

Histologic re-evaluation of a population-based series of renal cell carcinomas from The Netherlands Cohort Study according to the 2022 ISUP/WHO classification

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Abstract. The aim of the present study was to re-evaluate 457 renal cell carcinoma (RCC) cases from the Netherlands Cohort Study on Diet and Cancer (NLCS), a large population-based cohort, according to the new 2022 ISUP, Genitourinary Pathology Society and World Health Organisation (WHO) classifications to assess whether newly recognized subtypes of RCC could be found among these

cases. These cases were initially evaluated according to the 2004 WHO classification, the Fuhrman grading system and the 3rd version of the Tumor-Node-Metastasis (TNM). Data on tumor size, laterality and date of diagnosis, among other clinicopathological characteristics, were obtained through record linkage with the Netherlands Cancer Registry and the Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief. Digital slides from the NLCS were reviewed by two urogenital pathologists according to the new ISUP grading and the 2022 WHO classification (5th edition). Immunohistochemistry staining for carbonic anhydrase IX was performed on cases with ambiguous morphology. A total of 373 cases of clear cell RCC (ccRCC), 61 cases of papillary RCC (pRCC), 13 cases of chromophobe RCC, 3 cases of collecting duct carcinoma and 4 cases of oncocytoma were identified. The subtyping showed no discrepancy with the previous diagnoses. A comparison of the WHO/ISUP grading to the original Fuhrman grading showed a similar grading in 245 (56.5%) cases of the total ccRCC and pRCC cases. The staging according to the novel TNM classification 8th edition showed a restaging in 286 cases (65.5%). Lymphovascular (microvascular) invasion (LVI) and tumor necrosis (TN) were present in 14.4% and 33.5% of the total number of cases, respectively. Furthermore, the presence of sarcomatoid differentiation in 5.1% and rhabdoid differentiation in 4.2% of the cases was observed. In conclusion, none of the newly accepted and emerging/provisional RCC entities were identified in the NLCS cases, which could be attributed to the high mean age (71.4 years) at diagnosis of the patients included in the present study. A restaging of the NLCS cases using the TNM 8th edition and regrading using ISUP grading was performed, which showed that it is possible to report on newer features, such as sarcomatoid differentiation and LVI, even in an old sample collection.

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Abbreviations: RCC, renal cell carcinoma; ISUP, International Society of Urological Pathologists; WHO, World Health Organization; TNM, Tumor-Node-Metastasis; TN, tumor necrosis; LVI, lymphovascular (microvascular) invasion; NLCS, Netherlands Cohort Study on Diet and Cancer; GUPS, Genitourinary Pathology Society; NCR, Netherlands Cancer Registry; PALGA, Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief; ccRCC, clear cell RCC; pRCC, papillary RCC; chRCC, chromophobe RCC; FH, fumarate hydratase; FFPE, formalin-fixed-paraffin-embedded; RD, rhabdoid differentiation; CAIX, carbonic anhydrase IX; SDH, succinate dehydrogenase; UISS, University of California, Los Angeles integrated staging system

Key words: newly described RCC entities, LVI, necrosis, rhabdoid and sarcomatoid features, ISUP grading, TNM 8th edition

Introduction

Renal cell carcinoma (RCC) is a heterogeneous group of types of kidney cancer that arise from renal tubular epithelial cells. These types of cancer display divergent epigenetic and genetic abnormalities and are amongst the 10 most common types of cancer worldwide (1-4). Genetic factors such as the Von Hippel-Lindau and protein polybromo-1 genes have been associated with the pathogenesis of RCC (5). Over the last two decades, the classification of RCCs have undergone major changes based on histological presentation and molecular pathology and, in 2004, the World Health Organisation (WHO) classification recognized numerous histological RCC subtypes (6,7) with distinct genetic, biological and clinical behaviors (3,8). The most frequent subtypes of all cases of RCC are clear cell RCC (ccRCC, ~75%) (3,5), papillary RCC (pRCC, ~15%) (9,10) and chromophobe RCC (chRCC, ~5%) (11).

The growing understanding of the morphology, immunohistochemistry (IHC), genomics and epidemiology of RCC has allowed for an improved insight into the tumor biology and characterization of this disease (4,12-15). Therefore, in 2013, the International Society of Urological Pathology (ISUP) Vancouver consensus proposed a new classification of renal neoplasia including newly characterized RCC subtypes and other additional emerging/provisional entities (14-17). The new classification of RCC was revised by the WHO Renal Tumor Panel in 2015, with the results published in the 4th (14) and 5th (14,15) editions of the WHO Classification of Tumors of the Urinary System and Male Genital Organs Bluebook in 2016 and 2022 respectively.

Among the newly recognized epithelial renal tumors were fumarate hydratase (FH)-deficient RCC, succinate dehydrogenase (SDH)-deficient RCC, tubulocystic RCC, acquired cystic disease-associated RCC and clear cell papillary RCC (12,15,18,19). The aforementioned entities are now considered separate entities as their morphologies, immune-profiles and molecular characteristics have been adequately recognized (4,12,19,20). Previously, some of these entities were identified as unclassified types of RCC with aggressive features in younger adults with a mean age of ≤ 35 years (21-23). Other tumor types have been proposed as emerging/provisional entities; however, they have not yet been recognized by the WHO as separate entities due to a lack of sufficient evidence (14,19,24). Furthermore, certain oncocytic renal tumors have been described (19), such as eosinophilic vacuolated tumor and low-grade oncocytic renal tumor. High-grade oncocytic tumor has also been proposed to represent a potentially new renal entity (19,25). These new entities show divergent prognoses, varying from indolent to aggressive renal tumors (26) with correlated treatment implications. Moreover, correct diagnosis of the hereditary forms of these tumors has implications for affected family members (27). Therefore, accurate classification is important for prognosis, therapeutic treatment and genetic counselling (28).

In addition to the newly proposed and recognized subtypes, the Vancouver consensus proposed a new ISUP grading (16,29). The ISUP grading is similar to the well-established Fuhrman grading (30), i.e., it is also a four grade system, but relies on nucleolus identification to determine WHO/ISUP grade 1-3

and the presence of polymorphic giant tumor cells, sarcomatoid or rhabdoid differentiation (RD) features for assigning grade 4 (15,18). This new grading system was recommended to replace the Fuhrman grading as it is a more reproducible system (31,32).

Tumor-Node-Metastasis (TNM) staging has been considered the gold standard when predicting the prognosis of patients with RCC and for the guidance of patient management, surveillance and treatment (33). Moreover, it is continuously revised to improve its prognostic accuracy and predictive ability, and the 8th edition (12,34) is presently used by clinicians and pathologists (12,33,34).

In addition to TNM staging (35) and Fuhrman grading (36), tumor necrosis (TN) has been considered to be a prognostic factor (37) and evidence for this has been published for ccRCC and chRCC, independent of tumor stage and grade (14,37-39). Moreover, lymphovascular (microvascular) invasion (LVI), excluding that within the perinephric or renal fat which is already described in the pT3a part of TNM staging, has been reported to correlate with survival, independent of tumor size, grade or type (40,41).

Considering these developments, the aim of the present study was to review pathological slides of kidney tumors from the Netherlands Cohort Study on Diet and Cancer (NLCS) (42-44) in order to reclassify them by morphotype, TNM stage, ISUP grade, LVI and necrosis, according to the ISUP and the 2022 WHO classification (5th edition). A further aim was to assess whether newly accepted entities could be identified in this dataset of elderly patients. To the best of our knowledge, this is the first study to report such a re-evaluation on a large, unselected population-based series of RCCs with extensive clinical data.

Materials and methods

Study population. A total of 457 cases of RCC from the NLCS were reviewed. The NLCS is a prospective cohort study that has previously been described in detail (44-46). In summary, this series was initiated in September 1986 and included 120,852 men and women, aged 55-69 years at diagnosis. The collection of the samples was limited to cases diagnosed before the 31st of December 2008. Follow-up for cancer occurrence was available through computerized record linkage with the Netherlands Cancer Registry (NCR) and Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA), a national database of pathology reports (47) as described previously (46). After 22.3 years of follow-up, 659 histologically confirmed RCC cases were eligible for collection of formal in-fixed-paraffin-embedded (FFPE) tumor tissue from 51 pathology laboratories throughout The Netherlands. Data on tumor characteristics, such as laterality, date of diagnosis, TNM stage, initial treatment and other clinicopathological characteristics were obtained through record linkage with the NCR (46). Pathology reports were used to record the tumor size and to verify the staging information from the cancer registry.

Tissue collection. FFPE tumor tissues were collected after ethical approval by the Medical Ethical Committees of Maastricht University (Maastricht, The Netherlands), PALGA

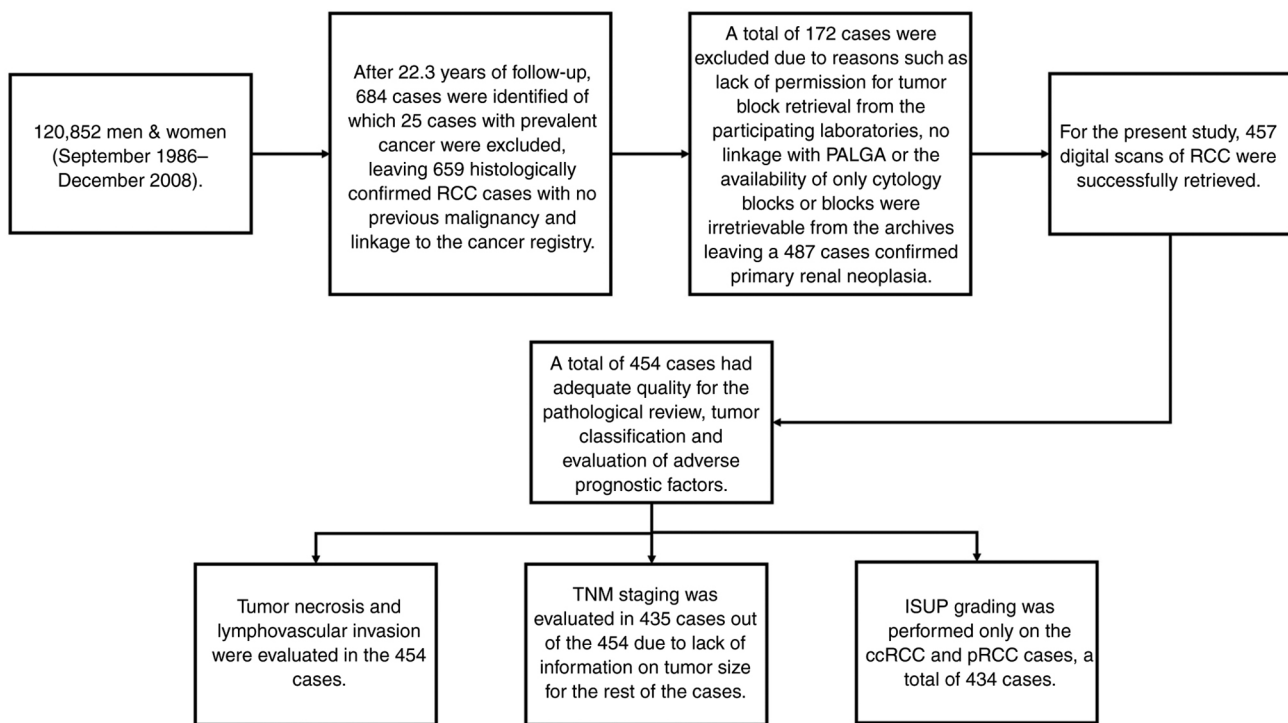


Figure 1. Flow chart showing the Netherlands Cohort Study on Diet and Cancer series from collection to the second pathology review performed in the current study. RCC, renal cell carcinoma; ISUP, International Society of Urological Pathologists; TNM, tumor-node-metastasis; ccRCC, clear cell RCC; pRCC, papillary RCC; PALGA, Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief.

and the NCR. Tissue collection was performed at the time of diagnosis for the cases diagnosed between 1986–1996 and was later extended to include cases diagnosed between 1997–2008 (45,47). Urothelial cell carcinomas were excluded and only histologically confirmed epithelial cancers were included. This resulted in the collection of a total of 487 cases of confirmed primary renal neoplasia.

Original pathology review. The original tumor blocks were retrieved from all participating laboratories and hematoxylin and eosin (H&E) slides were made at the laboratories of Maastricht University Medical Center (Maastricht, The Netherlands) and Radboud university (Nijmegen, The Netherlands). H&E-stained slides of all collected FFPE tumor tissues were assessed by two experienced urogenital pathologists, to confirm tumor histological subtype based on the 2004 WHO classification (33). Nuclear grading was performed according to the Fuhrman grading system. After revision, cases showing <10% malignant cells or cases that were reclassified as urothelial cell carcinomas were excluded, leaving a total of 487 confirmed RCC cases.

Second pathology review according to the new classifications of renal tumors, using the new ISUP grading and the 8th TNM edition. For the present study, 457 digital scans of RCC cases were retrieved for inclusion in the re-evaluation which applied the new 2022 ISUP/WHO classification. Fig. 1 shows the NLCS cases from the collection to the second pathology review. These scans were originally made from the tumor slides that were selected by the pathologists as those being the most representative of the tumor subtype. The scans were made

using a Ventana iScan HT scanner (series number, BI12N7070; Roche Tissue Diagnostics), in the diagnostics facility at the Department of Pathology at the Maastricht University Medical Center, which met an internal standard quality assurance check procedure (48). If the quality of the digital slides were inadequate for the evaluation of the nucleus details, the original H&E slides were re-scanned and evaluated for a second time. Two experienced urogenital pathologists both confirmed that all digital slides included in the present study were of the quality required for the re-evaluation process.

All tumors were re-evaluated and reassigned according to histological subtype, nuclear grade, TN, sarcomatoid differentiation, RD, novel nuclear ISUP grade and LVI, using the latest TNM version and, ISUP and WHO diagnostic criteria (4,15,16,20), independently by a urogenital pathologist at Maastricht University Medical Center and an expert urogenital pathologist from the Johns Hopkins University School of Medicine (Baltimore, USA). A total of two autopsy cases were included in the pathological revision but were excluded from the TNM reclassification. Furthermore, data on tumor size were used to assign the pathological T stage according to the TNM 8th edition. Both pathologists were blinded for the outcomes of previous pathological reviews.

IHC. IHC staining for carbonic anhydrase IX (CAIX) was performed on ccRCC cases that warranted further subtyping confirmation. The staining was performed on RCC tissue sections (3–4 μ m) from the FFPE tissue blocks. Firstly, the slides were deparaffinized at room temperature in xylene, rinsed in a decreasing alcohol concentration series and then rinsed in water. Samples were thereafter treated at room temperature with

0.3% hydrogen peroxide in methanol for 20 min. Afterwards, the slides were washed 3x at room temperature in 1X PBS. Subsequently, the antigen retrieval pretreatment was performed using 1X citrate antigen retrieval buffer (pH 6.0, 10X diluted in water; Dako; Agilent Technologies, Inc.; cat. no. S2369) in the microwave for 20 min at 600 watt. Staining was performed on an Autostainer Plus Link 48 System (Agilent Technologies, Inc.). All steps were performed at room temperature. Samples were covered with Endogenous Peroxidase Blocking Reagent (Agilent Technologies, Inc.) for 5 min and then washed with 1X PBS. The slides were then incubated for 20 min with rabbit anti-CAIX primary antibody (1:1,000; cat. no. NB100-417; Novus Biologicals, LCC) diluted in Agilent antibody diluent (cat. no. K8006; Agilent Technologies, Inc.). Secondary detection and visualization were performed using the EnVision FLEX+ detection system (cat. no. K8002; Agilent Technologies, Inc.). In brief, slides were incubated for 20 min with labelled polymer (EnVision FLEX-HRP; Agilent Technologies, Inc.) and then the slides were incubated for 10 min with substrate buffer and DAB chromogen solution. Counterstaining with hematoxylin for 90 sec at room temperature, subsequent dehydration in an increasing alcohol series and cover slips were added using a Leica Histocore (Leica Microsystems, Inc.). Slides were evaluated by a urogenital pathologist (IS) using a light microscope.

Statistical analysis. In the present study, the χ^2 test was performed using SPSS 28 (IBM Corp.).

Results

Patient characteristics. A total of 457 patients were included in the present study. There was a predominance of male patients (62.6%). The age at diagnosis of the included patients ranged from 56-88 years with a mean age of 71.4 ± 6.3 years. The mean tumor size was 67.2 ± 31.7 mm (Table I).

Pathological review and classification of the tumors. A total of three scans from the 457 cases were excluded due to poor quality and digital scans of the representative sections for 454 cases were available for revision. These scans were independently reviewed and classified based on morphological criteria by two pathologists based on the 2022 WHO classification, the ISUP recommendations and the Genitourinary Pathology Society (GUPS) update on renal neoplasia. The subtyping showed a 100% overlap with the previous diagnoses. The renal cell neoplasia included 373 ccRCC cases (82.1%), 61 pRCC cases (13.4%), 13 chRCC cases (2.9%), 3 cases of collecting duct carcinoma (0.7%) and 4 cases of oncocytoma (0.9%) (Table II). In 30 ccRCC cases, the diagnosis was confirmed by IHC staining for CAIX, which showed a box-like staining pattern (Fig. 2). Furthermore, none of the tumors demonstrated features compatible with FH-deficient RCC, SDH-deficient RCC, eosinophilic solid and cystic RCC or other recently described entities (12).

Tumor grade according to the new ISUP grading system and reporting on sarcomatoid and rhabdoid features. Initially, all 434 ccRCC and pRCC cases were graded according to the Fuhrman grading system by two pathologists (Tables I and III). Tumors were assigned grade 1 in 54 cases (12.4%), grade 2 in

Table I. Clinical characteristics of patients included in the present study.

Clinical characteristic	Number
Number of patients, n	457
Mean age at diagnosis (range), years	71.4 (56-88)
Sex, n (%)	
Male	286 (62.6)
Female	171 (37.4)
Mean tumor size \pm SD, mm	67.2 ± 31.7
Original histological review, n (%)	
Clear cell	375 (82.1)
Papillary	62 (13.6)
Chromophobe	13 (2.8)
Collecting duct carcinoma	3 (0.7)
Oncocytoma	4 (0.9)
Pathological T stage ^a , n, (%)	435 (95.2)
1	22 (5.1)
1a	1 (0.2)
1b	3 (0.7)
2	262 (60.2)
3	1 (0.2)
3a	73 (16.8)
3b	69 (15.9)
4	4 (0.9)
Fuhrman grade ^b , n (%)	434 (95.0)
1	54 (12.4)
2	196 (45.2)
3	143 (32.9)
4	41 (9.4)

^aClinical data on tumor size was available for 435 cases, ^bFuhrman grading was performed on clear cell and papillary cases only. T stage, tumor stage.

196 cases (45.2%), grade 3 in 143 cases (32.9%) and grade 4 in 41 cases (9.4%). All ccRCC and pRCC cases were regraded using the new WHO/ISUP grade by two pathologists blinded to the original Fuhrman grading (Tables II and III). This resulted in the assignment of ISUP grade 1 in 93 cases (21.4%), grade 2 in 191 cases (44.0%), grade 3 in 108 cases (24.9%) and grade 4 in 42 cases (9.7%). Comparison of the two grading systems showed the same tumor grade in 245 cases (56.5%), whereas a different grade was reported in 189 cases (43.5%) (Table III). Of the 454 cases histologically reviewed, sarcomatoid differentiation was identified in 23 patients (5.1%) and RD in 19 (4.2%).

Adverse prognostic factors. TN and LVI were also assessed and evaluated in the 454 cases. TN was identified in 152 cases (33.5%) and, tumor necrosis (TN) was evaluated as the percentage of tumor necrosis in relation to the total tumor volume as previously described (49,50). A total of 35 tumors (23.0% of the 152 cases) exhibited a TN of $\leq 5\%$, 84 tumors (55.3% of the 152 cases) showed 6-49% TN, 29 tumors (19.1% of the 152 cases) showed 50-89% TN, and 4 tumors (2.6% of

Table II. Clinical characteristic results of the re-evaluation of the histology of a population-based series of RCC cases from the NLCS 1986-2008, according to the 2022 ISUP grading systems and WHO classification.

Clinical characteristic	Number (%)
Histological review ^a , n (%)	454 (99.3)
Clear cell	373 (82.2)
Papillary	61 (13.4)
Chromophobe	13 (2.9)
Collecting duct carcinoma	3 (0.7)
Oncocytoma	4 (0.9)
Pathologic T stage ^b , n (%)	435 (95.8)
1a	94 (21.6)
1b	115 (26.4)
2	4 (0.9)
2a	50 (11.5)
2b	25 (5.7)
3	1 (0.2)
3a	73 (16.8)
3b	69 (15.9)
4	4 (0.9)
Sarcomatoid differentiation, n/total (%)	23/454 (5.1)
Rhabdoid differentiation, n/total (%)	19/454 (4.2)
Lymphovascular invasion, n/total (%)	64/454 (14.1)
Necrosis present, n (%)	152/454 (33.5)
Necrosis present per morphotype, n necrosis present/n total cases of the morphotype (%)	
ccRCC, n (%)	111/373 (29.8)
pRCC, n (%)	38/61 (62.3)
chRCC, n (%)	2/13 (15.4)
CDC, n (%)	1/3 (33.3)
Oncocytoma, n (%)	0/4 (0.0)
ISUP grade for ccRCC and pRCC ^c , n (%)	434 (95.6)
1	93 (21.4)
2	191 (44.0)
3	108 (24.9)
4	42 (9.7)

^a3 scans excluded due to poor quality, ^btumor size available for 435 cases. Cases noted as Tumor-Node-Metastasis 2 or 3 which were not further specified as 2a, 2b, 3a or 3b, lacked the needed information on tumor size and therefore the original staging information from the cancer registry was used. ^cISUP was performed on ccRCC and pRCC cases only. ccRCC, clear cell renal cell carcinoma; CDC, collecting duct carcinoma; ISUP, International Society of Urological Pathologists; NLCS, Netherlands Cohort Study on Diet and Cancer; pRCC, papillary RCC; T stage, tumor stage; WHO, World Health Organization.

the 152 cases) showed $\geq 90\%$ TN. TN was present more often in pRCC (38/61, 62.3%) compared with ccRCC (111/372, 29.8%) (χ^2 24.46, $P < 0.05$).

Table III. Comparison of nuclear grading classification according to the Fuhrman and ISUP grading systems on the Netherlands Cohort Study on Diet and Cancer, 1986-2008.

Grading system Fuhrman, n	ISUP, n				Total, n
	1	2	3	4	
1	34	17	3	0	54
2	58	111	26	1	196
3	1	61	70	11	143
4	0	2	9	30	41
Total, n	93	191	108	42	434

ISUP, International Society of Urological Pathologists.

LVI was identified in 64 renal neoplasms (14.1%). Invasion in the renal vein or its segmental branches was classified as a pT3a tumor (Table I). LVI was seen more often in ISUP grade 3 tumors (27/64 cases, 42.2%), followed by ISUP grade 2 (19/64 cases, 29.7%) and ISUP grade 4 (13/64 cases, 20.3%). Notably, LVI was seen in 5 cases of ISUP grade 1 ccRCC.

TNM staging according to the 8th edition. A total of 435 RCC cases were restaged according to the 8th edition of the TNM version as shown in Table II as information on tumor size was only available for 435 cases. This restaging resulted in the assignment of pT1a in 94 cases (21.6%), pT1b in 115 cases (26.4%), pT2 in 4 cases (0.9%), pT2a in 50 cases (11.5%), pT2b in 25 cases (5.7%), pT3 in 1 case (0.2%), pT3a in 73 cases (16.8%), pT3b in 69 cases (15.9%) and pT4 in 4 cases (0.9%). Comparison of the 8th edition of the TNM staging with the 3rd edition of the TNM staging that was originally applied to the NLCS cases showed a restaging in 65.5% of the cases. Table IV presented the comparison of the 3rd and the 8th edition of the TNM classification of the NLCS cases. The restaging in the present study showed that more cases were categorized in a lower TNM stage compared to the original classification, as 60.2% of the cases were originally assigned as pT2.

Discussion

In the present study, the aim was to review RCC cases from the large, prospective NLCS cohort according to the latest 2022 WHO classification and the latest updates of GUPS and ISUP on renal tumors, to evaluate the presence of newly recognized or emerging/provisional entities. Furthermore, an evaluation of whether recently accepted renal tumor subtypes can be identified in an existing cohort of patients with RCC was performed. Moreover, the present study also aimed to classify these cases according to the new ISUP grading, to assess TN, LVI and the presence of sarcomatoid differentiation and RD features, and to apply the latest TNM edition. None of the reviewed cases showed any of the newly described entities and all the cases showed the formerly well-recognized and most common RCC subtypes. The re-evaluation of the subtyping was completely in accordance with the previous diagnoses of these RCC cases.

Table IV. Comparison of the 3rd and 8th edition of the TNM classification on the Netherlands Cohort Study on Diet and Cancer, 1986-2008.

TNM edition TNM 3rd, n	TNM 8th, n									Total, n
	1a	1b	2	2a	2b	3	3a	3b	4	
1	22	0	0	0	0	0	0	0	0	22
1a	1	0	0	0	0	0	0	0	0	1
1b	1	1	0	0	1	0	0	0	0	3
2	70	114	4	50	24	0	0	0	0	262
3	0	0	0	0	0	1	0	0	0	1
3a	0	0	0	0	0	0	73	0	0	73
3b	0	0	0	0	0	0		69	0	69
4	0	0	0	0	0	0	0	0	4	4
Total, n	94	115	4	50	25	1	73	69	4	435

TNM, Tumor-Node-Metastasis.

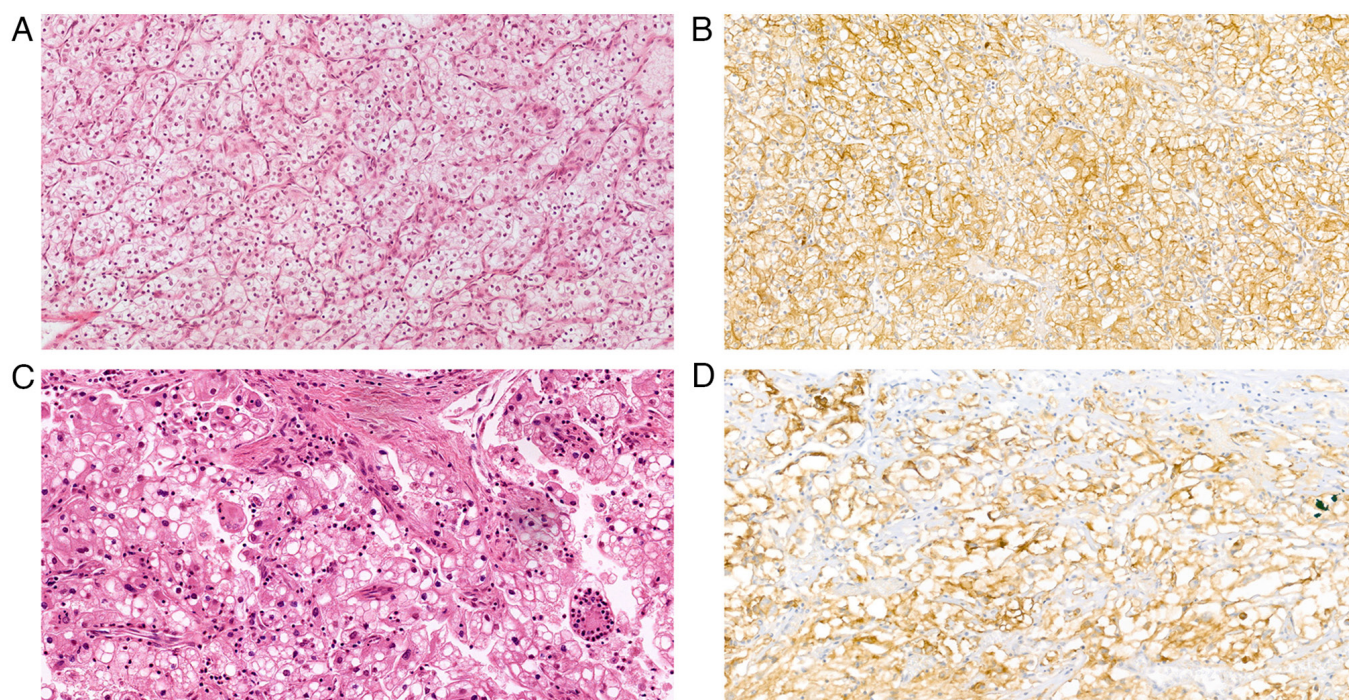


Figure 2. Histopathological images of representative cases of ccRCC. (A) A typical case of ccRCC with architectural features and clear cell morphology (H&E staining; magnification, x20). (B) IHC staining for CAIX showing a strong diffuse classical 'box-shape' staining pattern of ccRCC (magnification, x10). (C) A case of ccRCC with giant multinucleated cells (H&E staining; magnification, x20). (D) IHC staining with CAIX showing strong diffuse staining, confirming the diagnostics of ccRCC (magnification, x20). CAIX, carbonic anhydrase IX; ccRCC, clear cell renal cell carcinoma; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

Comparison of the ISUP and Fuhrman gradings on the ccRCC and pRCC cases showed a different grading in 43.5% of the cases. Evaluation of the presence of sarcomatoid differentiation and RD features revealed their presence in the NLCS cases. Furthermore, assessment of the presence of adverse prognostic features showed that TN was also present in 33.5% of the cases and that it was present more often in pRCC cases (62.3%) compared with other subtypes. Hemorrhage and necrosis, however, are known to be related to the pRCC subtype but not to prognosis, and therefore are not used as

adverse prognostic indicators in pRCC. Furthermore, LVI was similarly identified in few cases and was mostly detected in tumors with ISUP grade 3. Comparison of the TNM 8th edition and the previously applied TNM 3rd edition revealed a restaging in the majority of cases. However, this difference, as well as the difference seen between the Fuhrman and the ISUP grading, were to be expected as in both situations different grading criteria were applied.

In the present study, none of the newly described or emerging entities were identified. This could be explained

by the fact that the NLCS cohort included patients with a mean age of 71.4 years and some of the newly described entities, such as the SDH-deficient RCC subtype, have been reported to be particularly seen in younger adults (12). Thus, Kwon *et al* (51) reported a reclassification of a proportion (13%) of adults with unclassified RCC in a patient cohort with a mean age at presentation of 58 years old. Clemmensen *et al* (52) reclassified a subset of early onset RCC in patients aged <46 years. Li *et al* (23) re-evaluated oncocytic renal tumors in patients aged ≤35 years. These findings suggested a low chance of finding a newly described entity in the research databases and diagnostic archives consisting of an elderly patient cohort.

However, in the present study there were 30 cases with a differential diagnosis between ccRCC and the translocation-related RCC (such as Xp11 translocation RCC), based on the morphological images. Therefore, an additional test was necessary to confirm the diagnoses of these cases. According to the literature (53) and the widely used WHO 2022 diagnostic criteria, IHC CAIX staining is a specific and sensitive marker of ccRCC, since ccRCC has a 'box-shape' staining pattern and translocation-related RCC has a negative staining result. All tested cases showed a strong membranous 'box-shaped' expression of CAIX. Therefore, CAIX was used in the present study to confirm the diagnosis of ccRCC. It can therefore be postulated that using a limited IHC panel can also be sufficient to confirm the diagnosis, especially when reviewing a large cohort database that is used for multiple research purposes and where limited tissue availability can be a limitation.

The differences in grading that were seen for the originally applied Fuhrman grading on the NLCS cases and the recently applied ISUP can be explained by the differences between these two grading systems. Despite the fact that Fuhrman grading was accepted worldwide and has been employed for many years, several studies have reported its pitfalls, including questionable prognostic value and suboptimal interobserver reproducibility (24,31,54). This is due to the fact that Fuhrman grading relies on the simultaneous assessment of nuclear size, nuclear shape and nucleolar prominence and there is no further direction on how this should be handled when these three parameters provide conflicting information (29). In contrast, the ISUP grading system relies only on the size of the nucleolus for grading tumors 1-3 and on the presence of giant cells or, the presence of sarcomatoid differentiation or RD features for assigning grade 4 (18,29). Previously, several studies compared Furman grading with WHO/ISUP grading (55,56). In these studies, the WHO/ISUP grading was shown to provide superior prognostic information compared to Fuhrman grading (55,56). Therefore, the regrading of the kidney tumors in the research databases could be a reasonable procedure.

Additionally, reporting on adverse prognostic factors such as TN and LVI was also proposed in the ISUP consensus. TN has been reported to have prognostic significance for ccRCC and chRCC, independent of tumor stage and grade (50,57). However, pRCC tumors often contain areas of necrosis, the presence of which in this tumor type lacks the same significance (12). TN may also influence treatment efficacy as, for example, the response to VEGF/tyrosine kinase inhibitor-targeted therapy has been shown to be poor in patients with metastatic disease where there was ≥10% necrosis in

the primary ccRCC tumor (58). Therefore, assessment of the extent of tumor necrosis is recommended for reporting of kidney specimens by the International Collaboration on Cancer Reporting (59). LVI, either intratumoral, peritumoral or perirenal, has been reported to relate to metastasis rate and patient disease free survival, independent of tumor size, primary tumor category and grade (59). However, despite the fact that macroscopic tumor invasion into the renal and caval vein has been incorporated into the well-known American Joint Committee on Cancer and University of California, Los Angeles integrated staging system (UISS) (14), the predictive ability of LVI is debatable (40). This is due to the fact that certain studies have reported LVI as having been correlated with prognosis whereas others reported no association with prognosis (60-64).

The NLCS cases in the present study were originally evaluated using the TNM version that was applicable at the time of diagnosis, but all the cases were later converted to the 1987 3rd TNM version (65). However, there are significant differences between the 1987 TNM version and the currently used 8th edition, as the new TNM version has proven to be more concise and reproducible than all the previously published versions. The restaging performed in the present study showed that more cases were categorized to a lower TNM stage compared to the original classification. For example, 60.2% of cases were previously assigned pT2 while with restaging, only 11.6% of cases were assigned to pT2a and 5.8% to pT2b. Furthermore, a higher percentage in the pT1a and pT1b stages was seen, which were 21.6 and 26.4%, respectively, compared to the previously assigned staging (6% according to the TNM 3rd version). This could be explained by the major differences between the TNM classifications, namely the boundary values for assigning pT1a, pT1b, pT2a and pT2b. In the TNM 8th edition, T1a is assigned to tumors that are confined to the kidney and are <4 cm, and pT1b is assigned to tumors that are also confined to the kidney but are 4-7 cm. Furthermore, T2a is assigned to tumors that are limited to the kidney and are 7-10 cm and T2b is assigned to the tumors that are >10 cm but confined to the kidney; however, in the TNM 3rd edition, pT1 tumor was defined as <2 cm.

In the present study, despite the unique characteristics of the large population-based series with extensive clinical characteristics, there were certain limitations. Specifically, the age of the patients included in the analysis, including patients aged ≥55 years at baseline (46), hindered the possibility of reporting on some of the new entities that are mostly identified in younger patients. Another limitation was the lack of tumor size information for 5 cases, which impeded the conversion to the latest TNM version. This was hampered by the lack of access to the original clinical files and a reliance on the information obtained from the NCR and pathology reports. Furthermore, assessment was performed on the TN of representative digital slides and these slides were originally chosen by the pathologists as the ones being most representative of the tumor subtype and not necrosis. However, these scans should have been a reliable representation of tumor necrosis since a range of necrosis was observed in the cohort. If only viable tumor sections had been selected as representative slides, there would be fewer cases with tumor necrosis. Only one IHC marker, CAIX, was used to confirm the diagnosis of ccRCC in 30 cases, which was considered sufficient due to its specificity

and sensitivity (53). Furthermore, in large research cohorts, the application of extensive tests must be carefully considered when it comes to the use of tissues with limited availability and costs. Molecular testing was not performed on the revised slides as no recently described entities were identified based on the re-evaluation by two urogenital pathologists. Molecular studies are also mostly indicated when IHC is not conclusive and molecular studies are not routinely used.

In conclusion, to the best of our knowledge, the present study is the first to re-evaluate renal neoplasms from a large population-based prospective cohort that is extensively used for research purposes. The findings emphasize that the newly described entities are a minor component of the cases when analyzing a cohort of patients with a high average age. Moreover, the evaluation of additional prognostic factors in this existing cohort, such as ISUP, TNM 8th edition and rhabdoid/sarcomatoid features, allows for the updating of previously published prognostic models and comparison of these to other current prognostic models, such as the UISS and the Stage, Size, Grade and Necrosis system.

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Availability of data and materials

The datasets used and/or analyzed during the current are available from the corresponding author on reasonable request.

Authors' contributions

SO, IVS, LJS and KMS were responsible for the conception and design of the study and the acquisition of data. SO, IVS, AM, LJS and KMS were responsible for the analysis and interpretation of data. SO, IVS, LJS and KMS were responsible for drafting the manuscript and revising it critically for important intellectual content. JAAVDP, JVDM, GR, EG, MVE and AZH were responsible for the acquisition, analysis and interpretation of data, and drafting and critically revising the manuscript. MMLLB and CAHVDK were responsible for the conception and design of the study, drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final version of the manuscript. SO, IVS, LJS and KMS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Medical Ethical Committee of the Maastricht University (Maastricht, The Netherlands; approval nos. MEC 00-086.2 and MEC 85-012-8).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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