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Original Article

# Hilar fat infiltration: A new prognostic factor in metastatic clear cell renal cell carcinoma with first-line sunitinib treatment

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## Abstract

**Introduction:** The selection of patients with metastatic clear cell renal cell carcinoma (ccRCC) who may benefit from targeted tyrosine kinase inhibitors has been a challenge, even more so now with the advent of new therapies. Hilar fat infiltration (HFI) is a validated prognostic factor in nonmetastatic ccRCC (TNM 2009 staging system) but has never been studied in metastatic patients. We aimed to assess its phenotype and prognostic effect in patients with metastatic ccRCC treated with first-line sunitinib.

**Materials and methods:** In a multicentric study, we retrospectively included 90 patients and studied the corresponding ccRCC at the pathological, immunohistochemical, and molecular levels. Patient and tumor characteristics were compared using univariate and multivariate analysis. All the features were then studied by Cox models for prognostic effect.

**Results:** HFI was found in 42 patients (46.7%), who had worse prognosis (Heng criteria) ($P = 0.003$), liver metastases ($P = 0.036$), and progressive diseases at first radiological evaluation ($P = 0.024$). The corresponding ccRCC was associated with poor pathological prognostic factors that are well known in nonmetastatic ccRCC. For these patients, median progression-free survival was 4 months vs. 13 months ($P = 0.02$), and median overall survival was 14 months vs. 29 months ($P = 0.006$). In a multivariate Cox model integrating all the variables, only poor prognosis, according to the Heng criteria and HFI, remained independently associated with both progression-free survival and overall survival.

**Conclusion:** HFI was demonstrated for the first time to be an independent poor prognostic factor. Its potential role in predicting resistance to antiangiogenic therapy warrants further investigation. 

*Keywords:* Clear cell renal cell carcinoma; Sunitinib; Prognostic; Hilar fat infiltration

## 1. Introduction

Renal cell carcinoma (RCC) accounts for 3% of all adult cancers, with approximately 270,000 new cases and 110,000

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deaths per year worldwide [1]. Clear cell renal cell carcinoma (ccRCC) is the most frequent histological subtype of renal cancer, estimated to account for approximately 70%, with a poor prognosis [2]. Indeed, 20% of patients are metastatic at the time of diagnosis, and 30% will develop metastases. The 5-year overall survival (OS) is estimated to be 40% [1].

A better understanding of the oncogenic pathways involved in the oncogenesis of ccRCC, such as VHL/HIF/VEGF and PI3K/AKT/mTOR, led to the development of targeted therapies for metastatic patients. Sunitinib is an antiangiogenic tyrosine kinase inhibitor approved for use as a first-line treatment for metastatic ccRCC that is currently the most frequently administered [3,4]. However, approximately 20% of patients are inherently resistant to this therapy because they progress according to response evaluation criteria in solid tumors (RECIST) at first evaluation [5].

A new approach is based on targeted immunotherapy using checkpoint inhibitors (anti-PD-1, anti-PD-L1, or anti-CTLA4 antibodies) as ccRCC is considered an immunogenic tumor [6]. Recently, nivolumab, an anti-PD-1 antibody, has demonstrated efficiency as a second-line treatment vs. everolimus (mTOR inhibitor) [7]. Most notably, a phase 3 study comparing anti-PD-1 and anti-CTLA4 antibody association vs. sunitinib in first-line treatment is ongoing, highlighting questions about chronologic treatment strategies. Oncologists currently face the challenge of drug selection to treat their patients but lack reliable predictors of response or prognostic factors that may help choose the most appropriate therapy.

Hilar fat infiltration (HFI), also known as renal sinus fat infiltration, was previously described in several studies as a poor prognostic factor in nonmetastatic ccRCC [8]. Consequently, it was integrated in the TNM (Tumor Node Metastasis) staging system 2009, classifying tumors of at least T3a [9]. This stage also includes perirenal fat and venal invasion. Nevertheless, the effect of HFI has not been individually studied in metastatic ccRCC (m-ccRCC) thus far. In a retrospective cohort of metastatic patients with ccRCC treated by first-line sunitinib therapy, we aimed to correlate HFI with clinicopathological and molecular characteristics as well as clinical outcomes.

## 2. Patients and methods

### 2.1. Patient selection and classification

The study population included metastatic patients who were at least 18 years old with histologically proven ccRCC. Primary ccRCC specimens were collected from patients undergoing nephrectomy in 2 French University Hospitals (Rennes and Bordeaux, from UroCCR database). Patients with metastatic ccRCC received sunitinib (50 mg/d, 4 wk on/2 wk off) as first-line treatment (prior cytokine therapy was allowed) and completed at least one 28-day cycle of sunitinib. Tumor evaluation was realized according to Response Evaluation Criteria In Solid Tumors (RECIST 1.1) [10]. Follow-up chest/abdomen CT-scans were performed every 2 cycles of treatment (3 mo). For each patient, the following clinical and pathological information was gathered: age, sex, pTNM stage, tumor size, and nucleolar ISUP grade. HFI was suspected during gross examination and was confirmed by microscopy. Histopathologic assessment was performed by 3 experienced pathologists (S.F.K.J., M.Y., and N.R.L.). For each patient, formalin-fixed paraffin-embedded ccRCC were available. Informed consent was obtained from each patient, and institutional review board approval was obtained for this study (CNIL authorization receipt 1812601v0).

### 2.2. Immunohistochemical study

Protein expression patterns were assessed by immunohistochemistry using the following antibodies: anti-VEGFA (monoclonal, clone sp28, dilution, Spring Bioscience, California, USA), anti–carbonic anhydrase IX (CAIX; polyclonal, ab15086, dilution 1:1500, Abcam, Cambridge, UK), anti-PD-1 (anti-PD-1 antibody, clone NAT105, dilution 1:50; Abcam, Cambridge, UK), and anti-PD-L1 (anti-PD-L1 antibody, clone 130021, dilution 1:200; R&D Systems, Minneapolis, USA) [11–13]. The reactivity of antibodies was revealed with horseradish peroxidase–labeled polymer-conjugated secondary antibodies using diaminobenzidine as the chromogen (Sigma-Aldrich, France). Negative controls were performed by omitting the primary antibody. The tumor expression for each antibody was independently evaluated (S.F.K.J. and N.R.L.) without knowledge of the case. The cutoff for positive cases was 30% of tumor cells for VEGF and 85% for CAIX as previously described [12,14]. For PDL1, absent (0), weak (1), moderate (2), and strong expression (3) were reported, and cases were then subdivided into negative (score 0–1) or positive (score 2–3) subgroups [15]. For PD1, immunostaining density was evaluated in tumor-infiltrating lymphocytes and was semiquantified as absent, rare, moderate, or dense as previously reported [15].

### 2.3. VHL status

#### 2.3.1. Next-generation sequencing

For the *VHL* gene, the entire coding sequence and exon-intron junctions of exons 1, 2, and 3 were analyzed. Genomic DNA was extracted using Magtration System 12GC (Bionobis) according to the manufacturer's instructions. Genomic DNA from all samples was quantitated with the Quan-iT PicoGreen dsDNA assay kit (Thermo Fisher Scientific). DNA target preparation and enrichment were performed by amplification using the Access Array system (Fluidigm, San Francisco, USA). A 10-nucleotide "barcode" tag, specific to each sample and Illumina-specific sequencing adapters were attached using secondary polymerase chain reaction. Purified products were then pooled