Immunoexpression Patterns for Ki-67 in Renal Cell Carcinoma

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ABSTRACT

Introduction and Objectives. Renal cell carcinomas (RCCs) notoriously demonstrate unpredictable and pleomorphic clinical evolution patterns. The tumor volume doubling time must therefore be viewed as a variable, individual and case-specific RCC behavioral element. Originally, Ki-67 was identified in the nuclei of Hodgkin lymphoma cells, being highly expressed during the cell cycle, but markedly inhibited in the G0 phase. This characteristic has made Ki-67 an important clinical marker of cellular proliferation. The relationships between Ki-67 and tumor volume doubling rate and degree of differentiation have been very well studied for some human tumors, such as breast, gastric or malignant lymphomas. Data regarding this aspect in renal tumors are scarcer, the results are often controversial and the prognostic role uncertain.

Materials and Methods. In this study, we investigate the Ki-67 immunoexpression patterns in RCC tumor tissue and tumor-adjacent healthy renal parenchyma, using immunohistochemistry (IHC), i.e. a Ki-67-targeted monoclonal antibody, clone MM1. Our study population is comprised of a consecutive series of 134 RCC cases (Arad County Hospital, 2015-2018). We collected all the available and relevant clinical records. Paraffin-embedded, post-radical/partial nephrectomy, tumor specimens were obtained and resampled. After hematoxylin-eosin (HE) staining, pathological reevaluation allowed for RCC diagnosis and morphological subtyping. The study inclusion criteria for Ki-67 IHC analysis was artefact free, viable RCC tumor tissue slides, lacking extensive areas of necrosis. For the Ki-67-targeted immunoreaction, we used an external positive control (multiple in vivo healthy skin samples). A positive Ki-67 immunoreaction was considered valid only when the final reaction product was strictly nuclear restricted, without cytoplasmic staining. Anisocarias and mitoses were evaluated and quantified.

Results. In total, 120 RCC samples met the study inclusion criteria and were stained for Ki-67. Regarding the tumor-adjacent renal tissue, very rare cells (<1%) with Ki-67-positive nuclei were seen (in the mesangium, tubular system and inflammatory infiltrate). RCC Ki-67 immunoreactivity was characterized by marked inter and intratumoral

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heterogeneity, with some areas being showing major agglomerations of positive nuclei. Ki-67 positivity was more evident at the periphery of the tumor, in the immediate vicinity of the proliferation front, and around neoformation blood vessels. Practically, each case presented defined values of the proliferation index, and thus Ki-67 becomes an individual prognostic marker. Only 12 RCC cases showed negative Ki-67 tumor tissue. When mitoses were absent and anisokarya was present, the Ki-67 proliferation rate was typically ranked between 2-10%, whereas marked mitoses and anisokarya was associated with an increased Ki-67 labeling index. No statistically significant correlation was seen between RCC nuclear grade and Ki-67 immunoreactivity. Poorly differentiated and/or sarcomatoid RCCs had an increased Ki-67 immunoreactivity, whereas, in papillary and microcystic clear cell RCCs, Ki-67 was underexpressed.

Conclusions. In conclusion, our data demonstrates a relatively low rate of cellular proliferation within the RCCs evaluated, particularly in the microcystic clear cell and papillary RCC subtypes. However, Ki-67 immunoreactivity did not correlate with the degree of RCC differentiation. Ki-67 should be viewed as an individual and independent prognostic marker for RCCs. Additionally, Ki-67 immunoreactivity may prove useful for treatment response assessment, as an objective indicator of the effectiveness of systemic humanized growth factor inhibition therapy. Most likely, Ki-67 will at one point become a part of the standard pathological evaluation of all renal tumors.

Key-words: Ki-67, renal cell carcinoma, immunohistochemistry, cellular proliferation rate, biomarkers, pathological assessment, risk stratification, treatment response assessment.

INTRODUCTION AND OBJECTIVES

RCCs notoriously demonstrate unpredictable and pleomorphic clinical evolution patterns, as seen in a significant majority of the RCC cases that allowed for the observation of natural disease evolution, i.e. managed paleatively with the watchful waiting approach. Many RCCs, depending on specific subtype, may display indolent local growth profiles, with considerably delayed systemic dissemination rates. Initially, early stage small renal tumors will most often be asymptomatic, with their detection being, implicitly, incidental and/or accidental. The tumor volume doubling time must therefore be viewed as a variable, individual and case-specific RCC behavioral element. Even so, most authors agree that these early stage small renal tumors will most likely be able to evolve locally for years, before becoming symptomatic and clinically apparent. These clinical observations corroborate the amounting histopathological reports, focusing on the particularities of RCCs and their constitutive cellularity. Herein, the remarkable uniformity of replicative cells in the highly prevalent proliferative variants of RCCs must be noted. In fact, poorly differentiated and anaplastic RCCs represent the rarest morphological presentations. Conversely, even though these rare RCC presentations are characterized by a remarkable, local and later-on systemic, clinical aggressiveness, their cellularities do not generally display the intuitively expected abundance of replicative modifications (anisokarya, anisocytosis and mitoses - typical or atypical), which are actually and surprisingly represented in only a minority of cases.

This disparity, i.e. the lack of mitoses and/or anaplastic transformation in otherwise clinically aggressive RCCs, can be attributed primarily to the relatively long cell cycle of these transformed RCC cells, with a longer life span in interkinesis, and a short division cycle. Thus, the characteristic and quantifiable moments of cellular division are more difficult to synchronize with and capture through conventional pathological staining methods, on RCC sample preparations made from formalin-fixed specimens. Furthermore, cytology studies performed on RCCs, which use native cell samples, untreated with alcohol or toluene solutions, were similarly incapable of providing a clear conclusion regarding the division capabilities of RCC cellularity. For this purpose, a histochemical method based on silver salts, which identifies nucleolar organizers (AgNOR), was developed and introduced many years ago. AgNORs have been shown to be more numerous the higher the RCC proliferation rate is. The method can also highlight tumor cells in mitotic or atypical division, as is the case in most malignant tumors. Unfortunately, the histochemical reaction has proven to be technically capricious and it is very difficult to standardize this procedure for studies on a large number of cases.

Upon this humble early histochemical background, after 1990, immunohistochemical markers of cell proliferation were introduced. The first marker in this category, to report reproducible results was proliferating cell nuclear antigen (PCNA). However, clinico-pathological observations, made on large series of patients with malignant tumors, have indicated that the PCNA immunoreaction results had consistently overestimated the actual data regarding division, because of the innate limitation of staining both the nuclei of actively dividing cells, but also the nuclei of inactive cells that have divided within the last 48 hours. Under these conditions, the results obtained for PCNA did not correlate with clinical-pathological prognostic parameters and were not useful for the therapeutic strategy applied in malignant tumors. For these reasons, after several years of use, most authors have given up on PCNA identification and quantification.

Originally, Ki-67 was identified in the nuclei of Hodgkin lymphoma cells [1], being highly expressed during the cell cycle, but markedly inhibited in the G0 phase [2]. This characteristic has made Ki-67 an important clinical marker of proliferation, with utility in refining the degree of differentiation in many human cancers [3,4]. The prognostic value of this marker has been demonstrated in large patient series [5,6]. Although clinically useful in a large number of human malignancies, much less data is available regarding the molecular functions of Ki-67. A protein found in all vertebrates, Ki-67 is encoded by the MKI67 gene in humans. Two proteins with different molecular weights were initially described, and three variants

with different expression patterns were identified in humans. Ki-67 isoforms may differentially impact cell proliferation. The relationships between Ki-67 and tumor volume doubling rate and degree of differentiation have been very well studied for some human tumors, such as breast, gastric or malignant lymphomas. Data regarding this aspect in renal tumors are scarcer, the results are often controversial and the prognostic role uncertain. For these reasons, as a part of the current thesis, we aimed to analyze the results of the immunoreaction for Ki-67, by comparison with the histopathological form and the degree of differentiation.

MATERIALS AND METHODS

After obtaining adequate Institutional Review Board approval (26/28 September 2018), and using IHC as an investigative method, we proceeded to analyze RCC biological samples. The global RCC population of the current study is comprised of a consecutive series of 134 RCC cases, managed clinically at Arad County Hospital, within the Urology Department, from 2015-2018. All patients had undergone partial or radical nephrectomy directly. Neoadjuvant systemic therapy was not administered in any of the RCC cases. The resulting native RCC specimens were further processed and paraffin-embedded within the local Morphopathology Department. Pathological evaluation offered the definitive and complete RCC diagnosis, alongside the pathological T stage. For this global RCC population, we collected all the available relevant clinical records.

Paraffin-embedded, post-radical/partial nephrectomy, tumor specimens were obtained. A microtome was used to obtain multiple, 3 µm thick, sections for each case. These sections were further processed, by being positioned on albumin-treated, silanized tissue glass slides and stretched out in a liquid environment (one drop of distilled water was added), in order to avoid artefact formation. After the excess distilled water was removed, the slide was thermally treated (thermostat, 58 degrees Celsius, for a minimum of 30 minutes). After a preliminary deparaffination stage (benzene, 58 degrees Celsius, for 30 minutes), the tissue slides are stained with HE, in an automated manner, using the Leica Autostainer XL (Leica Biosystem Newcastle Ltd, Balliol Business Park West, Benton Lane, New Castle Upon Tyne NE 12 EW, United Kingdom), following the well-established and standardized technical protocol. After HE staining, all of the resulting RCC tissue slides were reevaluated morphologically, using a Nikon E600 photon microscope.

The study inclusion criteria for Ki-67 IHC analysis was artefact free, viable RCC tumor tissue slides, lacking extensive areas of necrosis. For the Ki-67-targeted immunoreaction, control specimens were external, being comprised of multiple in vivo healthy skin samples. Within these samples, the epidermis was constantly positive in the basal and para-basal layers for Ki-67. A positive Ki-67 immunoreaction was considered valid only when the final reaction product was strictly nuclear restricted, without cytoplasmic staining.

After preliminary evaluation, the cases which met the inclusion criteria were further classified, based on predominant morphological growth pattern, utilizing the classic 3-tier system for conventional subtypes was used: 1 – clear cell (cc)RCC, 2 – papillary (p)RCC and 3 – chromophobe (ch)RCC, with 4 - sarcomatoid dedifferentiation variants of RCC (svRCC) being reported separately. An additional morphological distinction is made, within the ccRCC subgroup, between solid and microcystic variants. Cellular aggressiveness was assessed, based on predominant nuclear traits, quantified using the WHO/ISUP 2017 grading system: G1 - absent/inconspicuous, basophilic, nucleoli, at 400×; G2 - visible eosinophilic nucleoli at 400×, but not prominent at 100×; G3 - prominent eosinophilic nucleoli at 100× magnification; G4 - extreme nuclear pleomorphism, giant neoplastic cells and/or any degree of sarcomatoid/rhabdoid dedifferentiation.

Regarding the Ki-67-targeted IHC protocol, pre-treatment (20 minutes with ER2 – Bond Epitope Retrieval Solution 2, Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK) and peroxide blocking, was followed by incubation with the primary antibody, a commercially available, mouse monoclonal antibody raised against recombinant

human MKI67, clone MM1. Visualization was achieved by using the Bond Polymer Refine Detection System, with 3,3-diaminobenzidine dihydrochloride being used as a chromogen and hematoxylin as a counterstain. The entire Ki-67 targeted immunohistochemical procedure was performed with the Bond Max autostainer (Leica Biosystems) and our method was additionally validated using an external positive control (human skin). The Ki-67 targeted, IHC stained, slide evaluation, was performed using a Axiocam 506 color, Zeiss, Jena, Germany. All slides included in the study database were scanned and digitalized using the Desk Pannoramic Scanner (3D Histech, Budapest, Hungary), then stored in the Histology Department's Digital Slides Library Case Center. The quantitative assessment, i.e. the density of Ki-67 positive cellularity, was documented, using the following protocol:

- <1% of cells with positive nuclei - marked with 0;
- 2-10% of cells with positive nuclei - marked with +1;
- 11-25% of cells with positive nuclei - marked with +2;
- >25% of cells with positive nuclei - marked with +3.

The above-mentioned protocol for Ki-67 immunoreaction quantitative evaluation was utilized on a single representative microscopic field, under 200x magnification. Anisocarias were also evaluated and noted for each case, using the following labels: absent - 0, mild - 1, moderate - 2, severe - 3. Mitoses observed within Ki-67 positive RCC tumor sections were graded as absent (0), rare (1–2/field), average (3–5/field), or frequent (more than 5/field).

RESULTS

For morphological analysis and to establish the histopathological subgroup, we used the classic HE staining technique. On the material we studied, and which come from 134 cases of malignant renal tumors, we identified the tumor lesion in 120 cases, with only 73 cases including the RCC/normal tissue interface. Exclusively normal renal parenchyma was seen in 4 sampled cases and solely modified renal parenchyma bordering tumors in 5 cases. Another 5 RCC slides were deemed to be too low in histological quality, manifesting extensive artefactual and/or necrotic distortion. Thus, only 120 RCCs could be included in the Ki-67 IHC cohort.

The normal renal parenchyma was characterized by the presence of renal corpuscles, with afferent filtration spaces, normal cellularity and tubular systems, without detectable morphological changes. Conversely, the renal parenchyma adjacent to the tumor tissue displayed altered morphologies, with varying degrees of severity, but rather consistently, even though the patients had no history of chronic glomerulonephritis. Although unspecific, we describe some of these encountered alterations below.

In most cases, we identified a peritumoral stroma richly infiltrated with lymphocytes, especially at the border between the tumor tissue and the stroma. Sporadically, foamy macrophages were also identified. There were also cases in which the peritumoral stroma showed pseudocartilaginous metaplasia, collagenization and even isolated calcification. Renal glomeruli showed a wide range of changes, from completely hyalinized atrophic aspects, to glomeruli with proteinaceous deposits in the Bowman's space, to glomeruli with normal morphological appearance. Renal tubules also showed changes, such as hyalinized tubules, with intratubular hyaline cylinders. In some cases, we found pseudocalcifications and hemosiderin pigment in the renal capsule (Img. 1a-d). The blood vessels in the peritumoral stroma also showed changes, as we identified completely thrombosed vessels, as well as vessels with tumor emboli and inflammatory infiltrate.

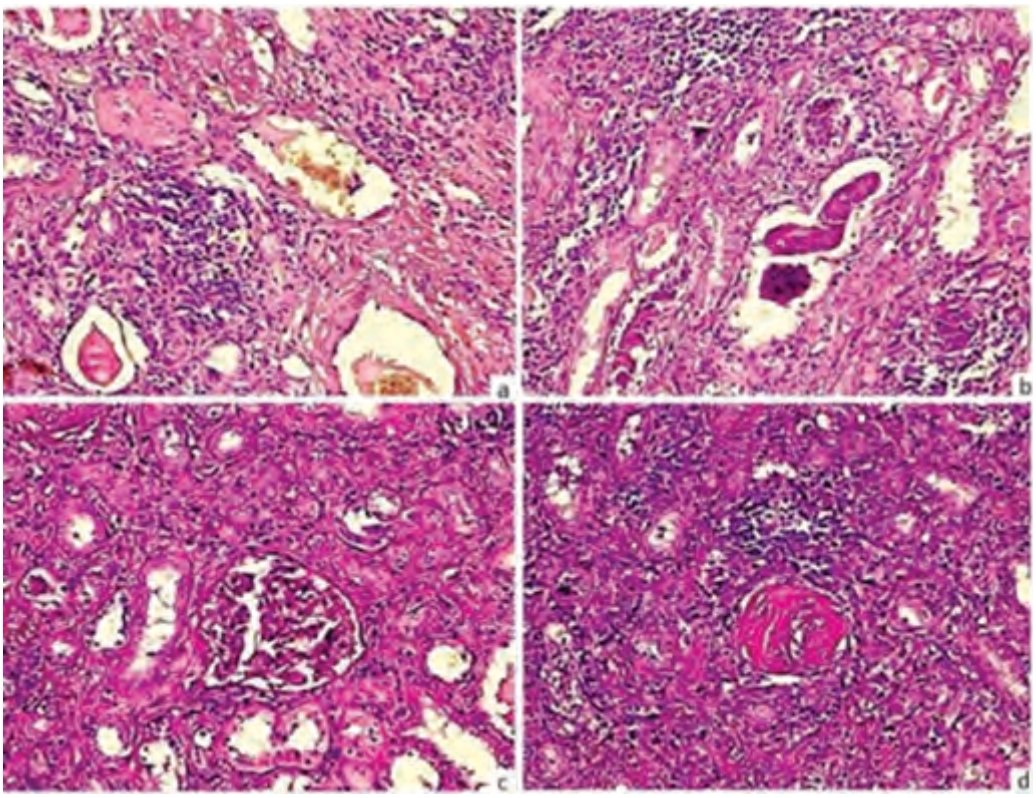


Image 1. Renal parenchyma adjacent to RCC tumor tissue, HE staining: (a) 200×, Tubules with hialine cylinders and stromal inflammatory infiltrate (mostly lymfocites) ; (b) 200×, Degenerated tubules with granulary cylinders; (c) 200×, Renal corpuscules with minimal changes – partial pensation of the urinary filtration space; (d) 200×, Renal corpuscule with complete hyalinization and inflammatory infiltrate in the cortical interstitium.

Out of the 120 RCCs evaluated, the majority of cases were ccRCC (98 cases). These RCCs were characterized by the presence of round or polygonal cells, with abundant and clear cytoplasm, due to the increased content in glycogen, with nuclei in different degrees of evolution - from small nuclei without nucleoli, to large, globular nuclei with multiple nucleoli. Some cases showed large areas of necrosis, with hemorrhagic suffusions, cells with granular cytoplasm and hemosiderin content, with or without invasion of the renal capsule, and various degrees of inflammatory infiltrate at the border between tumor tissue and normal renal tissue.

A particular aspect was presented by the cases of ccRCC with a microcystic appearance, which were characterized by multiple microcysts, hemorrhagic suffusions, with tumor cells arranged in islands and nests (Img. 2a-d). Thus, these morphologies constituted a distinct microcystic ccRCC subgroup (Img. 2c) within the current study (9 cases), as opposed to solid ccRCCs (the remaining 89 cases, Img. 2a). Rarely, we identified ccRCC morphologies demonstrating pseudocartilaginous metaplasia, and numerous vessels with intraluminal calcifications. Moreover, we also identified cases of ccRCCs, which presented cells with eosinophilic cytoplasm, sometimes granulated, with an increased content in mitochondria.

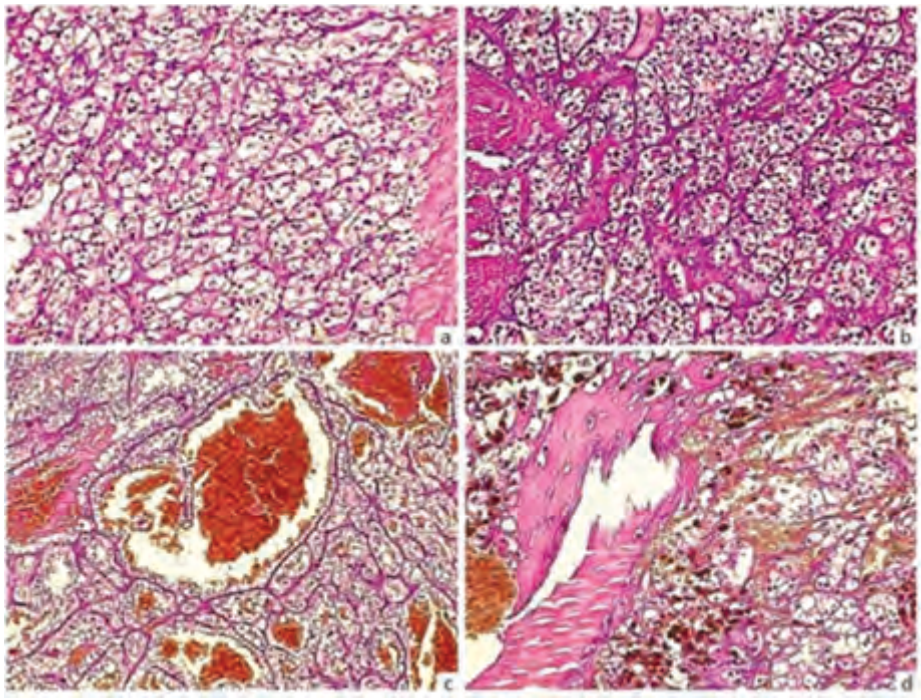


Image 2. ccRCC tumor tissue, HE staining: (a) 200×, Typical solid ccRCC morphology, with tumor cells arranged in small nests and cords; (b) 200×, Intratumoral stroma, rich in small blood vessels; (c) 100×, Microcystic ccRCC - pseudocysts with hemorrhagic suffusions; (d) 200×, Pre-existent blood vessel and hemosiderinic pigment accumulation between RCC cells.

The pRCC subgroup (11 cases) was characterized by tumor cells growth being arranged along well-vascularized connective axes (Img. 3a-b). Cellular traits allowed for the distinction between pRCC subtypes, namely: Type 1 pRCC (4 cases) - a clearer basophilic cytoplasm, with low-grade nuclei; Type 2 pRCC (7 cases) - an eosinophilic cytoplasm, granular, with high-grade nuclei.

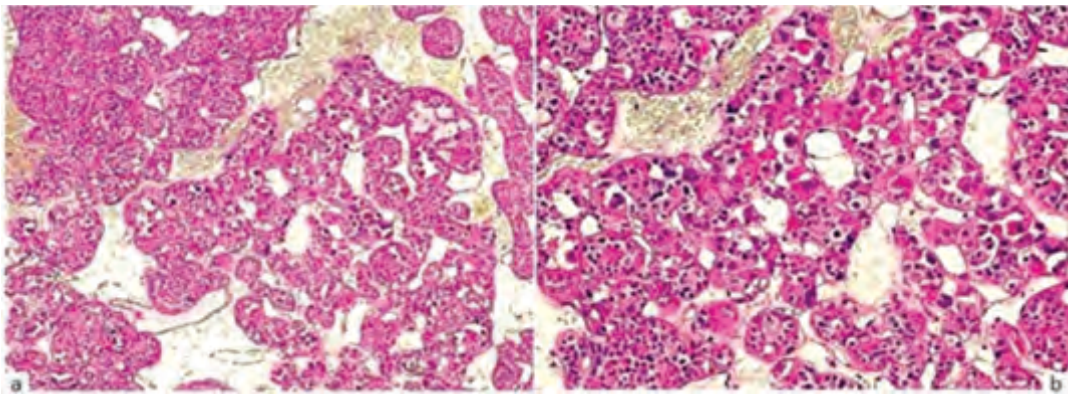


Image 3. pRCC tumor tissue, HE staining: (a) 200×, Typical pRCC morphology: papillae and pseudorosettes, with large and irregular vascular spaces; (b) 400×, Detail with numerous anaplastic cells and atypical mytoses.

Regarding chRCCs, we found 6 cases, which were characterized by a mixture of eosinophilic cells and classic chromophobe cells, with a perinuclear halo (Img. 4).

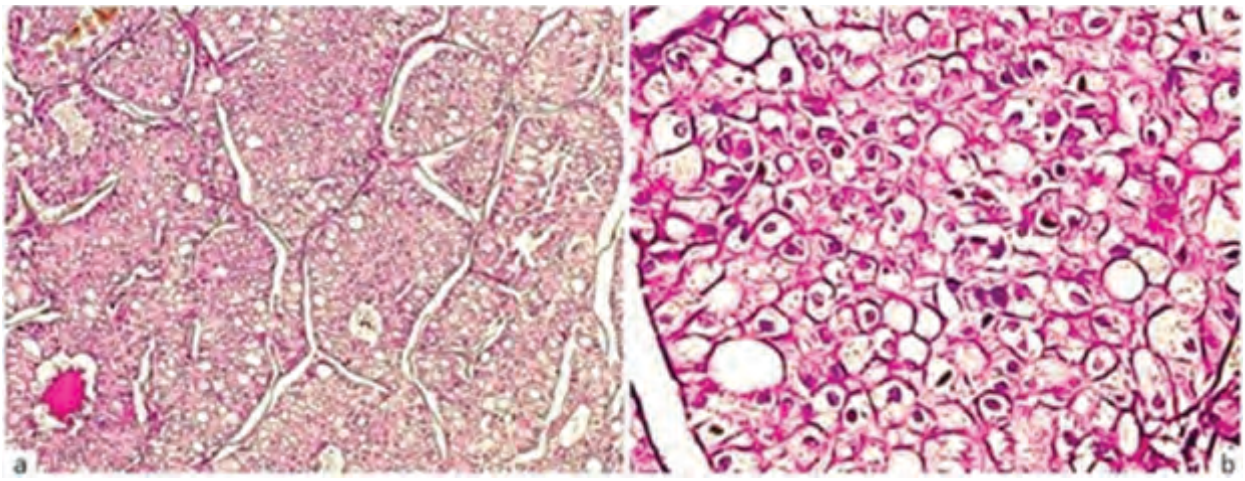


Image 4. chRCC tumor tissue, HE staining: (a) 100×, Overall perspective; (b) 400×, Detail of classic chRCC cells – clear citoplasm with a chromophobe perinuclear halo.

The 5 cases of svRCCs identified, demonstrated a completely altered structure: cells with a rhabdoid / sarcomatoid appearance, pleomorphic cells with acidophilic cytoplasm and longitudinal striations, but also cells with a stellate appearance and thick cytoplasmic extensions, surrounded by abundant stromal myofibroblasts, all cellular elements showing frequent interconnections (Img. 5).

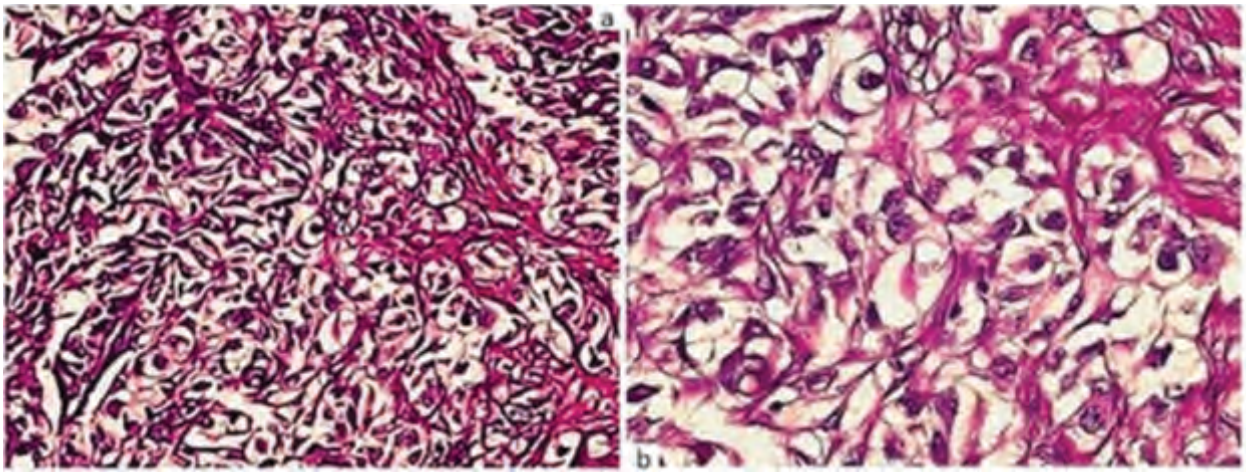


Image 5. svRCC tumor tissue, HE staining: (a) 200×, Overall perspective; (b) 400×, Detail of svRCC cells – anaplasia, anisocytosis, anisokarya and macronucleoli.

The same WHO/ISUP 2017 grading system further classified these RCC subtypes, based on cellular aggressivity (Tabel 1):

Table 1. Distribution of the Ki-67 RCC subpopulation regarding subtype and cellularity.

Subtype	WHO/ISUP 2017 grading system			
	G1	G2	G3	G4
Solid ccRCC	5	50	33	1
Microcystic ccRCC	0	3	6	0
Type 1 pRCC	0	3	1	0
Type 2 pRCC	0	2	5	0
chRCC	0	0	5	1
svRCC	0	0	0	5

After initial morphological assessment, 120 RCC specimens were stained for Ki-67 expression using IHC. Regarding the tumor-adjacent renal tissue, seen in within most RCC slides (73 cases), very rare cells with Ki-67-positive nuclei were seen within the mesangium, the tubular system of the nephron and as isolated elements of the inflammatory infiltrate, if present in the stroma. In fact, cells with positive nuclei were below 1% in all tumor-adjacent renal tissues evaluated, regardless of their microscopic subtype.

The Ki-67 immunoreaction within the positive RCC tumor cells differed in density and intensity from case to case, and was characterized by marked intratumoral heterogeneity, with some areas being characterized by major agglomerations of positive nuclei. Ki-67 positivity was more evident at the periphery of the tumor, in the immediate vicinity of the proliferation front. Also, a large number of positive cells was observed in the vicinity of neoformation blood vessels. Practically, each case presented defined values of the proliferation index, and thus Ki-67 becomes an individual prognostic marker.

A number of 12 cases were negative in the sense that they did not present cells with Ki-67 positive nuclei in the tumor area. In cases where mitoses were absent and anisokarya was present, the Ki-67 proliferation rate was typically ranked between 2-10%. The presence of marked mitoses and anisokarya was associated with an increased Ki-67 labeling index (Img. 6a-d).

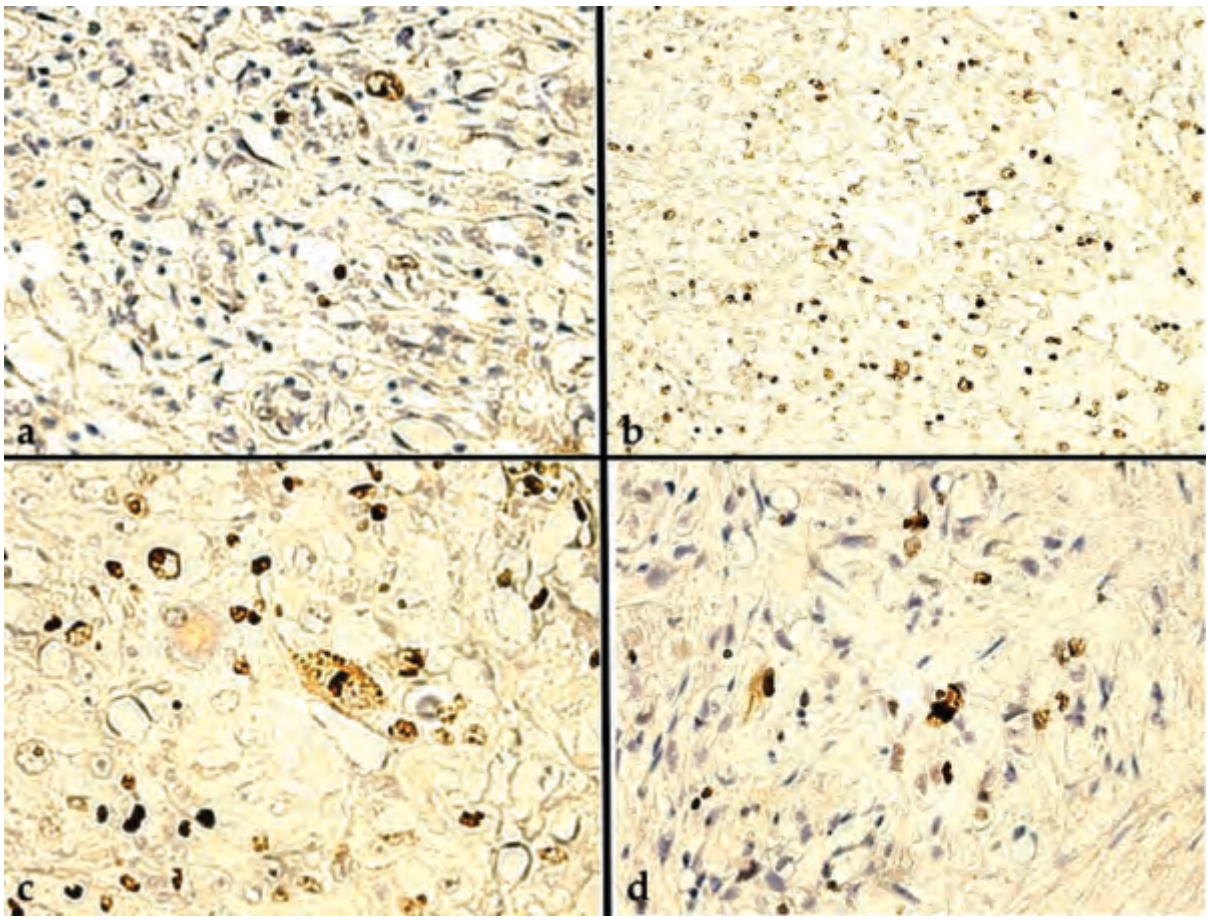


Image 6. Immunoreaction for Ki-67: (a) 400×, Negative reaction, less than 1% positive RCC cells; (b) 200×, Positive reaction +1, with less than 10% positive cells; (c) 400×, Positive cells, one being in active mitosis; (d) 400×, Multiple atypical divisions.

We observed a higher density of Ki-67 positive cells in tumor areas located in the vicinity of the proliferation front and in those proximal to chronic inflammatory infiltrates, sometimes present within the tumor area. Tumors with a high density of cells with Ki-67 positive nuclei were mostly grade G2, G3, and G4. No statistically significant correlation was seen between these two elements.

The relationship between Ki-67 expression and RCC histopathological subtype was further assessed (see Table 2). As expected, the most common cases which scored +3 on Ki-67 immunoreaction evaluation were poorly differentiated and/or sarcomatoid carcinomas. Surprisingly, we obtained very low values for papillary carcinomas. Median values of the proliferation index were observed in solid variant ccRCCs and chRCCs. The microcystic variant clear cell carcinoma showed only rare positive cells, scored as +1 (Img. 7a-d).

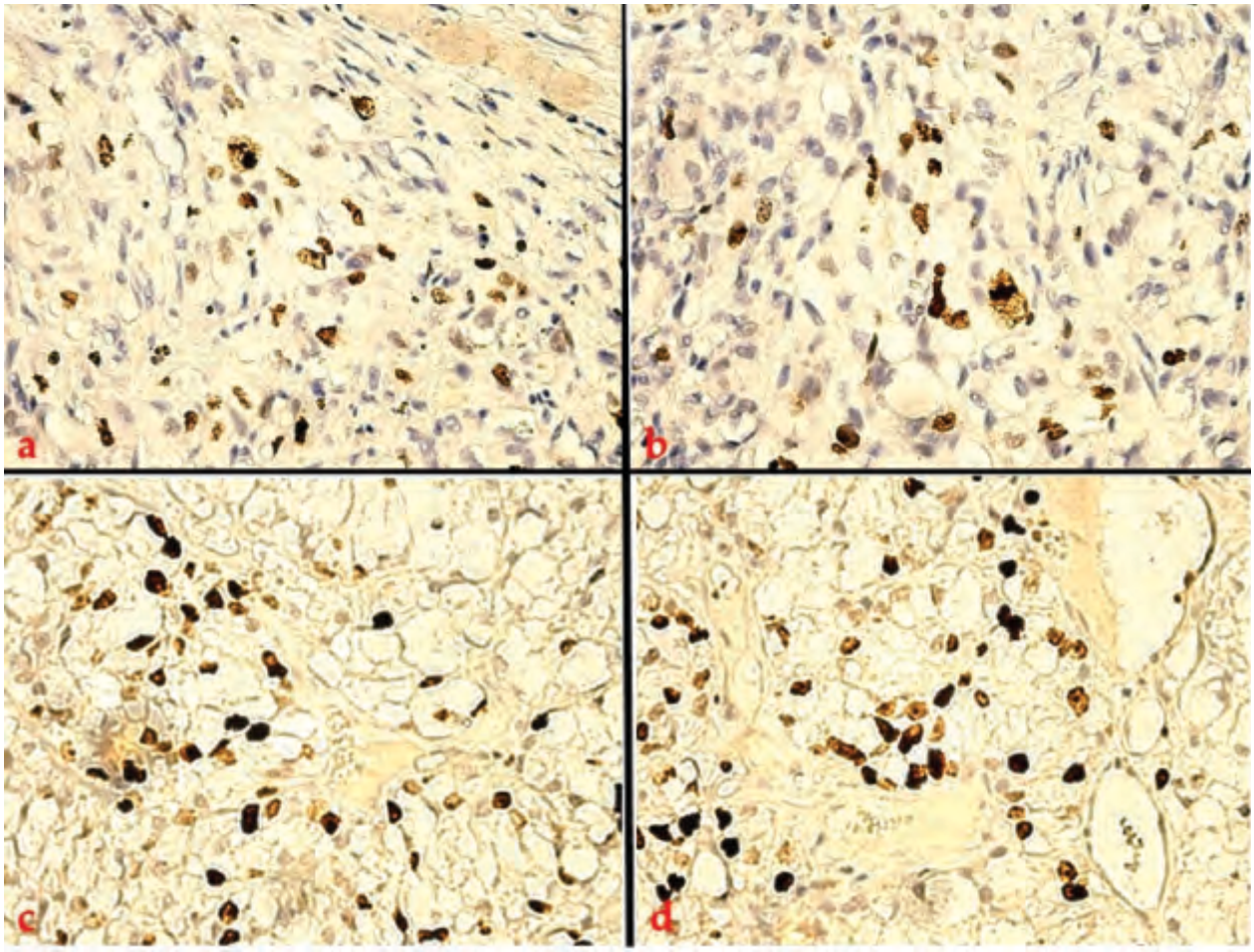


Image 7. Ki-67 expression in RCC subtypes, 400x. (a,b) svRCCs; (c,d) Solid ccRCCs.

Table 2. Ki-67 scores for RCC subtypes.

Subtype	Total no.	0	+1	+2	+3
Solid ccRCC	89	7	19	49	14
Microcystic ccRCC	9	4	5	-	-
pRCC	11	1	9	1	-
chRCC	6	-	1	3	2
svRCC	5	-	-	1	4

Lastly, we present the variability of Ki-67 staining intensity, within the same microscopic field, generally encountered among Ki-67 positive RCCs (Img. 8).

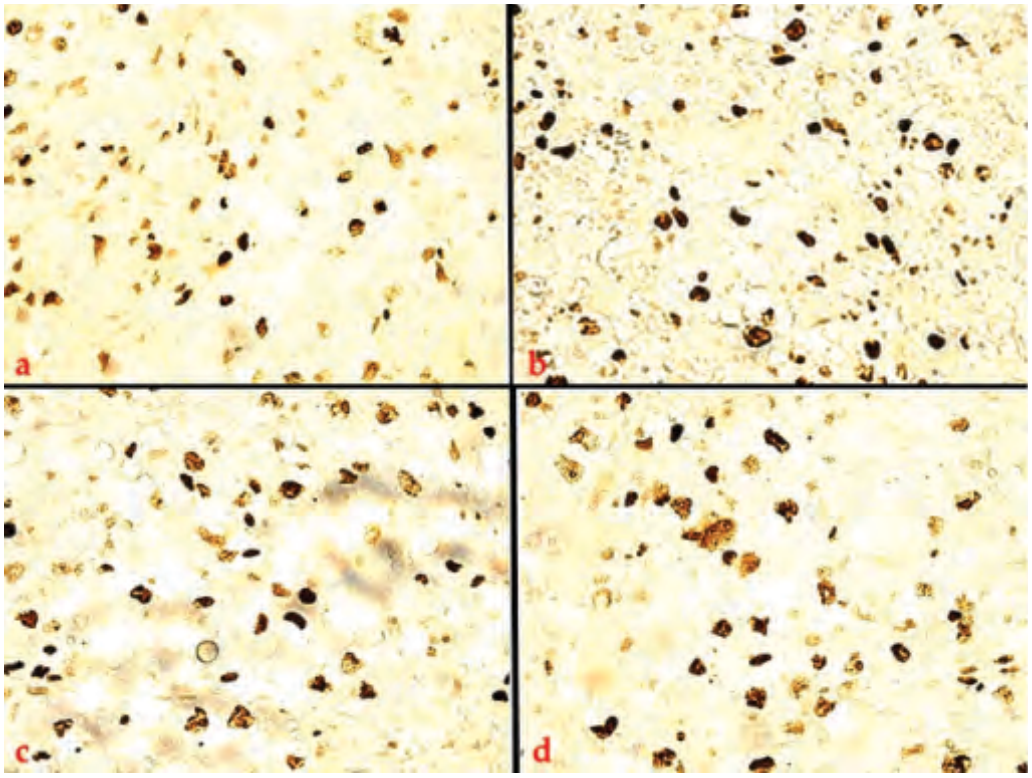


Image 8. Ki-67 expression in RCC specimen, 400x. (a-d) Intensity differences between tumor cell nuclei in the same microscopic field.

DISCUSSIONS

Since being firstly identified more than 3 decades ago, within an investigation by Gerdes et al. [2,3], focusing on lymphomas, the Ki-67 nuclear protein has been thoroughly analyzed and well-characterized as a central molecular marker for the accurate quantification of cellular proliferation. The resulting clinical application for Ki-67, as an IHC target, which reliably reflects cellular proliferation rates, constitutes, implicitly, a very useful tool within the process of pathological tumor assessment.

The underlying molecular basis for this clinically relevant Ki-67 oncological management applications is represented by the distinctive Ki-67 nuclear immunoreactivity patterns, seen during specific phases of the cellular cycle. Specifically, in metabolically viable cells, undergoing active proliferation, i.e. throughout the G1, S, G2, and M stages, Ki-67 expression is constantly detectable, whereas mitotically inactive, metabolically dormant cells, stuck in the G0 phase, will characteristically lack Ki-67 immunoreactivity. Apparently, Ki-67 is involved in the fundamental process of ribosomal RNA synthesis, essential for cellular mitosis. Thus, Ki-67 nuclear expression seemingly constitutes a molecular prerequisite for cells to be able to initiate and undergo proliferation, as exemplified within certain malignancies, and their more biologically aggressive cellular subpopulations [7].

In light of this remarkable ability, additional investigations were developed to further assess Ki-67 as an oncological proliferation marker, in a wide variety of cancers, spawning from a plethora of tissues / cellularities. Being easily implementable in routine clinical evaluation and consistently showing high sensibility and specificity, this biomarker has long been view as a very attractive

and potentially impactful predictive element for oncological risk stratification, as well as a promising novel therapeutic target for systemic oncological treatment.

To date, multiple reports have linked Ki-67 IHC expression patterns with overall oncological outcomes. Statistically significant positive correlations have been identified between Ki-67 immunoreactivity of primary tumors and subsequent clinical aggressivity, adequately predicting prognosis and offering improved risk stratification abilities for multiple malignant tumors, i.e. urothelial carcinomas, in both lower and upper urinary tract localizations, breast, cervical, prostate, hepatic and lung carcinomas, as well as gliomas and malignant lymphomas [8-13]. In spite of these amounting reports, prognostic/clinical/pathological value of Ki-67 expression assessment in the context of RCC clinical management remains controversial.

Many recent investigations have reported that an increased Ki-67 expression in RCCs is indicative of a proportionally unfavorable prognosis and poorer clinical outcomes [14-17]. Even so, there are lingering contradictions, as divergent results have also emerged, stating that Ki-67 immunoexpression is irrelevant for the assessment RCC prognosis, being unable to accurately predict RCC clinical behavior and disease outcome [18,19]. This being said, a very recent, extensive meta-analysis, reporting on a total of 20 studies, which included 5,398 individual RCC patients, found that an increased Ki-67 immunoexpression in RCCs did in fact imply poorer outcomes and survival rates, while also correlating with overall TNM stages, pT stage, metastatic status and Fuhrman nuclear grade, concluding that Ki-67 had predictive value as a prognostic biomarker for RCCs [20].

In an effort to address the discrepancies in the literature, regarding the predictive capabilities of Ki-67 IHC expression in RCC prognosis assessment, we designed our current investigation, aiming to analyze the relationship between nuclear Ki-67 immunoexpression and other well-established, relevant clinical and pathological prognostic factors. Within the current cohort, most of the RCC specimens evaluated displayed a relatively low rate of proliferation. Higher rates were encountered amongst the microcystic variants of ccRCCs and pRCCs. Even so, it seems that Ki-67 immunoexpression did not correlate with the degree of cellular differentiation. Further analysis is need for a definitive conclusion, yet it is becoming apparent that Ki-67 should be viewed as an individual and independent prognostic marker for RCC. Additionally, Ki-67 may prove useful in treatment response assessment, being able to reflect the effectiveness of humanized growth factor inhibition therapy.

CONCLUSIONS

In the current investigation, we studied the proliferation rate of RCC tumor cells, using morphological assessment and Ki-67 immunoexpression comparatively. Our data has demonstrated a relatively low rate of cellular proliferation within the RCC specimens evaluated, more evident in the microcystic morphological variants of ccRCCs and pRCCs. However, Ki-67 immunoreactivity did not apparently correlate with the degree of RCC differentiation. Although further statistical analysis is need to validate these observations and formulate a definitive conclusion, it is becoming increasing clear that Ki-67 should be viewed as an individual and independent prognostic marker for RCCs. Additionally, Ki-67 immunoreactivity based treatment response assessment, as an objective indicator of the effectiveness of systemic humanized growth factor inhibition therapy, should be further investigated. So far, our data supports the integration of Ki-67 IHC quantification in routine clinical practice, constituting a relevant addition to the currently approved panel diagnostic/prognostic IHC antibodies. Most likely, Ki-67 will at one point become a part of the standard pathological evaluation of all renal tumors.

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