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# The prognostic value of erythrocyte polyamines in the preoperative evaluation of patients with renal cell carcinoma

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ABSTRACT

*Introduction:* Polyamines, spermine and spermidine, are ubiquitous polycationic structures, which are essential for cell proliferation and differentiation. We tested whether spermine and spermidine could improve the prognostic ability of six established preoperative predictors of cancer-specific mortality (CSM) after partial or radical nephrectomy for renal cell carcinoma (RCC).

*Materials and methods:* Overall, 385 patients with clinical stages $T_{1-3}$, $M_{0-1}$ RCC were treated with radical or partial nephrectomy at a single institution between 1990 and 2007. Kaplan–Meier plots depicted CSM after stratification according to spermine and spermidine levels (dichotomised to above and below the median value). Univariable and multivariable Cox regression models tested the prognostic ability of continuously coded spermine and spermidine levels in preoperative CSM predictions. Covariates consisted of pre-treatment T stage, M stage, age, gender and symptom classification.

*Results:* The 5-year CSM-free survival of patients with spermine levels $\leqslant$4.5 and >4.5 nmol/$8 \times 10^9$ erythrocytes were, respectively, 79.5% and 65.0%. Similarly, the 5-year CSM-free survival of patients with spermidine levels $\leqslant$9.0 and $>$ 9.0 nmol/$8 \times 10^9$ erythrocytes were, respectively, 81.1% and 63.7%. In multivariable analyses addressing CSM after surgery, both spermine ($p \leqslant 0.002$) and spermidine ($p \leqslant 0.001$) achieved independent predictor status and improved the accuracy of established preoperative CSM predictors by 2.1% ($p < 0.001$).

*Conclusions:* Circulating polyamine levels may significantly improve the prognostic value of established determinants of CSM in patients with RCC of all stages prior to nephrectomy. External validation of our findings is required prior to implementation in clinical practice.



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## 1. Introduction

The natural history of renal cell carcinoma (RCC) is highly variable, and, to date, only one pre-treatment prognostic model showed adequate ability to predict cancer-specific mortality (CSM) after partial or radical nephrectomy.[1] This model was based on age, gender, clinical-determined T and M stages, tumour size and symptom classification and achieved 84.2–88.1% accuracy in an external validation cohort.[1] Although the predictions of this model are very robust and informative, they are not perfect.[1] Therefore, the search for additional predictors that could improve existing models continues.

Polyamines, spermine and spermidine, are ubiquitous polycationic structures that originate from putrescine, which is synthesised from ornithine through a reaction catalysed by ornithine decarboxylase.[2] The function of polyamines is driven by electrostatic interactions with macromolecules such as nucleic acids (RNA and DNA), nucleotides (ATP), proteins and membrane components such as phospholipids. Polyamines are essential for normal cell proliferation and differentiation as they play a role in DNA synthesis and stabilisation.[2–4] Moreover, polyamines are released by dead cells and are also produced during physiological and tumoural cell proliferation.[5] Free circulating polyamines are transported by erythrocytes.[2,4,5]

In the current study, we hypothesised that two specific circulating polyamines, spermine and spermidine, may improve the predictive accuracy of our previously validated preoperative model.[1] Our hypothesis was based on promising data that originated from prostate cancer,[6–9] acute leukaemia[10] and supratentorial malignant glioma studies,[11,12] where polyamines achieved statistical significance and independent predictor status in the prognostic stratification of patients with those malignancies. Additionally, renal tissue-derived polyamines levels were able to stratify the survival of patients after radical nephrectomy for RCC and were predictors of metastatic progression.[13]

Our tests relied on multivariable models. They focused on the ability of erythrocyte polyamines to improve the prognostic accuracy of the previously externally-validated pre-nephrectomy nomogram predicting CSM after partial or radical nephrectomy. Predictive accuracy, defined by the concordance index (c-index), was used as a benchmark. Specifically, we quantified the gain in accuracy (increase in c-index values) related to the consideration of the two polyamines, relative to a model without these two variables.

## 2. Materials and methods

### 2.1. Patient population

After review board approval, data were retrieved from an institutional database and yielded 385 assessable patients treated with partial or radical nephrectomy between 1990 and 2007 at Rennes University Hospital. Preoperative-determined variables consisted of patient age, gender, clinical T stage, presence of distant metastases, tumour size, symptom classification and erythrocyte spermine and spermidine levels.

Patients were staged preoperatively with computerised tomography of the abdomen and pelvis, with computerised tomography of the chest, or with chest X-rays. The largest tumour dimension was used to define tumour size. Symptoms were prospectively recorded and defined as asymptomatic, local (lumbar pain, haematuria, palpable mass) and systemic (fever, fatigue, weight loss, night sweats).[14]

Follow-up consisted of one postoperative baseline visit and was then performed every 6 months for a minimum of 2 years. Subsequently, minimum follow-up consisted of annual visits. At each visit, a CT of the chest or a chest X-ray accompanied a CT of the abdomen. The cause of death was either obtained from the medical chart and recorded prospectively or obtained from the death certificate in a retrospective fashion. RCC-specific mortality included deaths that were directly attributed to RCC.

### 2.2. Erythrocyte polyamine level determination

Informed consent for blood sample testing was obtained in all cases. Erythrocyte spermine and spermidine levels were determined preoperatively from patient blood samples, as described by Cipolla et al.[8] Five millilitres (mL) blood samples were collected preoperatively in blood collection tubes containing a 0.129 M (M) buffered sodium citrate solution and were centrifuged at 2500 relative centrifugal force (*g*). After the removal of plasma and buffy coat layer, the red blood cell pellet was washed with 0.14 M NaCl solution. One millilitre of the packed red blood cell pellet was removed and haemolysed with 2 mL of distilled water. Proteins were removed from the hemolysate by the addition of 2 mL of ice-cold 10% HClO. After centrifugation at 3000 g, free polyamines contained in red blood cells were measured from 1 mL perchloric supernatant. For each sample, 0.5 mL of aqueous sodium carbonate saturated solution was added with 1 mL of dansyl chloride dissolved in acetone. The samples were kept in the dark during 16 h, under a slight air depression for allowing a selective evaporation of acetone. Dansylated derivatives of polyamines were recovered by two successive dry extractions using cyclohexane. The residue was then resuspended in 1 mL of acetonitrile. Ten microlitres of this extract were used for high performance liquid chromatography (HPLC) analysis. The supernatant fluid was stored frozen at –40 °C. HPLC polyamine determination was performed as previously described.[8,9] In the following determinations, circulating polyamine values were expressed in nmol/$8 \times 10^9$ erythrocytes.

### 2.3. Statistical analyses

Spermine and spermidine blood levels were dichotomised according to the median. Kaplan–Meier plots were used to graphically illustrate the CSM rates for the entire cohort, as well as the CSM rates according to dichotomised spermine and spermidine levels. Differences between spermine and spermidine groups above and below the median were assessed using the pairwise log-rank test.

Univariable and multivariable Cox regression models tested the effect of continuously coded spermine and spermidine on CSM. Covariates consisted of established preoper-

ative CSM predictors, namely patient age, gender, tumour size, symptom classification and preoperative T and M stages.[1]

Subsequently, Cox regression coefficients were used to quantify the univariable accuracy of continuously coded erythrocyte polyamine levels in the prediction of CSM. Moreover, we quantified the multivariable accuracy gain related to the addition of spermine, spermidine or both variables to the established clinical predictors of CSM that consisted of age, gender, tumour size, symptom classification and preoperative T and M stages.[1] In accuracy analyses, a value of 100% indicates perfect prediction whereas 50% is equivalent to the toss of a coin. Predictive accuracy is usually quantified with receiver operating characteristics-derived area under the curve (AUC) estimates. In Cox regression models, the AUC is substituted with Harrell's concordance index (c-index),[15] which was used in the current analyses. The Mantel–Haenszel test was used to assess the statistical significance of the accuracy gain related to the addition of continuously coded spermine and spermidine blood levels to the other predictors. Two hundred bootstrap resamples were used to reduce overfit bias and for internal validation of all accuracy estimates.

Finally, since the predictive value of circulating spermine and spermidine may vary with tumour stage, all steps of the analyses were then repeated in patients with non-metastatic ($M_0$) RCC, as well as in patients with metastatic RCC ($M_1$). All statistical tests were performed using either the Statistical Package for Social Science, version 16.0 (SPSS, Chicago, IL) or the S-PLUS Professional, version 1 (Mathsoft, Seattle, WA). All tests were two-sided with a significance level set at 0.05.

## 3. Results

Overall, 385 patients were treated with either radical nephrectomy ($n = 287$) or partial nephrectomy ($n = 98$) for RCC between 1990 and 2007 (Table 1). Most of them were male (59.2%). Mean age was 62.6 (±12.8) years. Overall, 25.5%, 18.4%, 15.6% and 40.5% patients had, respectively, clinical stages $T_{1a}$, $T_{1b}$, $T_2$ and $T_3$ disease. Distant metastases were observed in 58 (15.1%) patients. Median levels of spermine and spermidine were 4.5 and 9.0 nmol/$8 \times 10^9$ erythrocytes.

Fig. 1 shows CSM-free survival for the entire cohort. CSM-free survival rates at 1, 2 and 5 years after surgery were, respectively, 88.9%, 81.1% and 72.0%. After stratification according to dichotomised circulating polyamines levels, the 5-year CSM-free survival rates were, respectively, 79.5% versus 65.0% for, respectively, patients with spermine levels of $\leqslant$4.5 and >4.5 nmol/$8 \times 10^9$ erythrocytes (log-rank test: $p = 0.001$) (Fig. 2A). Similarly, the 5-year CSM-free survival rates were 81.6% versus 67.0% for, respectively, patients with spermidine levels of $\leqslant$9.0 and >9.0 nmol/$8 \times 10^9$ erythrocytes (log-rank test: $p < 0.001$) (Fig. 2B). When survival analyses were limited to patients with non-metastatic RCC ($M_0$), the 5-year CSM-free survival rates were, respectively, 88.1 versus 70.1% for patients with spermine levels of $\leqslant$4.5 and >4.5 nmol/$8 \times 10^9$ erythrocytes (log-rank test: $p < 0.001$) (Fig. 2C). In the same patient subgroup, the 5-year CSM-free survival rates were 84.9% versus 72.8% for, respectively, patients with spermidine levels of $\leqslant$9.0 and >9.0 nmol/$8 \times 10^9$ erythrocytes (log-rank test: $p = 0.01$) (Fig. 2D).

Continuously coded spermine ($p < 0.001$) and spermidine ($p < 0.001$) achieved statistical significance in univariable analyses predicting CSM rates after surgery (Table 2). Similarly, continuously coded spermine ($p \leqslant 0.002$) and spermidine

**Table 1 – Characteristics of the study population of patients treated with partial or radical nephrectomy for renal cell carcinoma between 1990 and 2007 ($n = 385$).**

| Characteristics of the study population ($n = 385$) | |
|---|---|
| *Clinical variables* | |
| Age (years) | |
| Mean (±SD) | 62.6 (±12.8) |
| Gender | |
| Male | 228 (59.2%) |
| Female | 157 (40.8%) |
| Tumour size (cm) | |
| Mean (±SD) | 7.2 (±4.0) |
| Circulating spermine level (nmol/$8 \times 10^9$ erythrocytes)[a] | |
| Mean (±SD) | 6.3 (±7.6) |
| Circulating spermidine level (nmol/$8 \times 10^9$ erythrocytes)[b] | |
| Mean (±SD) | 10.9 (±6.2) |
| Surgery type | |
| Partial nephrectomy | 98 (25.5%) |
| Radical nephrectomy | 287 (78.7%) |
| Symptom classification | |
| Asymptomatic | 198 (51.4%) |
| Local | 105 (27.3%) |
| Systemic | 82 (21.3%) |
| Clinical T stage | |
| $T_{1a}$ | 98 (25.5%) |
| $T_{1b}$ | 71 (18.4%) |
| $T_2$ | 60 (15.6%) |
| $T_3$ | 156 (40.5%) |
| M stage | |
| $M_0$ | 327 (84.9%) |
| $M_1$ | 58 (15.1%) |
| *Pathological variables* | |
| RCC histological subtypes | |
| Clear-cell | 329 (85.5%) |
| Papillary | 37 (9.6%) |
| Chromophobe | 19 (4.9%) |
| Fuhrman grade | |
| I | 13 (3.4%) |
| II | 155 (40.3%) |
| III | 149 (38.7%) |
| IV | 68 (17.7%) |
| Follow-up of censored patients (months) | |
| Mean (±SD) | 42.3 (±40.6) |

SD: standard deviation.
RCC: renal cell carcinoma.
[a] Circulating spermine level, median: 4.5 nmol/$8 \times 10^9$ erythrocytes.
[b] Circulating spermidine level, median: 9.0 nmol/$8 \times 10^9$ erythrocytes.

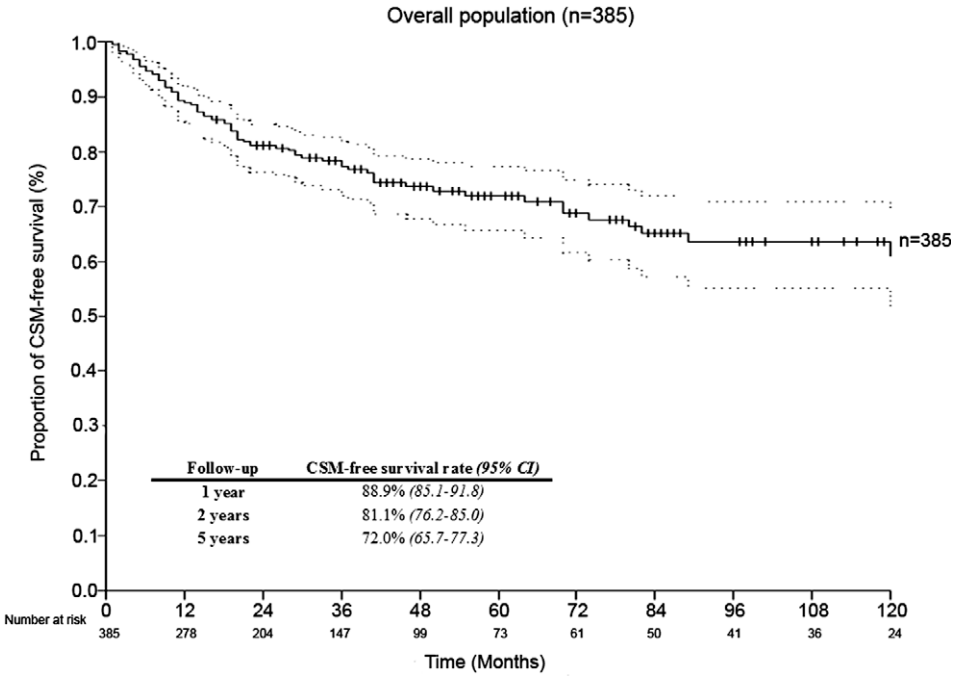


**Fig. 1 – Kaplan–Meier survival curve depicting cancer-specific mortality after partial or radical nephrectomy in the overall population of renal cell carcinoma patients (*n* = 385).**

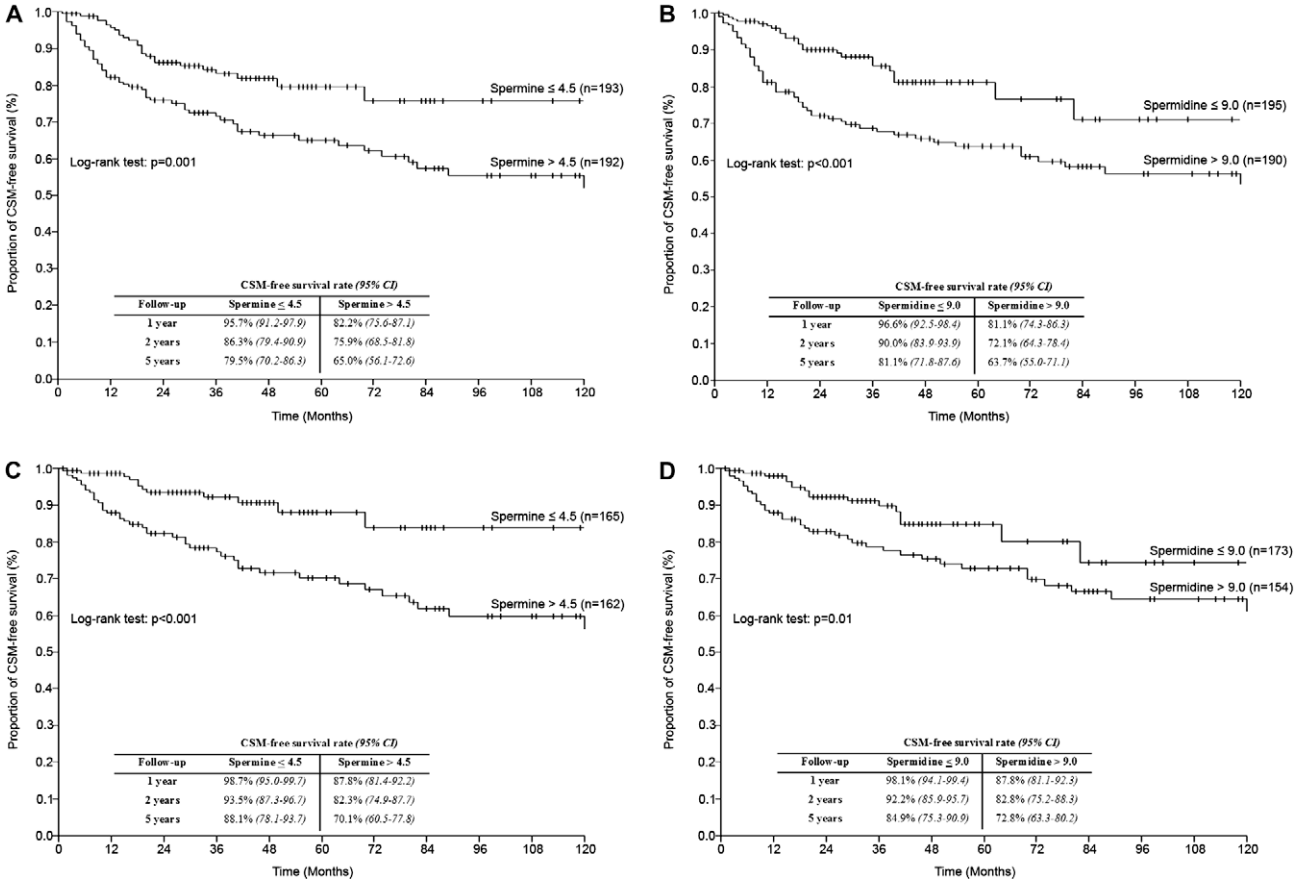


**Fig. 2 – Kaplan–Meier survival curves depicting cancer-specific mortality in the overall population (A and B) and in patients with non-metastatic renal cell carcinoma only (C and D), stratified according to the median spermine (A and C) and spermidine (B and D) values.**

**Table 2 – Univariable and multivariable analyses predicting the probability of cancer-specific mortality after surgery in the overall population of patients with renal cell carcinoma treated with partial or radical nephrectomy ($n$ = 385). The area under the curve (AUC) reflects the prognostic value of individual variables (columns), as well as of the multivariable models in predicting the probability of cancer-specific mortality.**

| Predictors | Univariable analysis | | Multivariable analysis | | | |
|---|---|---|---|---|---|---|
| | $p$-Value HR (95% CI) | AUC of individual predictor variables | Full model without spermine and spermidine $p$-Value HR (95% CI) | Full model with spermine $p$-Value HR (95% CI) | Full model with spermidine $p$-Value HR (95% CI) | Full model with spermine and spermidine $p$-Value HR (95% CI) |
| Continuously coded spermine | $p < 0.001$<br>1.04 (1.03–1.06) | 69.1% | – | $p < 0.001$<br>1.03 (1.02–1.05) | – | $p = 0.002$<br>1.03 (1.01–1.04) |
| Continuously coded spermidine | $p < 0.001$<br>1.07 (1.05–1.09) | 69.2% | – | – | $p < 0.001$<br>1.06 (1.03–1.08) | $p = 0.001$<br>1.05 (1.02–1.08) |
| Symptom classification<br>Local versus asymptomatic<br>Systemic versus asymptomatic | $p < 0.001$<br>5.56 (2.74–11.3)<br>13.54 (6.77–27.1) | 76.3% | $p < 0.001$<br>3.08 (1.40–6.61)<br>5.58 (2.63–12.0) | $p < 0.001$<br>2.81 (1.31–6.03)<br>4.83 (2.22–10.5) | $p < 0.001$<br>3.012(1.43–6.37)<br>5.32 (2.47–11.4) | $p = 0.001$<br>2.85 (1.35–6.03)<br>4.64 (2.14–10.1) |
| T stage<br>$T_{1b}$ versus $T_{1a}$<br>$T_2$ versus $T_{1a}$<br>$T_3$ versus $T_{1a}$ | $p < 0.001$<br>2.21 (0.55–8.83)<br>6.13 (1.75–21.5)<br>13.94 (4.38–44.4) | 72.6% | $p = 0.02$<br>1.56 (0.42–6.41)<br>2.35 (0.61–9.42)<br>4.17 (1.12–15.3) | $p = 0.03$<br>1.65 (0.40–6.82)<br>2.31 (0.57–9.29)<br>4.02 (1.09–14.8) | $p = 0.03$<br>2.07 (0.49–8.65)<br>3.02 (0.74–12.3)<br>4.69 (1.29–17.0) | $p = 0.04$<br>2.15 (0.51–9.03)<br>2.96 (0.72–12.1)<br>4.63 (1.27–16.9) |
| M stage<br>$M_1$ versus $M_0$ | $p = 0.001$<br>5.34 (3.33–8.58) | 64.5% | $p = 0.001$<br>2.34 (1.42–3.91) | $p < 0.001$<br>2.76 (1.62–4.69) | $p = 0.002$<br>2.33 (1.37–3.98) | $p < 0.001$<br>2.69 (1.56–4.63) |
| Tumour size | $p < 0.001$<br>1.13 (1.08–1.18) | 70.0% | $p = 0.5$<br>1.02 (0.91–1.13) | $p = 0.5$<br>1.02 (0.96–1.09) | $p = 0.6$<br>1.02 (0.95–1.08) | $p = 0.8$<br>1.01 (0.95–1.09) |
| Age | $p = 0.3$<br>1.01 (0.99–1.03) | 51.1% | $p = 0.4$<br>1.01 (0.91–1.03) | $p = 0.4$<br>1.01 (0.99–1.03) | $p = 0.2$<br>1.01 (0.99–1.03) | $p = 0.2$<br>1.01 (0.99–1.03) |
| Gender<br>Female versus male | $p = 0.3$<br>0.79 (0.51–1.23) | 51.6% | $p = 0.4$<br>1.20 (0.82–1.93) | $p = 0.5$<br>0.84 (0.54–1.32) | $p = 0.6$<br>0.88 (0.56–1.39) | $p = 0.5$<br>0.87 (0.55–1.37) |
| AUC of multivariable models | – | – | 79.8% | 81.0% | 81.6% | 81.9% |
| Gain in predictive accuracy (Mantel–Haenszel, $p$-value) | – | – | – | +1.2%<br>($p < 0.001$) | +1.8%<br>($p < 0.001$) | +2.1%<br>($p < 0.001$) |

AUC: area under the curve.
HR: hazard ratio.

**Table 3 – Univariable and multivariable analyses predicting the probability of cancer-specific mortality after surgery in the subgroup of patients with non-metastatic renal cell carcinoma ($M_0$) ($n = 327$). The area under the curve (AUC) reflects the prognostic value of individual variables (columns), as well as of the multivariable models in predicting the probability of cancer-specific mortality.**

| Predictors | Univariable analysis | | Multivariable analysis | | | |
|---|---|---|---|---|---|---|
| | *p*-Value HR (95% CI) | AUC of individual predictor variables | Full model without spermine and spermidine *p*-Value HR (95% CI) | Full model with spermine *p*-Value HR (95% CI) | Full model with spermidine *p*-Value HR (95% CI) | Full model with spermine and spermidine *p*-Value HR (95% CI) |
| Continuously coded spermine | $p < 0.001$<br>1.05 (1.03–1.06) | 73.9% | – | $p < 0.001$<br>1.03 (1.01–1.04) | – | $p = 0.02$<br>1.02 (1.01–1.04) |
| Continuously coded spermidine | $p < 0.001$<br>1.08 (1.05–1.10) | 68.4% | – | – | $p < 0.001$<br>1.07 (1.03–1.11) | $p = 0.001$<br>1.07 (1.03–1.11) |
| Symptom classification<br>Local versus asymptomatic<br>Systemic versus asymptomatic | $p < 0.001$<br>6.83 (2.98–15.6)<br>13.74 (5.80–32.5) | 76.4% | $p < 0.001$<br>3.71 (1.53–9.02)<br>7.38 (2.87–18.9) | $p = 0.001$<br>3.46 (1.41–8.46)<br>6.52 (2.50–17.0) | $p < 0.001$<br>3.79 (1.56–9.22)<br>7.80 (3.01–20.2) | $p < 0.001$<br>3.66 (1.50–8.94)<br>6.87 (2.61–18.1) |
| T stage<br>$T_2$ versus $T_1$<br>$T_3$ versus $T_1$ | $p < 0.001$<br>4.98 (1.81–13.7)<br>9.89 (4.19–23.4) | 74.1% | $p = 0.02$<br>2.37 (0.75–7.48)<br>3.73 (1.38–10.1) | $p = 0.04$<br>2.24 (0.70–7.15)<br>3.44 (1.25–9.44) | $p = 0.1$<br>1.89 (0.58–6.16)<br>2.67 (0.96–7.45) | $p = 0.1$<br>1.84 (0.61–6.01)<br>2.62 (0.93–7.33) |
| Tumour size | $p < 0.001$<br>1.15 (1.10–1.22) | 69.7% | $p = 0.5$<br>1.03 (0.95–1.12) | $p = 0.5$<br>1.03 (0.94–1.12) | $p = 0.3$<br>1.05 (0.96–1.16) | $p = 0.4$<br>1.04 (0.96–1.13) |
| Age | $p = 0.2$<br>1.02 (0.99–1.04) | 53.5% | $p = 0.2$<br>1.02 (0.99–1.04) | $p = 0.2$<br>1.02 (0.99–1.04) | $p = 0.2$<br>1.02 (0.99–1.04) | $p = 0.2$<br>1.02 (0.99–1.04) |
| Gender<br>Female versus male | $p = 0.5$<br>0.82 (0.48–1.41) | 50.6% | $p = 0.3$<br>0.72 (0.41–1.26) | $p = 0.3$<br>0.73 (0.42–1.28) | $p = 0.2$<br>0.69 (0.39–1.20) | $p = 0.2$<br>0.67 (0.39–1.18) |
| AUC of multivariable models | – | – | 79.2% | 80.0% | 81.1% | 81.5% |
| Gain in predictive accuracy (Mantel–Haenszel *p*-value) | – | – | – | +0.8%<br>($p = 0.002$) | +1.9%<br>($p < 0.001$) | +2.3%<br>($p < 0.001$) |

AUC: area under the curve.
HR: hazard ratio.

($p \leqslant 0.001$) achieved independent predictor status in multivariable Cox regression analyses (Table 2).

In analyses that quantified predictive accuracy, when each variable was examined continuously spermidine and spermine ranked, respectively, 4th (69.2%) and 5th (69.1%) (Table 2). When spermine was added to T and M stages, tumour size, symptom classification, age and gender, accuracy increased by 1.2% (Mantel–Haenszel test: $p < 0.001$). When spermidine was added to the same CSM predictors, accuracy increased by 1.8% (Mantel–Haenszel test: $p < 0.001$). When spermine and spermidine were added to T and M stages, tumour size, symptom classification, age and gender, accuracy increased by 2.1% (Mantel–Haenszel test: $p < 0.001$).

Virtually the same results were obtained when univariable and multivariable Cox regression analyses were limited to patients with non-metastatic RCC ($M_0$) (Table 3). Both spermine ($p \leqslant 0.02$) and spermidine ($p \leqslant 0.001$) achieved independent predictor status. In accuracy analyses, the inclusion of both spermine and spermidine increased the AUC of the baseline model by 2.3% (Mantel–Haenszel: $p < 0.001$).

After restricting the analysis to metastatic RCC patients, spermine ($p = 0.006$) and spermidine ($p = 0.03$) achieved statistical significance in univariable Cox regression models addressing CSM (table not shown). In multivariable models, spermine ($p = 0.02$) remained an independent predictor of CSM, but not spermidine ($p = 0.09$).

## 4. Discussion

The natural history of patients with RCC treated with nephrectomy is highly variable. Individuals with $T_1N_0M_0$ tumours may not require any form of therapy due to the indolent nature of their disease.[16,17] Conversely, patients with $T_3N_0M_0$ RCC may rapidly succumb to their disease. Similarly, heterogeneity exists in patients with nodal metastases.[18] As many as 50% of patients may remain alive at 5 years. Conversely, others may die within 2 years after nephrectomy. Finally, patients with metastatic RCC may also show important variability in CSM.[19] Long-term survivors may not succumb to RCC for several years or even decades without active chemotherapy. Conversely, some patients with metastatic RCC may die within less than one year after cytoreductive nephrectomy.

Several prognostic models have been devised to assist clinicians with risk stratification prior to nephrectomy. Those models may help with sub-stratification of patients with all RCC stages. They may help with the identification of patients with aggressive variants of $T_1N_0M_0$ RCC, and they may also help discriminating between rapidly versus slowly evolving metastatic RCC variants. To date, three models have been devised with the intent of predicting RCC recurrence or CSM prior to nephrectomy.[1,20,21] The two recurrence models demonstrated poor accuracy (65–67%).[20,21] Conversely, the CSM model showed 84–88% accuracy in external validation.[1] To date, no additional studies attempted to improve the accuracy of this model. In the current study, we describe the results of multivariable accuracy tests that quantified the gain in predictive accuracy related to the inclusion of two circulating polyamines, spermine and spermidine to the previously devised model.

Our results showed that spermine and spermidine can enhance the accuracy of the pre-nephrectomy nomogram in the prediction of CSM. The nomogram was 79.8% accurate in the current cohort. The addition of spermine and spermidine resulted in a 2.1% gain, which was statistically significant ($p < 0.001$). It is noteworthy that virtually the same results were observed when accuracy analyses were limited to patients with non-metastatic RCC. In this patient subgroup, the accuracy gain related to the addition of circulating polyamines to the baseline prognostic model was 2.3% ($p < 0.001$).

From a practical perspective, a 2.1–2.3% gain implies that between 21 and 23 additional patients will be correctly classified out of 1000. Although this may appear trivial on an individual basis, it is of significance in RCC chemotherapy trials, where hundreds or even thousands of patients may be stratified. The biological rationale for the observed prognostic ability of spermine and spermidine stems from the functional role of those two polyamines that are critical for cell proliferation and differentiation, and are involved in DNA, RNA and protein synthesis.[22,23]

Several other serum markers capable of predicting the natural history or the prognosis of RCC patients have been proposed. Vascular endothelial growth factor (VEGF) was found to be related with RCC grade and stage.[24] However, in multivariable analyses addressing CSM, VEGF failed to achieve the independent predictor status.[24,25] Circulating interleukin-6 was found to be an independent predictor of survival in patients with metastatic RCC.[26] Li et al. showed that serum carbonic anhydrase IX level is associated with postoperative RCC recurrence.[26] Similarly, in patients with RCC, Vasudev et al. observed that low preoperative sodium concentration was associated with worse survival.[27] However, the gain in predictive accuracy related to the addition of such markers to established CSM predictors has not been tested. Previous analyses showed that independent predictor status does not always result in a predictive accuracy gain, which represents the cornerstone of the prognostic qualities of a variable of interest.[28]

To date, only C-reactive protein showed an improvement in the predictive accuracy of pathological and clinical disease characteristics after nephrectomy.[29] Our model confirms that highly accurate markers, such as spermine and spermidine, can also improve the predictive accuracy, but can be applied preoperatively, without the knowledge of histological subtype or Fuhrman grade.

The prognostic value of circulating polyamines was confirmed both in the overall population and in the non-metastatic patient subgroup. Conversely, in patients with metastatic RCC, only spermine achieved the independent predictor status. It is noteworthy that, in the current study, all patients with metastatic RCC were diagnosed and treated prior to the advent of tyrosine-kinase inhibitors. Interferon-alpha and interleukin-2 were administered to some of these patients. However, as shown by numerous previous studies, they failed to significantly improve the survival of most patients with metastatic RCC.[30] In consequence, the available treatment options unlikely affected CSM in the current patient population.

In spite of its promising results, our study has some limitations. Its retrospective nature is shared with all previous

tumour markers analyses.[24,25,27,29,31–33] An additional limitation consisted of the inability of the current study to perform a head-to-head comparison between circulating polyamine levels and other promising serum markers. Unfortunately, the current dataset did not contain alternative novel markers to generate such comparisons. However, lack of independent external validation represents the most important limitation, and is required in future studies of those two promising markers. Additionally, spermine and spermidine measurements require inter-institutional standardisation to ensure stable performance in future studies evaluating the prognostic value of these biomarkers in RCC patients.

In conclusion, spermine and spermidine erythrocyte levels may significantly improve the accuracy of established determinants of CSM in the preoperative prognostic stratification of patients with RCC. Independent external validation of our findings is required, prior to implementation in clinical practice.

## Conflict of interest statement

None declared

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