models for mRCC includes the Heng score linking platelet and neutrophil count with shorter survival in patients treated with VEGF–targeted therapy[7]. Incorporation of additional potential prognostic biomarkers might improve the predictive accuracy of these risk-assessment tools.

Inflammation is known to have impact on several steps in tumor development[8,9] and different inflammatory markers, such as neutrophil-lymphocyte ratio (NLR), have been proposed as prognostic factors in various malignancies including prostate[10], gastric[11], lung[12], hepatocellular[13], colorectal[14] and cervical cancer[15]. Likewise, NLR has been indicated as a prognostic marker in both localized and metastatic RCC[16–21]. An elevated NLR indicates an increased neutrophil inflammatory reaction as well as a reduced lymphocyte mediated antitumor immune response, which may contribute to more aggressive tumor biology, progression and poor prognosis[22]. This is supported by previous findings associating elevated NLR with more aggressive histologic subtypes, higher Fuhrman grade and higher T stage at time of nephrectomy[23,24]. Although a meta-analysis including 15 cohorts containing 3.357 patients found elevated NLR to be associated with a 1.8 fold increase in overall mortality[16], results regarding NLR and recurrence-free survival are conflicting[25–27]. Since up to 30% of patients with localized RCC later will experience recurrence with a 5-year survival rate of less

than 10%[28], identify better prediction of patients who, at time of initial diagnosis, are most likely to suffer from disease recurrence will be of clinical importance.

Since NLR is easily measured and cheap, it could serve as a cost-effective prognostic marker. Large population-based data assessing both the role of NLR as a prognostic factor at time of RCC diagnosis and at time of RCC recurrence and additionally NLR as an indicator of RCC recurrence risk are sparse. Therefore, using data from Danish medical registries, we conducted a large population-based cohort study including patients with localized or metastatic RCC to examine the prognostic value of NLR measured at time of initial diagnosis and at time of RCC recurrence. We hypothesized that patients with elevated NLR at RCC diagnosis and at RCC recurrence have poorer prognosis compared with patients with low NLR and that elevated NLR is a predictor of RCC recurrence.

Methods

Study population and data collection

We performed a population-based cohort study including all patients above 18 years of age diagnosed with localized or metastatic RCC in 1999-2015 in the North and Central Denmark Regions (~1.9 million residents, representing one-third of Denmark's population). The Danish health care system provides tax-supported health care services to all residents, guaranteeing free access to all general practitioners and hospitals. Furthermore, the Danish Civil Registration System (CRS) assigns a unique ten-digit civil person registration (CPR) number to all inhabitants in Denmark at birth or upon immigration. This number is used in all Danish registries and allows unambiguous linkage of information from multiple data sources[29]. We linked data from the CRS to the Danish National Pathology Registry (DPR), the Danish Cancer Registry (DCR), the Danish National Patient Registry (DNPR) and the Clinical Laboratory Information System Research Database (LABKA).

The DPR, fully established in 1997, has registered detailed descriptions of all pathological specimens using Danish SNOMED codes[30]. We identified our study population through SNOMED codes (see supplementary) with date of diagnosis being the date of pathology requisition. DPR further provided us with information on specimen morphology, tumor size, Leibovich score, Fuhrman grade and presence of sarcomatoid differentiation or tumor necrosis. Reporting to the DPR is based on national guidelines for uniform registration and since reporting is obligatory, the proportion of missing data should be extremely low[31,32]. We obtained information regarding tumor stage from

DCR. It contains records of all incidences of malignant neoplasms in the Danish population since 1943 and reporting to DCR has been mandatory since 1987[33]. DNPR, a nationwide register established in 1977, contains information on inpatient admissions to Danish somatic hospitals since 1977 and outpatient visits since 1995. From DNPR, we retrieved information regarding date of hospital arrival and departure, diagnosis types (ICD-10 codes) and type of operation and treatment[34]. LABKA, initiated in 1990, contains all results of analyzed blood samples drawn from patients from hospital departments and medical practices in the Northern and Central Regions of Denmark, with full coverage since 1997 in the North Region and 2000 in the Central Region. Depending on the year of analysis, data are either coded according to the international NPU (Nomenclature, Properties and Units) coding system or specific analysis numbers[35]. From this database, we obtained information regarding exposure levels and biochemical values included as covariates. The nationwide CRS database, established in 1968, is updated daily and is virtually complete and has high accuracy[29]. It provided us with information on sex, birthdates, emigration and vital status.

Exposure and outcome

NLR was defined as the absolute neutrophil count ($x10^9$/L) divided by the absolute lymphocyte count ($x10^9$/L) assessed within 30 days prior to date of diagnosis. If several values existed in this 30 day-period, we used the latest before date of diagnosis. We excluded patients without a NLR measurement in this time period. Likewise, we obtained NLR measures within 30 days prior to date of RCC recurrence.

Primary outcomes were 1- and 5-year survival after initial RCC diagnosis. The exact date of death was obtained from the CRS database and we followed patients from date of diagnosis until death, emigration or up to five years. Among patients with localized disease at time of initial diagnosis who underwent curative-intended surgical resection, secondary outcomes were RCC recurrence and 1- and 3-year survival after first RCC recurrence. We defined recurrence as first occurrence of either a pathology diagnosis of RCC recurrence recorded in DPR or a hospital contact to an oncology department with a kidney cancer diagnosis (DC64*) more than 120 days after date of nephrectomy and within 3 years of date of RCC diagnosis.

Covariates

Based on existing literature, we included the following covariates: sex, age (≤60 vs. >60 years), Charlson Comorbidity Index (CCI) score (0, 1-2 and >3), TNM stage (I-IV), histological subtypes

(clear cell and non-clear cell), tumor size (<4, 4-7 and >7cm), Fuhrman grade (1-2 vs. 3-4), presence of tumor necrosis or sarcomatoid differentiation, Leibovich score (0-2, 3-5 and >6) and selected baseline biochemical values stratified according to the upper or lower limits of normal (ULN and LLN, respectively)[36], included hemoglobin level (female, <7.3 vs. ≥7.3 mmol/L; male, <8.3 vs. ≥8.3 mmol/L), platelet count (female, ≤400 vs. >400x10^9/L; male, ≤350 vs. >350x10^9/L), sodium level (<137 vs. ≥137 mmol/L), LDH level (18-70 years, ≤205 vs. >205 U/L; >70 years, ≤255 vs. >255 U/L), CRP level (<8.0 vs. ≥8.0 mg/L) and albumin (18-70 years, <36 vs. ≥36 g/L; >70 years, <34 vs. ≥34 g/L). As a measure of abnormal calcium level, we used ionized calcium (≤1.32 vs. >1.32 mmol/L). If ionized calcium was not recorded, we used pre-calculated serum corrected calcium (≤10 vs. >10 mg/dL). If neither ionized calcium nor serum corrected calcium were recorded, we constructed a serum corrected calcium as: total serum calcium + (0.8*(4-serum albumin)).

Statistical analysis

Patients' characteristics at time of diagnosis with RCC were described according to exposure status. We predefined NLR cut-off as 3.0 and examined other cut-offs in sensitivity analyses. To examine the association between NLR at time of diagnosis and mortality, we estimated survival probabilities using Kaplan-Meier curves and hazard ratios (HR) with 95% confidence intervals (95% CI) using Cox proportional hazards regression. *A priori* we selected three multivariate models: 1) Adjusting for age, sex, stage and CCI, 2) also including albumin, sodium, calcium and LDH and 3) including hemoglobin, CRP, and platelets as well. We stratified analyses according to hemoglobin, CRP, and platelets levels, since we hypothesized that they all could be a product of the same inflammatory mechanisms as NLR. Additionally, we restricted the analyses to 1) patients with confirmed clear cell RCC and 2) patients with localized RCC at time of initial diagnosis, respectively. We similarly analyzed the association between NLR at time of RCC recurrence and mortality. To examine time to RCC recurrence according to NLR at time of initial diagnosis, we estimated cumulative incidence rates considering death as a competing risk and hazard ratios (HR) with 95% confidence intervals (95% CI) using Cox proportional hazards regression. We assessed the assumption of proportional hazards by log-minus-log plots and unless stated otherwise, we found it not to be violated. If violated, we additionally used a pseudo value analysis[37].

Due to missing data, we did not include Tumor size, Fuhrman grade, Leibovich score, and presence of tumor necrosis or sarcomatoid differentiation in the analyses. We categorized remaining missing data in every variable as unknown and explored the impact of having missing data with sensitivity

analyses comparing our results with a worst-case scenario replacing all missing values among patients with NLR≤3.0 with the best prognostic categorical value, whereas missing values among patients with NLR>3.0 were given the worst prognostic value.

All the statistical analyses were conducted using STATA version 14.2 software package. The study was approved by the Danish Data Protection Agency (Jr. number: 2014-54-0922).

Results

We identified 2.849 patients in the two regions with a pathology verified RCC diagnosis in the period from 1999 until 2015. Of these, 979 patients had a recorded NLR at time of RCC diagnosis and were included in the study. We had virtually complete follow-up (one person ended follow-up due to emigration) with a median follow-up time of 2.3 years. Median age at time of diagnosis was 66.6 years.

Median NLR at diagnosis was 3.4, 416 had a NLR ≤3.0 and 563 had a NLR >3.0. Patients with elevated NLR were older, more often male, more likely to have stage IV disease and higher CCI level, and less likely to have clear cell RCC. Regarding biochemical values, patients with elevated NLR tended to have low hemoglobin, sodium and albumin levels and high calcium, LDH, CRP, leucocytes and platelet levels. Additionally, patients with elevated NLR at time of RCC diagnosis were less likely to have surgery performed (table 1A).

Association between NLR at time of initial RCC diagnosis and mortality

During follow-up, 439 (44.8%) patients died with 105 (23.9%) patients in the NLR ≤3.0 group and 334 (76.1%) patients in the NLR >3.0 group, respectively. The 1-year survival rate was 59.4% in RCC patients with elevated NLR compared with 87.7% in patients with low NLR, and 5-year survival rates were 69.4% in patients with elevated NLR and 35.2% in patients with NLR ≤3.0 (figure 1). Compared with NLR ≤3.0, an elevated NLR at time of RCC diagnosis was associated with poorer prognosis, unadjusted HR = 3.1 (95% CI, 2.5;3.9) and adjusted HR = 2.4 (95% CI, 1.9;3.0), adjusted for sex, age, stage and CCI. Additionally including calcium, albumin, sodium and LDH HR was 2.0 (95% CI, 1.6;2.6). After including other markers of inflammatory activity (hemoglobin, platelets and CRP) as well, NLR remained associated with an almost two-fold increased mortality (HR=1.8 (95% CI, 1.4;2.2)) (table 2). Re-analyzing our data with different cut-off values ranging between 2.0-5.0 similarly found elevated NLR to be associated with poorer prognosis compared to patients with low NLR. However, the association was weaker in the outer-range of these cut-off

values (results not shown). Examining the impact of having missing data in a worst case scenario substantially attenuated the association with in HR adjusted for all covariates of 1.1 (95% CI, 0.8;1.4).

Restricting the analysis to patients with clear cell RCC showed a slightly stronger association in each adjusted analysis while restricting to localized RCC yielded results similar to those in our main analysis (table 2). In the stratified analyses (Table 3), elevated NLR was associated with a nearly two-fold higher mortality in patients with low hemoglobin at time of initial diagnosis and a nearly three-fold increased mortality in patients who presented with a hemoglobin level above lower limit of normal even after taking age, sex and comorbidity into account. Although less pronounced, stratifying by low CRP level or by low platelets level resulted in different stratum-specific estimates as well (table 3).

Association between NLR at time of initial RCC diagnosis and recurrence risk

Among 705 patients who received curative-intended surgical resection, 444 had known localized disease at time of initial RCC diagnosis and were followed for recurrence. Table 1B summarizes baseline clinical and pathological characteristics of the 444 RCC patients stratified according to NLR level.

In total, 76 patients (17.1%) experienced recurrence within three years of follow-up. However, to obtain proportional hazards, we had to restrict this analysis to the first year of follow-up. In the first year, 62 recurrences were observed. Figure 2 shows cumulative incidence proportions (CIP) within one year of follow-up according to NLR group. The 1-year risk of RCC recurrence was marginally higher for patients with elevated NLR at time of initial RCC diagnosis (CIP=14.4% (95% CI, 10.0;19.7)) than for patients with low NLR (CIP=13.6% (95% CI, 9.6;18.2)). However, the difference was not apparent after adjustment (table 4). Using pseudo values method similarly showed that NLR at time of RCC diagnosis was not associated with RCC recurrence risk (results not shown).

Association between NLR at time of RCC recurrence and mortality

Out of the 444 localized RCC patients who underwent surgery, 76 patients (17.1%) had RCC recurrence within three years of follow-up. Of these, 59 had NLR measured at time of diagnosis with RCC recurrence and were included in this analysis. To obtain proportional hazards, we restricted follow-up to two years.

In total, 32 (54.2%) patients had a NLR ≤ 3.0 and 27 (45.8%) had a NLR >3.0 at time of diagnosis with RCC recurrence. The 1-year survival rate was 66.5% in RCC patients with elevated NLR compared with 84.4% in patients with low NLR, and 2-year survival rates were 54.2% and 78.0%, respectively (figure 3). An elevated NLR at time of RCC recurrence was associated with poorer prognosis with unadjusted HR = 2.5 (95% CI, 1.0;6.4) and adjusted HR = 2.1 (95% CI, 0.8;5.7) (adjusting for sex and age at time of diagnosis of RCC recurrence).

Discussion

Elevated NLR at time of initial RCC diagnosis was associated with poorer prognosis overall and in clear cell RCC and localized RCC as well. Likewise, elevated NLR at time of RCC recurrence predicted a poorer survival. At time of initial RCC diagnosis, patients with elevated NLR differed from those with low NLR in various clinical, pathologic and biochemical parameters, supporting that an increased NLR indicates worse clinical behavior of RCC. We did not find NLR at time of initial RCC diagnosis to be associated with recurrence risk.

The link between inflammation and cancer is evident and several inflammatory cells pose a significant role in the development of cancer and its progression[8,9]. Neutrophils, the most abundant circulating leukocyte, has both tumor-promoting and anti-tumor effects[38]. Studies have shown that neutrophils contribute to tumor progression, metastases and angiogenesis, whereas others have shown that neutrophils play an important role in prevention of cancer progression[39]. These versatile functions of neutrophils could represent therapeutic targets. Especially, therapies suppressing the pro-tumor functions while optimizing the anti-tumor effects of neutrophils would be ideally. However, despite the evidence of neutrophils involvement in cancer development, much remain unknown. It is not completely understood how tumors induce neutrophilia and if there exist several subsets of neutrophils with pro-tumor and anti-tumor functions, respectively. The existence of different surface markers to distinguish between such subsets could give rise to optimal therapy targets[40]. Lymphocytes, on the other hand, has a certain anti-tumor function by producing cytokines that suppress tumor progression[41]. Hence, an increased amount of neutrophils and a reduced amount of lymphocytes may be an indicator of cancer progression and prognosis and combining these to biomarkers in NLR could be a stronger predictor than either of them considered alone. In addition, studies have evaluated whether NLR could improve the predictive accuracy of well-established prognostic models for RCC and they found an improvement if NLR was added to these models[22,42].

Several previous studies have examined the association between NLR and RCC progression and prognosis; our findings support these previous findings of an association between elevated NLR at time of initial RCC diagnosis and an increased overall mortality and likewise an elevated NLR at time of RCC recurrence and poorer survival rate[16–19]. A noteworthy limitation in these studies is the small sample sizes. In contrast with some previous findings, we could not confirm an association between elevated pretreatment NLR and RCC recurrence[16,19,22,27,42,43]. This is however in line with previous findings of no association between NLR and progression-free survival[25,26]. Our findings should be interpreted with cautions, however. RCC recurrence registration in DPR is not complete and it may have affected our results[3]. We believe that a recurrence rate of 17% seems low; thus, our dataset may have missing recurrences.

Accordingly, it is possible that patients with elevated NLR would benefit from more frequent post-operative surveillance, enabling earlier detection of recurrent disease and the possibility of curative salvage surgery. Other investigators also outlined an importance of NLR with regard to therapy response[19]. Our results showed that 25% of patients with an elevated NLR were dead after 4 months, which could support NLR's clinical importance in treatment decisions. NLR could be helpful in stratifying patients into those who will benefit from treatment and those who would be better off without any treatment due to high risk of side effects and low chance of treatment effect.

Existing studies have used a NLR cut-off ranging between 2.0-5.0[18,44]. Although we had predefined a cut-off of 3.0, our sensitivity analysis showed that changing the cut-off within this range did not alter the conclusion that an elevated NLR was associated with poorer prognosis compared to patients with a low NLR. From the point of clinical practice, using a fixed cut-off is preferred for offering patients a simple risk classification into "high" versus "low". However, the fact that studies have used several different cut-offs and found an association between an elevated NLR and poorer prognosis regardless of the NLR cut-off may suggest that using 3.0 as a fixed threshold may be too rigor since patients with NLR levels between 2.0 and 5.0 also have increased mortality. Furthermore, the natural variability of neutrophil and lymphocyte levels in patients further challenge the determination of the best NLR cut-off. One may speculate whether changes in NLR over time may offer better prognostic information[39]. Unfortunately, our data did not allow us to examine this any further.

The main strengths of this study are its population-based design and the use of region- and nation-wide administrative and medical registries with prospectively collected data preventing selection bias and allowing for a virtually complete follow-up of all patients. Another noteworthy strength is the relatively large sample size. However, we do acknowledge some limitations. First, despite the fact that reporting to DPR is based on national guidelines with obligatory reporting, we had missing data on some pathology information. Consequently, we were not able to take tumor size, Fuhrman grade, Leibovich score and presence of tumor necrosis or sarcomatoid differentiation into account as potential confounding factors. Thus, it is possible that residual confounding by these factors may explain at least some of the increased mortality. Likewise, the potential missing data on RCC recurrence limit our findings in the association between NLR at time of recurrence and mortality. Second, we also had missing data on the remaining covariates and included them as an unknown category in each variable, which may also bias our results. We used a worst-case scenario to examine whether missing data could explain our findings. Although only a 10% increased mortality remained in this worst-case scenario we find it to be rather unlikely that missing data entirely explains the increased mortality in our study. Third, we did not take into account that drug intake or conditions such as active infection, inflammatory diseases, stress or smoking behavior at the time of blood collection may have affected the NLR level[25].

Nonetheless, even considering these limitations, our data clearly indicate that an elevated NLR at time of initial RCC diagnosis and at time of RCC recurrence is associated with poorer prognosis. NLR at time of initial RCC diagnosis might not be associated with recurrence risk. Clinicians should consider incorporating NLR in traditional prognostic models and use NLR to stratify patients for postoperative surveillance and as guidance in decisions of therapy treatments. Future studies should indeed clarify NLR's role as predictor of RCC recurrence and furthermore, explore the underlying biology of neutrophils and lymphocytes in cancer development with a view to develop new potential therapeutic agents.

SUPPLEMENTARY

Background

Kidney cancer

Renal cell carcinoma (RCC), originating in the proximal convoluted tubule in the renal cortex, is the most common type of kidney cancer in adults and comprises 90%. Other types, such as urothelial carcinoma, lymphoma and sarcoma comprise the remaining kidney and kidney pelvic cancers. RCC histologic subtypes includes clear cell, papillary, chromophope, oncocytic and collecting duct with clear cell RCC being the most common subtype. Although, the pathogenesis is not completely understood, several risk factors exist. Smoking, hypertension, obesity, occupational exposure to toxic compounds and some genetic disorders are examples of well-established risk factors[45]. Regarding clinical manifestations, many patients remain asymptomatic until the disease is advanced. However, due to a more widespread use of radiological imaging procedures, the incidental diagnosis of RCC is increasing, currently comprising approximately 50% of all cases. Presenting symptoms include hematuria, pain, an abdominal mass, weight loss and paraneoplastic symptoms and are only seen in 10% of patients with localized disease[46]. In patients with primary metastatic RCC (mRCC) presenting symptoms are metastatic symptoms from brain, lung and bone metastases. Imaging methods can support the diagnosis and subsequently biopsy or nephrectomy can give a definitive diagnosis.

RCC is among the most therapy-resistant cancers, especially mRCC, which is a serious problem since 20-30% will have metastatic disease at the time of diagnosis. Treatment of mRCC can include nephrectomy in selected patients and subsequently targeted therapy or immune therapy. Standard treatment of localized RCC is nephrectomy, which can be radical or partial, and robotic, laparoscopic or open surgery. Post-operative surveillance is every 6-12 months within five years after operation[47].

Methodological considerations

Study design

A cohort study is an observational study and includes two or more groups of individuals defined by differences in exposure. Every individual are then followed for a period of time to observe if the incidences of one or more outcome of interest differ by exposure status[48]. We conducted a historical population-based cohort study using prospectively collected registry data to answer the aim of our research project. Our choice of study design has several advantages. First of all is the external va-

lidity, which is regarded as one of the main advantages of a population-based cohort study[49]. Secondly, a historical cohort study is efficient, easy to conduct and an inexpensive design compared to e.g. a cohort study with ongoing prospective data collection because data are already collected in the databases. At last, the registration of a huge amount of different information in Danish registries provides data on potential confounding factors, which can be included as covariates in the analysis phase. However, this design has some disadvantages as well. There might be some differences between the exposed and unexposed patients besides the exposure, which could confound the examined association if some of these differences are related to the outcome of interest. However, confounding in cohort studies can be limited by taking certain precautions in the analysis phase of the study, which will be elaborated on later. Another disadvantage to this design is the fact that we are limited to the use of the information recorded in the databases, which means that we cannot adjust for factors not recorded[48]. Residual confounding, the consequence of unmeasured confounding, is unavoidable in cohort studies. Whereas a successful randomized control trial design results in an evenly distribution of both known and unknown confounders between study groups. This type of design is, however, not possible in our project. We cannot randomly assign study participants to have a low or an elevated NLR.

Data sources and covariates

Additional comments and considerations on the registries used in this study together with some remarks on advantages and limitations will be briefly reviewed in the following sections.

Information on pathology

We identified RCC patients using the pathologic SNOMED code T71 in combination with M80103-M958x3, except M89603 (nephroblastoma) and M81203 (urothelial carcinoma), or the specific code ÆF4510 from DPR. Additional information on pathology were identified using the SNOMED-classification and pathology coding guidelines[50]. Information on tumor stage from DCR were classified according to TNM-classification and then divided into stage group I-IV[51]. A noteworthy limitation to the DPR, is the lack of information on "non-existing findings" e.g. no presence of tumor necrosis or sarcomatoid differentiation. In register research, it would be ideal to have a "not existing" and "unknown" code, since the current system only register a "yes", it is impossible to tell if there in fact was no tumor necrosis or if it was not investigated or if the "yes" code

is actually missing. Unfortunately, we did not have access to pathology journals. Otherwise, we could have validated our pathology information and perhaps gained further pathology information.

Biochemical values

Using the LABKA database, there are certain considerations to take in to account[52]. First of all is missing measurements. Out of 2.849 patients identified with a RCC diagnosis in the time period 1999-2015, we were only able to find a NLR measurement in 979 patients. We believe that these missing NLR measurements are simply due to the fact that the blood sample was not taken and not caused by errors in the LABKA database. This missing values problem is also discussed under *selection bias.* Information in the LABKA database is obtained directly, electronically, and without filtering from the LABKA systems in the North Denmark region and the Central Denmark region, which makes the proportion of missing data extremely low[35]. Furthermore, we examined possible variations in blood sample names (component names), NPU codes and analysis codes to make sure that every eligible blood sample was included. "Analysefortegnelsen.dk", "labterm" and "Region Nord laboratorievejledning" were all inspected. Afterwards the chosen NPU codes and analysis coded were examined with regard to comparability to every biochemical marker. For example, we might have five different codes or component names for a hemoglobin sample. Units, means, minimum and maximum were tabulated in STATA for each biomarker code and then compared and additionally, each biomarker was examined for each year of the study period to look for remarkable variations. We excluded analysis codes or NPU codes that were incomparable to the others, and in the existence of different units for one biomarker, we converted one of them. For future studies using LABKA database, it would be ideal if a comprehensive list of comparable analysis codes and NPU codes were devised. Furthermore, a noteworthy limitation in LABKA is the fact that there is no information on the underlying reason for requesting the blood samples. This is also addressed further in *selection bias.*

Charlson comorbidity index

As a measure of overall comorbidity, we determined CCI score from ICD-codes in DNPR within 10 years before cancer diagnosis. We did not include metastatic solid tumor or a kidney cancer diagnosis in any tumor. Three comorbidity levels were defined: low (score of 0), medium (score of 1-2), and high (score of $\geq$3)[53].

Statistical analysis

Kaplan-Meier survival curves

We used Kaplan-Meier estimates to plot survival curves showing the estimated probability of surviving in a given length of time. To derive the Kaplan-Meier estimates, we consider the risk sets of individuals (n) still being studied at each time (t), at which an event occurs (d) e.g. death.

Survival probability (s) at time (t) is: $S_t = (n_t - d_t) / n_t$

The survival probability remains 1 until an event occurs and it does not change during intervals in which no one dies; it is recalculated only when an event occurs. If patients are lost to follow-up during the time of interest, they are referred to as censored and will not be included when calculating the survival probability estimates. We evaluated the three assumptions for using this method. Firstly, the Kaplan-Meier method only works with independent right censoring (assumption 1), which means that a study subject is followed for a period and then at one point is no longer observed because of e.g. end of follow-up or loss to follow-up. This individual could experience the event of interest in the future, but we do not know when[54]. And when censoring is independent, the probability of being censored is not related to the risk of the event occurring. In the present study, we censored individuals who emigrated during follow-up or were failure-free at the administrative end of follow-up. Secondly, to use Kaplan-Meier curves, the event studied (e.g. death) must happen at a specified time (assumption 2), which was possible due to the exact date of death obtained from the CPR register. Thirdly, we assumed that the survival probabilities were the same for subjects recruited early and late in the study (assumption 3)[55].

Finally, the survival curve can be displayed and is a stepwise function; the curve is horizontal until an event occurs which will be displayed as a vertical drop at the curve[48,56]. We presented crude Kaplan-Meier curves, i.e. not including covariates, since this is more traditional than using adjusted Kaplan-Meier curves.

Cox proportional hazard regression analysis

This regression method can be used in the analysis of survival data. We evaluated the two assumptions for using this analysis:

1) The censoring is independent given covariates.
2) The ratio of hazards comparing different exposure groups remains constant over time.

Independent censoring cannot be checked statistically but must be assumed. The second assumption, known as the proportional hazard assumption, can be evaluated in different ways. We used

log-minus-log plots. The proportional-hazards assumption is not violated when the curves are parallel. A possible analysis option to handle non-proportional hazards is to split the follow-up time into different time periods. In our examinations of the association between NLR at time of initial RCC diagnosis and recurrence risk and between NLR at time of RCC recurrence and mortality, we restricted the analysis time period to obtain acceptable proportional hazards.

Competing risks

In some follow-up analyses, the occurrence of an event influences the risk of another event. In our case, when studying the rate of RCC recurrence, patients who die without having had RCC recurrence are no longer at risk for developing recurrence. Death, in this situation, is considered as a competing risk and should be taken into account when examining the cumulative risk of recurrence; this as called a competing-risk analysis. Using Kaplan-Meier estimator would treat death as censoring. That is, people who died during follow-up would still be at potential risk of RCC recurrence, which would be absurd[56]. Cox regression, however, can be applied in the presence of competing risks since the association measure is hazard ratios.

Pseudo values

As a sensitivity analysis, we used pseudo values method to examine the association in question if the assumption of proportional hazards in the cox regression analysis was violated. The method can be used when competing risks are present. In our examination of the association between NLR at time of initial RCC diagnosis and recurrence risk, using pseudo values resulted in similar results as in the cox regression analysis. NLR at time of RCC diagnosis was not associated with RCC recurrence risk.

The idea in the pseudo values approach is to generate a transformation of the survival data – called pseudo observations – and use the transformed observations for analysis. Explaining the underlying theory for this model is beyond the scope of this report. However, the assumptions for using the model was evaluated[37]:

1) The censoring is independent (not given covariates).
2) The censoring distribution does not dependent of the covariates in the regression model.

Errors, bias and confounding

Interpretation of results in every epidemiologic study should include the consideration of potential errors. Two types of error are described in the literature: random error and systematic error.

Random error is normal variability in the data due to chance that results in measurement variation. It is influenced by study size; with increasing study size random error is reduced. Several Danish registries are regional- or nationwide enabling large population sizes to be included in studies, which is an advantage of our study. Statistically, the confidence interval reflects the extent of random error of an estimate with a narrow interval indicating a small amount of random error and vice versa[48,57]. For example: In the analysis examining the association between NLR at time of initial RCC diagnosis and mortality, we had 979 patients included and the adjusted estimates had a more narrow confidence interval compared to the analysis examining the association between NLR at time of RCC recurrence and mortality with only 59 patients included, a more wide confidence interval was observed.

Systematic error, on the other hand, is insensitive to increasing study size. It is also called bias and consists of selection bias, information bias and confounding[57]. These three parts determine the internal validity of a study and will be discussed in relation to the present study in the following.

Selection bias

This can arise in the selection of study participants at the level of entry into study and/or through loss to follow-up, if the association between exposure and outcome differs between study participants and non-participants[57].

As stated previously, we were not able to include every patient with a pathology verified RCC diagnosis due to no NLR measurement at the time of diagnosis. We had expected to have fewer patients without an NLR measurement, since a complete blood count is usually a part of the RCC diagnosing. It is not possible for us to know if the patients with missing NLR had an elevated or low NLR and as mentioned earlier, we had no information on the underlying reason for requesting blood samples. Without knowing the indication, it is more difficult to assess the possibility of selection bias.

The risk of selection bias, however, is substantially reduced due to the design of the study with use of Danish registries. All residents in Denmark have free access to all general practitioners and hospitals due to the Danish health care system providing tax-supported health care services, and CPR numbers are given to all inhabitants in Denmark by the CPR system, which is daily updated on information on migration and vital status allowing for complete long-term follow-up of patients. We had virtually complete follow-up (one person ended follow-up due to emigration).

Information bias

Another way to introduce systematic error is erroneous collection of information. This is often referred to as misclassification if the variable is measured on a categorical scale. Hence error will arise if a person is placed in an incorrect category. This misclassification can be either non-differential or differential and it is especially important to consider a possible misclassification of two key variables; namely exposure and outcome. Non-differential misclassification occurs when the misclassification is unrelated to exposure or outcome and when the exposure is dichotomized, this mostly leads to an underestimation of the association. Whereas differential misclassification arises when the misclassification is related to exposure or outcome and this can induce bias in either direction[57]. Misclassification of covariates can occur as well, which may result in residual confounding.

In our study, the exposure was NLR, the main outcome was death and secondary outcome included RCC recurrence. Considering the exposure, measurements error of NLR is possible, but we have no reason to suppose that these errors were related to our outcomes, death and RCC recurrence, since data were registered prospectively and independently of the study outcomes. Accordingly, we expect non-differential misclassification. Considering the outcome death, rationally thinking, this is very unlikely to be misclassified. We defined RCC recurrence as first occurrence of either a pathology diagnosis of RCC recurrence recorded in DPR or a hospital contact to an oncology department with a kidney cancer diagnosis. Our results therefor depend on the validity of the RCC recurrence diagnosis in DPR or the contact diagnosis to an oncology department in DNPR. As mentioned earlier, due to our findings of a low RCC recurrence rate, we believe that out dataset may have missing recurrences. However, we expect any potential misclassification to be non-differential in regard to the exposure. If this is the case, then our relative estimates will be unbiased.

Confounding

The literature often describes confounding as a mixing of effects, whereby the effect of one variable is attributed to another and thereby leading to bias. In other words, a confounding variable is a third variable that wholly or partially accounts for the observed effect of an exposure. For a variable to be a confounder, three criteria must be met[57]:

1) A confounder must be associated with the exposure.
2) A confounder must be associated with the outcome of interest.

3) A confounder must not be an intermediate step of the causal pathway from exposure to disease.

There are multiple ways to deal with confounding both in the phase of designing the study and during data analysis[48]. In the analysis phase of our study we controlled for confounding by using stratification and multivariable adjustment in regression analyses. We decided which covariates to include based on previous studies. As mentioned earlier, we had missing data on a large amount of pathology information, which compromised the inclusion of potential confounders in our analyses with the possibility of residual confounding by these factors. Furthermore, drug intake or conditions such as active infection, inflammatory diseases, stress or smoking behavior at the time of blood collection may have affected the NLR. Since, we did not have information on these, we could not adjust for it in our analyses.

External validity

After discussing the intern validity of a study, the external validity is next to be evaluated. External validity, also referred to as generalizability, is the degree to which the findings of a study hold true in other settings, e.g. other populations, time periods and geographical places[48]. Our study population was population-based, which enables the generalizability of our findings to the rest of the Danish regions. However, one may speculate whether our study population reflects a special subpopulation of patients with RCC, since they have a measurement of NLR. Patients without an NLR measurement may have some unknown reasons for why they did not have the same blood samples drawn.

Additional analyses and results

NLR as a continuous variable

To obtain a clearer picture of the relation between elevated NLR at time of RCC diagnosis and RCC survival, we also assessed NLR as a continuous variable. QQ-plots and histograms confirmed linearity of ln(NLR) and therefore, we fitted the Cox model with lnNLR. Using Cox proportional hazards regression, we identified ln(NLR) as a continuous variable to be a prognostic factor with an unadjusted HR of 2.1 (95% CI, 1.9;2.4) and adjusted HR of 2.1 (95% CI, 1.8;2.4) (adjusted for age, sex, stage and CCI). The estimate did not change by more than 10% when additionally adjusting for 1) albumin, sodium, calcium, LDH and 2) hemoglobin, platelets, CRP (results not shown).

The combined prognostic effect of NLR and sodium at time of initial RCC diagnosis

As seen in previous studies[58–60], low sodium level at time of diagnosis has been associated with poor survival in both localized and metastatic RCC. Combining the prognostic information of these two biomarkers, NLR and sodium, may contribute to a more valid stratification of mortality risks.

We, therefore, in an additional analysis examined the combined prognostic effect of NLR and sodium levels at time of initial RCC diagnosis. Patients were divided into four groups according to their NLR (≤ 3.0, >3.0) and sodium below lower limit of normal (LLN) or above LLN values (sodium normal values for a person above 18 years are 137-145 mmol/L). We estimated survival probabilities using Kaplan-Meier curves and hazard ratios with 95% confidence intervals using Cox proportional hazards regression.

In total, we included 970 patients who had a measurement of both variables at time of initial diagnosis. Among these, 352 patients had a NLR ≤3.0 and sodium levels ≥137 mmol/L and 181 patients had a NLR >3.0 and low sodium levels (supplemental table 5). Our results showed that 25% of patients with an elevated NLR and low sodium levels were dead after 2-3 months. Furthermore, the 5-year survival rate was 21.7% in RCC patients with an elevated NLR (>3.0) combined with a low sodium level (<137 mmol/L) compared with 73.2% in patients with NLR ≤ 3.0 and normal sodium levels (supplemental figure 4). Comparing these two patient groups, we found that an elevated NLR combined with a low sodium level were associated with a higher mortality compared to having a NLR ≤ 3.0 and normal sodium levels (unadjusted HR = 5.6 (95% CI, 4.2;7.4) and adjusted HR= 4.1 (95% CI, 3.1;5.5) (adjusted for age, sex, stage and CCI)). For further results, see supplemental table 5.

Association between leucocytes levels at time of initial diagnosis of RCC and mortality

Since neutrophils are the most abundant leukocyte in human circulation, accounting for 50–70% of circulating leukocytes, we made an additional analysis examining the prognostic effect of leucocyte levels at time of initial RCC diagnosis. Among 2.849 patients initially identified with a RCC diagnosis, 1.559 patients had a measurement of leucocytes and were above 18 years at time of diagnosis and were included in the analysis. Normal values of leucocytes levels in blood for persons above 18 years are 3,5-10,0 × 10^9/L. We divided patients according to having leucocytes levels above upper limit of normal (ULN) values or not and found that 476 had leucocytes levels >ULN and 1.083 had leucocytes levels ≤ULN. The 1-year survival rate was 59.4% in RCC patients with leucocytes levels > ULN compared with 78.1% in patients with leucocytes levels ≤ ULN, and 5-year survival rates

were 35.4% and 55.9% respectively (supplemental figure 5). The unadjusted HR was 2.0 (95% CI, 1.7;2.3) and the adjusted HR was 1.7 (95% CI, 1.5;2.0), when adjusting for sex, age, stage, and CCI. When including calcium, albumin, sodium, and LDH, we found a HR of 1.5 (95% CI, 1.3;1.8) and at last when further including hemoglobin, platelets, and CRP in the multivariate analysis, leucocytes level >ULN remained associated with an increased mortality (HR=1.4 (95% CI, 1.2;1.7)) (supplemental table 6). Although a less strong association, these findings are similar to the results from the association between NLR at time of initial diagnosis of RCC and mortality, supporting our findings. However, NLR seems to be a stronger predictor of survival than leucocytes. Supplemental table 6 and 7 present results from restricted and stratified analysis, respectively.

Sensitivity analysis

Dealing with missing data on NLR-levels: A comparison of clinical and pathological characteristics

In total, we identified 2.849 patients with a pathology verified RCC diagnosis. However, due to missing NLR information, we only included 979 patients in our study. We compared the prevalence of clinical and pathological characteristics between our study population of 979 patients and patients excluded due to missing NLR values (N=1.870). RCC patients without a NLR measurement at time of diagnosis had similar age, sex, and CCI distribution. Concerning surgery, the same percentage of patients without an NLR measurement had nephrectomy done, but with a higher percentage of open surgery, compared to patients with a measurement of NLR. RCC patients without a NLR measurement tended to have a higher prevalence of localized disease at time of diagnosis. The remaining variables were difficult to compare, since RCC patients without a NLR measurement tended to have a higher amount of missing values (results not shown).

Additional discussion and clinical perspectives

Using NLR as a prognostic marker for RCC, we should consider the best way to incorporate it in clinical practice; dichotomized with a defined cutoff, as a continuous variable or by examining patients' individual changes in NLR over time. This report focuses on NLR as a dichotomized prognostic marker. However, we did examine NLR as a continuous variable (see additional analyses and results) and found that elevated NLR at time of initial RCC diagnosis was associated with shorter overall survival as well. This is supportive of previous studies findings of similar results[61]. Considering changes in NLR over time, a rise in NLR could indicate a higher recurrence risk and

poorer prognosis, whereas a drop in NLR could indicate a chance of a better prognosis[39]. Studies have examined the importance of changes in NLR in relation to mRCC therapy treatment. Patients that experienced an early decrease in NLR after therapy initiation had better outcomes[61] and similarly, patients with a low pretreatment NLR that was maintained during targeted therapy treatment experienced a more favorable outcome from sequential targeted therapy[62]. Likewise, the absolute neutrophil count and the absolute lymphocyte count have each separately been suggested as indicators of the response to surgery and therapy treatment[63–65]. Patients with an increased amount of neutrophils or a decreased amount of lymphocytes might have a lower chance of benefitting from surgery and therapy treatment. Unfortunately, our data did not allow us to examine individual changes in NLR over time due to a high proportion of missing values.

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Tables and figures

Table 1: Clinical and pathological characteristics of A) patients with RCC and B) patients with localized disease at time of initial diagnosis who underwent curative-intended surgery. Both stratified according to NLR level.

Table 1A: Clinical and pathological characteristics of RCC patients stratified according to NLR level

	NLR ≤3.0		NLR >3.0		Total	
	n	%	n	%	n	%
Total	416	100.0	563	100.0	979	100.0
Age (years)						
≤60	147	35.3	152	27.0	299	30.5
>60	269	64.7	411	73.0	680	69.5
Sex						
Female	165	39.7	198	35.2	363	37.1
Male	251	60.3	365	64.8	616	62.9
Stage						
I	143	34.4	109	19.4	252	25.7
II	40	9.6	60	10.7	100	10.2
III	70	16.8	70	12.4	140	14.3
IV	56	13.5	181	32.1	237	24.2
Unknown	107	25.7	143	25.4	250	25.5
Localized vs. metastatic RCC						
Localized	253	60.8	239	42.5	492	50.3
Metastatic	56	13.5	181	32.1	237	24.2
Unknown	107	25.7	143	25.4	250	25.5
Histologic subtype						
Undiff./unclass. RCC	148	35.6	331	58.8	479	48.9
Non-clear cell RCC	47	11.3	56	9.9	103	10.5
Clear cell RCC	221	53.1	176	31.3	397	40.6
Tumor size						
<4 cm	49	11.8	17	3.0	66	6.7
4-7 cm	56	13.5	30	5.3	86	8.8
>7 cm	65	15.6	70	12.4	135	13.8
Unknown	246	59.1	446	79.2	692	70.7
Fuhrman's grade						
Grade 1	24	5.8	19	3.4	43	4.4
Grade 2	115	27.6	77	13.7	192	19.6
Grade 3	75	18.0	76	13.5	151	15.4
Grade 4	17	4.1	35	6.2	52	5.3
Unknown	185	44.5	356	63.2	541	55.3
Presence of tumor necrosis						
Yes	44	10.6	61	10.8	105	10.7
No/Unknown	372	89.4	502	89.2	874	89.3
Sarcomatoid differentiation						
Yes	7	1.7	15	2.7	22	2.2
No/unknown	409	98.3	548	97.3	957	97.8

Leibovich score						
0-2	33	7.9	11	2.0	44	4.5
3-5	43	10.3	13	2.3	56	5.7
>6	27	6.5	35	6.2	62	6.3
Unknown	313	75.2	504	89.5	817	83.5
Type of nephrectomy						
Partiel	89	21.4	47	8.3	136	13.9
Radical	272	65.4	297	52.8	569	58.1
None nephrectomy	55	13.2	219	38.9	274	28.0
Method of surgery						
Laparoscopic	235	56.5	183	32.5	418	42.7
Open	126	30.3	161	28.6	287	29.3
No surgery	55	13.2	219	38.9	274	28.0
Low hemoglobin level						
No	240	57.7	192	34.1	432	44.1
Yes	176	42.3	370	65.7	546	55.8
Unknown	0	0.0	1	0.2	1	0.1
Low sodium level						
No	352	84.6	378	67.1	730	74.6
Yes	59	14.2	181	32.1	240	24.5
Unknown	5	1.2	4	0.7	9	0.9
Low albumin level						
No	287	69.0	269	47.8	556	56.8
Yes	99	23.8	254	45.1	353	36.1
Unknown	30	7.2	40	7.1	70	7.2
High Calcium level						
No	281	67.5	306	54.4	587	60.0
Yes	60	14.4	139	24.7	199	20.3
Unknown	75	18.0	118	21.0	193	19.7
High LDH level						
No	259	62.3	253	44.9	512	52.3
Yes	60	14.4	123	21.8	183	18.7
Unknown	97	23.3	187	33.2	284	29.0
High CRP level						
No	160	38.5	97	17.2	257	26.3
Yes	172	41.3	407	72.3	579	59.1
Unknown	84	20.2	59	10.5	143	14.6
High leucocytes level						
No	348	83.7	352	62.5	700	71.5
Yes	67	16.1	210	37.3	277	28.3
Unknown	1	0.2	1	0.2	2	0.2
High platelets level						
No	229	55.0	289	51.3	518	52.9
Yes	64	15.4	190	33.7	254	25.9
Unknown	123	29.6	84	14.9	207	21.1
Charlson Comorbidity Index						
Score 0	251	43.4	327	56.6	578	59.0
Score 1-2	132	42.9	176	57.1	308	31.5
Score 3+	33	35.5	60	64.5	93	9.5

Table 1B: Clinical and pathological characteristics of patients with localized disease at time of initial diagnosis who underwent curative-intended surgery. Stratified according to NLR level.

	NLR ≤3.0		NLR >3.0		Total	
	n	%	n	%	n	%
Total	243	100.0	201	100.0	444	100.0
Age (years)						
≤60	96	39.5	55	27.4	151	34.0
>60	147	60.5	146	72.6	293	66.0
Sex						
Female	95	39.1	68	33.8	163	36.7
Male	148	60.9	133	66.2	281	63.3
Stage						
I	135	55.6	90	44.8	225	50.7
II	40	16.5	53	26.4	93	20.9
III	68	28.0	58	28.9	126	28.4
Localized vs. metastatic RCC						
Localized	243	100.0	201	100.0	444	100.0
Histologic subtype						
Undiff./unclass. RCC	82	33.7	98	48.8	180	40.5
Non clear-cell RCC	23	9.5	23	11.4	46	10.4
Clear-cell RCC	138	56.8	80	39.8	218	49.1
Tumor size						
<4 cm	37	15.2	15	7.5	52	11.7
4-7 cm	37	15.2	22	10.9	59	13.3
>7 cm	41	16.9	33	16.4	74	16.7
Unknown	128	52.7	131	65.2	259	58.3
Fuhrman's grade						
Grade 1	14	5.8	4	2.0	18	4.1
Grade 2	76	31.3	37	18.4	113	25.5
Grade 3	52	21.4	44	21.9	96	21.6
Grade 4	6	2.5	12	6.0	18	4.1
Unknown	95	39.1	104	51.7	199	44.8
Presence of tumor necrosis						
Yes	22	9.1	23	11.4	45	10.1
No/Unknown	221	90.9	178	88.6	399	89.9
Sarcomatoid differentiation						
Yes	2	0.8	6	3.0	8	1.8
No/unknown	241	99.2	195	97.0	436	98.2
Leibovich score						
0-2	21	8.6	6	3.0	27	6.1
3-5	24	9.9	6	3.0	30	6.8
>6	11	4.5	15	7.5	26	5.9
Unknown	187	77.0	174	86.6	361	81.3
Type of nephrectomy						
Partiel	64	26.3	35	17.4	99	22.3
Radical	179	73.7	166	82.6	345	77.7

Method of surgery						
Laparoscopic	164	67.5	121	60.2	285	64.2
Open	79	32.5	80	39.8	159	35.8
Low hemoglobin level						
No	151	62.1	95	47.3	246	55.4
Yes	92	37.9	106	52.7	198	44.6
Unknown	0	0	0	0	0	0
Low sodium level						
No	215	88.5	148	73.6	363	81.8
Yes	26	10.7	53	26.4	79	17.8
Unknown	2	0.8	0	0.0	2	0.5
Low albumin level						
No	171	70.4	116	57.7	287	64.6
Yes	54	22.2	71	35.3	125	28.2
Unknown	18	7.4	14	7.0	32	7.2
High Calcium level						
No	167	68.7	123	61.2	290	65.3
Yes	22	9.1	30	14.9	52	11.7
Unknown	54	22.2	48	23.9	102	23.0
High LDH level						
No	157	64.6	97	48.3	254	57.2
Yes	28	11.5	28	13.9	56	12.6
Unknown	58	23.9	76	37.8	134	30.2
High CRP level						
No	106	43.6	56	27.9	162	36.5
Yes	86	35.4	118	58.7	204	45.9
Unknown	51	21.0	27	13.4	78	17.6
High leucocytes level						
No	208	85.6	143	71.1	351	79.1
Yes	34	14.0	58	28.9	92	20.7
Unknown	1	0.4	0	0.0	1	0.2
High platelets level						
No	130	53.5	101	50.2	231	52.0
Yes	29	11.9	47	23.4	76	17.1
Unknown	84	34.6	53	26.4	137	30.9
Charlson Comorbidity Index						
Score 0	156	64.2	114	56.7	270	60.8
Score 1-2	74	30.5	68	33.8	142	32.0
Score 3+	13	5.3	19	9.5	32	7.2

Table 2: The association between NLR at time of initial RCC diagnosis and mortality. Results of A) Complete case analysis and when restricting the analysis to patients with B) Clear cell carcinoma histologic subtype and C) Localized disease at time of initial diagnosis.

NLR ≤3.0 5-year survival % (95% CI)	NLR>3.0 5-year survival % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
A) Total (n=979)					
69.4 (63.7-74.4)	35.2 (30.7-39.6)	3.1 (2.5-3.9)	2.4 (1.9-3.0)	2.0 (1.6-2.6)	1.8 (1.4-2.2)
B) Restricted to clear cell RCC (n=397)					
75.0 (64.3-82.9)	45.1 (34.2-55.3)	3.4 (2.3-5.0)	2.7 (1.8-4.0)	2.3 (1.5-3.5)	2.1 (1.3-3.1)
C) Restricted to localized disease at time of initial RCC diagnosis (n=492)					
80.0 (72.8-85.5)	58.1 (50.6-64.8)	2.6 (1.8-3.9)	2.2 (1.5-3.3)	1.9 (1.3-2.9)	1.9 (1.3-2.9)

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium and LDH

[c]Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium, LDH, hemoglobin, CRP and platelets

Table 3: The association between NLR at time of initial RCC diagnosis and mortality. Results from stratified analysis.

	NLR ≤3.0 5-year survival % (95% CI)	NLR>3.0 5-year survival % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
Low hemoglobin						
Yes	51.1 (42.1-59.5)	27.9 (23.0-33.0)	2.2 (1.7-2.9)	1.9 (1.4–2.4)	1.7 (1.3-2.2)	1.5 (1.2-2.0)
No	83.7 (77.1-88.5)	(38.7-56.5)	3.9 (2.6-5.9)	2.9 (1.9–4.5)	2.6 (1.7-4.0)	2.4 (1.5-3.7)
High CRP						
Yes	57.6 (49.1-65.2)	26.3 (21.7-31.1)	2.5 (1.9-3.2)	2.1 (1.6–2.7)	1.8 (1.4-2.4)	1.8 (1.4-2.4)
No	76.3 (63.9-84.9)	63.9 (48.0-76.2)	2.1 (1.2-3.7)	2.1 (1.1–3.8)	2.2 (1.1-4.1)	2.1 (1.1-4.1)
Low Platelets						
Yes	44.0 (30.2-57.0)	21.1 (15.1-27.9)	2.1 (1.4-3.1)	2.0 (1.3-3.0)	2.0 (1.3-3.1)	2.0 (1.3-3.0)
No	71.8 (64.4-78.0)	39.4 (33.1-45.7)	3.1 (2.3-4.2)	2.4 (1.8-3.4)	2.0 (1.4-2.7)	1.7 (1.2-2.3)

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium and LDH

[c]Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium, LDH and hemoglobin, CRP and platelets (excluding the variable being stratified by).

Table 4: Cumultative incidence and hazard ratios showing the association between NLR at time of initial diagnosis of RCC and risk of recurrence within 1 year after operation.

	RCC reccurence, n	Cumultative incidence, % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
NLR ≤ 3 (n=243)	33	13.6 (9.6-18.2)	ref.	ref.	ref.	ref.
NLR > 3 (n=201)	29	14.4 (10.0-19.7)	1.1 (0.7-1.8)	1.1 (0.6-1.8)	1.0 (0.6-1.8)	1.0 (0.6-1.8)

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium and LDH

[c] Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium, LDH and hemoglobin, CRP and platelets

Figure 1: Kaplan-Meier curves with 95%-CI for overall survival according to NLR group at time of initial RCC diagnosis.

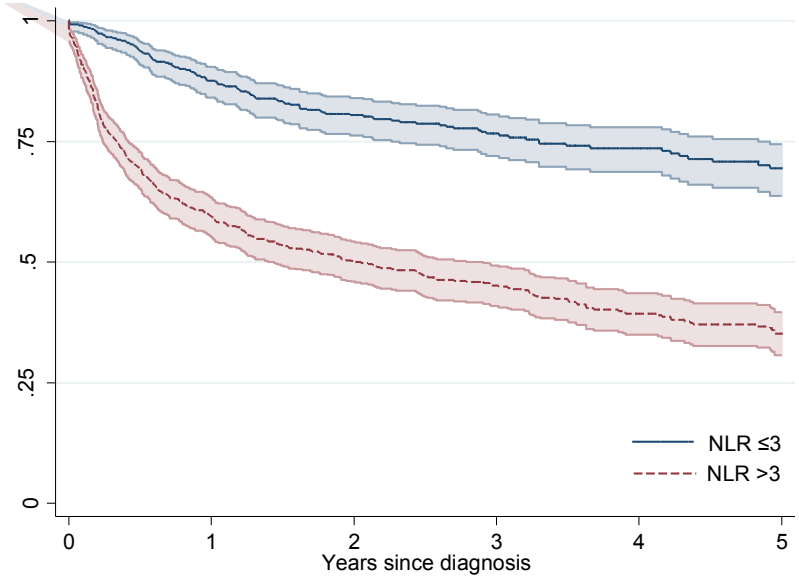


Figure 2: Cumultative incidence of RCC recurrence according to NLR group up to 1 year after day of operation.

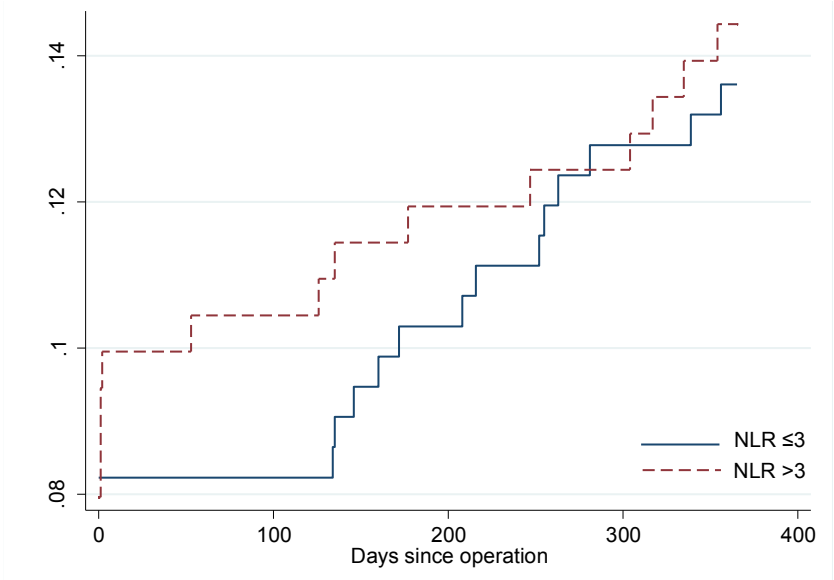
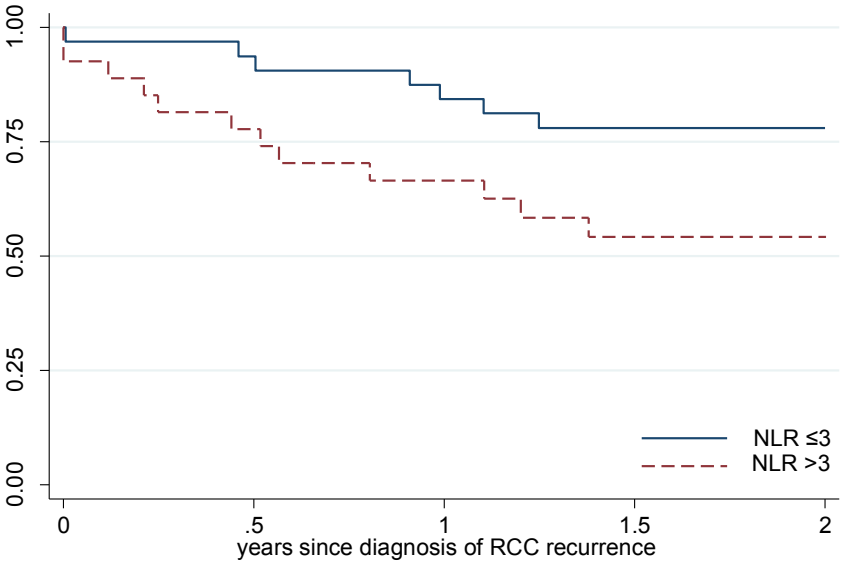


Figure 3: Kaplan-Meier curves for overall survival according to NLR group at time of RCC recurrence.



Supplemental tables and figures

Supplemental table 5: The combined prognostic effect of NLR and sodium at time of initial RCC diagnosis.

NLR & sodium levels	n	5-year survival % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
NLR≤3 & Na ≥ULN	352	73.2 (67.1-78.4)	ref.	ref.	ref.	ref.
NLR≤3 & Na <ULN	59	45.3 (29.7-59.7)	2.7 (1.7-4.1)	2.2 (1.4-3.4)	2.0 (1.3-3.1)	1.8 (1.2-2.8)
NLR>3 & Na ≥ULN	378	41.9 (36.2-47.5)	3.1 (2.4-4.0)	2.3 (1.8-3.0)	2.1 (1.6-2.8)	1.9 (1.4-2.4)
NLR>3 & Na <ULN	181	21.7 (15.4-28.7)	5.6 (4.2-7.4)	4.1 (3.1-5.5)	3.5 (2.6-4.7)	2.8 (2.1-3.8)

NLR = neutrophil-lymphocyte ratio. Na = sodium.

ULN: Upper limit of normal. Sodium normal values for a person above 18 years are 137-145 mmol/L.

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin and LDH

[c]Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, LDH, hemoglobin, CRP and platelets

Supplemental table 6: The association between leucocyte levels at time of initial RCC diagnosis and mortality. Results of A) Complete case analysis and when restricting the analysis to patients with B) Clear cell carcinoma histologic subtype and C) Localized disease at time of initial diagnosis.

Leucocytes ≤ULN 5-year survival % (95% CI)	Leucocytes >ULN 5-year survival % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
A) Complete case analysis (n=1.559)					
55.9 (52.5-59.2)	35.4 (30.9-40.1)	2.0 (1.7-2.3)	1.7 (1.5-2.0)	1.5 (1.3-1.8)	1.4 (1.2-1.7)
B) Restricted to clear cell RCC (n=501)					
67.4 (60.0-73.8)	42.4 (30.9-53.4)	2.5 (1.8-3.5)	2.1 (1.5-3.0)	1.9 (1.3-2.7)	1.9 (1.3-2.7)
C) Restricted to localized disease at time of initial RCC diagnosis (n=786)					
72.6 (68.3-76.5)	56.6 (48.7-63.7)	1.9 (1.5-2.5)	1.8 (1.3-2.3)	1.6 (1.2-2.1)	1.5 (1.1-2.0)

ULN: Upper limit of normal (leucocyte normal values for a person above 18 years are 3,5-10 $x10^9$/l)

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium and LDH

[c]Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium, LDH, hemoglobin, CRP and platelets

Supplemental table 7: The association between leucocyte levels at time of initial RCC diagnosis and mortality. Results from stratified analysis.

	Leucocytes ≤ULN 5-year survival % (95% CI)	Leucocytes >ULN 5-year survival % (95% CI)	Unadjusted HR (95% CI)	Adjusted HR[a] (95% CI)	Adjusted HR[b] (95% CI)	Adjusted HR[c] (95% CI)
Low hemoglobin						
Yes	43.2 (38.7-47.6)	30.5 (25.1-36.0)	1.5 (1.3-1.8)	1.5 (1.2-1.8)	1.4 (1.1-1.6)	1.3 (1.1-1.6)
No	70.2 (65.3-74.5)	44.6 (36.2-52.7)	2.6 (2.0-3.5)	2.1 (1.6-2.8)	1.9 (1.4-2.6)	1.8 (1.3-2.4)
High CRP						
Yes	43.9 (39.6-48.1)	30.9 (26.0-36.0)	1.6 (1.3-1.9)	1.5 (1.2-1.7)	1.4 (1.2-1.7)	1.4 (1.2-1.7)
No	75.1 (66.6-81.7)	46.1 (28.6-62.0)	2.7 (1.6-4.4)	2.5 (1.5-4.3)	2.4 (1.3-4.1)	2.3 (1.3-4.1)
Low Platelets						
Yes	35.1 (27.8-42.5)	21.3 (15.0-28.3)	1.7 (1.3-2.1)	1.7 (1.3-2.2)	1.8 (1.4-2.4)	1.9 (1.4-2.4)
No	56.7 (52.3-60.8)	40.9 (34.2-47.5)	1.7 (1.4-2.1)	1.5 (1.2-1.9)	1.3 (1.03-1.6)	1.2 (1.0-1.5)

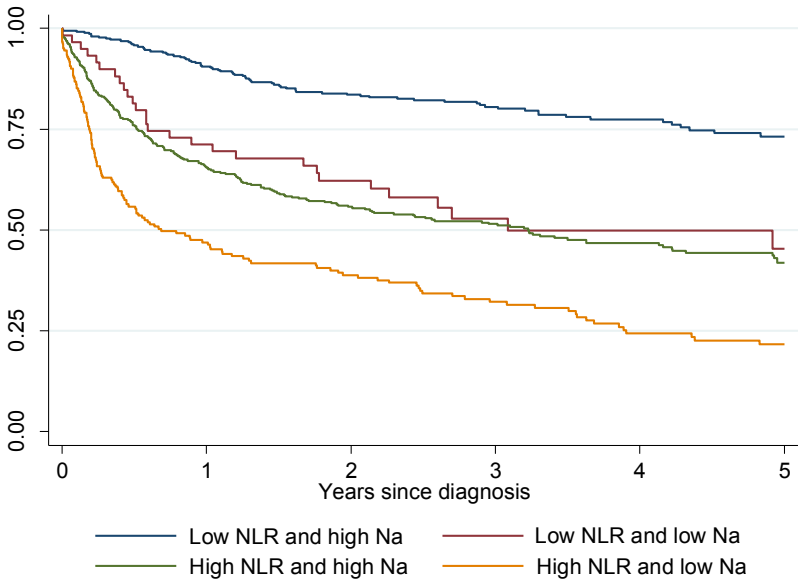
ULN: Upper limit of normal (leucocyte normal values for a person above 18 years are 3,5-10 $x10^9$/l)

[a]Model 1 = Adjusted for age, sex, stage and Charlson Comorbidity Index

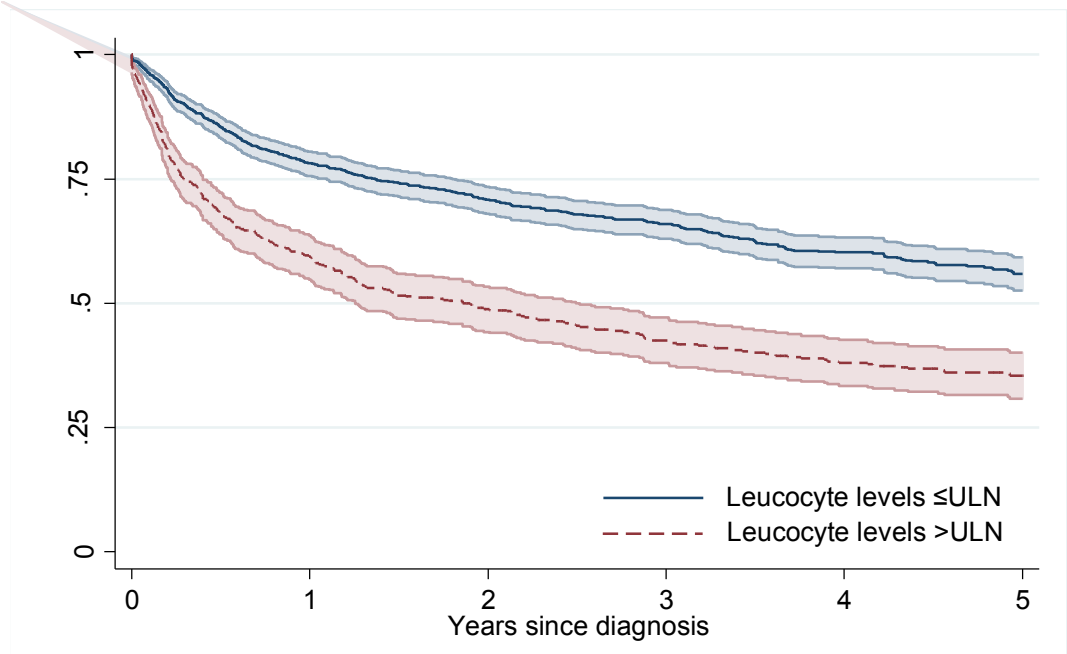
[b]Model 2 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium and LDH

[c]Model 3 = Adjusted for age, sex, stage, Charlson Comorbidity Index, calcium, albumin, sodium, LDH, hemoglobin, CRP and platelets

Supplemental figure 4: Kaplan-Meier curves for overall survival according to NLR and sodium (Na) groups at time of initial RCC diagnosis.



Supplemental figure 5: Kaplan-Meier curves with 95%-CI for overall survival according to leucocyte level group at time of initial RCC diagnosis.



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28. Vivian Langagergaard: Birth outcome in Danish women with breast cancer, cutaneous malignant melanoma, and Hodgkin's disease. PhD thesis. *2007*.

29. Cynthia de Luise: The relationship between chronic obstructive pulmonary disease, comorbidity and mortality following hip fracture. PhD thesis. *2007*.

30. Kirstine Kobberøe Søgaard: Risk of venous thromboembolism in patients with liver disease: A nationwide population-based case-control study. Research year report. *2007*.

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32. Mette Skytte Tetsche: Prognosis for ovarian cancer in Denmark 1980-2005: Studies of use of hospital discharge data to monitor and study prognosis and impact of comorbidity and venous thromboembolism on survival. PhD thesis. *2007*.

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37. Use of medical databases in clinical epidemiology. *2008*.

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39. Blodprop i hjertet - forekomst og prognose. En undersøgelse af førstegangsindlæggelser i Region Nordjylland og Region Midtjylland. *2008*.

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44. Lars Pedersen: Use of Danish health registries to study drug-induced birth defects – A review with special reference to methodological issues and maternal use of non-steroidal anti-inflammatory drugs and Loratadine. PhD thesis. *2009*.

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