The Urinary Sarcosine/Creatinine Ratio is a Potential Diagnostic and Prognostic Marker in Prostate Cancer

AB 1 Meng Wang*
DEG 2 Lihui Zou*
DF 3 Jing Liang
C 4 Xin Wang
CF 4 Dalei Zhang
B 2 Yuan Fang
CF 2 Junhua Zhang
A 2 Fei Xiao
AG 4 Ming Liu

* These authors contributed equally to this work
Corresponding Authors: Ming Liu, e-mail: liuming3222@bjhmoh.cn, Fei Xiao, e-mail: xiaofei3965@bjhmoh.cn

Source of support: This study was supported in part by Grant No. 7112120, Beijing Natural Science Foundation (to M. L); Grant No. 2011-4011-04, Capital Health Research and Development of Special (to F. X.), and Grant No. 7172193, Beijing Natural Science Foundation (to L.Z.)

Background: The aim of this study was to evaluate the role of the urinary sarcosine/creatinine ratio in the diagnosis and prognosis of prostate cancer, using a sarcosine oxidase assay.

Material/Methods: Urine samples were obtained from 203 patients with benign prostate hyperplasia (BPH) and 209 patients with prostate cancer. Levels of urinary sarcosine were measured using the sarcosine oxidase method. The urinary sarcosine/creatinine ratios were compared between the group of patients with BPH and the patients with prostate cancer. In the two patients groups, the urinary sarcosine/creatinine ratio was compared with the measurement of serum prostate-specific antigen (PSA) and the free/total (F/T) PSA ratio. Correlations between of the urinary sarcosine/creatinine ratio and the Gleason grade and stage of prostate cancer were analyzed.

Results: There was a significant difference between the urinary sarcosine/creatinine ratio in the BPH group and prostate cancer group (P<0.01), which was independent of serum PSA. The receiver-operating characteristic (ROC) curve, and area under the curve (AUC) for the urinary sarcosine/creatinine ratio was significantly higher compared with the serum PSA and the F/T PSA ratio. There was a significant difference in the urinary sarcosine/creatinine ratio in patients with prostate cancer with Gleason score ≤6, 7, and ≥8, and between patients with metastatic and non-metastatic prostate cancer.

Conclusions: The urinary sarcosine/creatinine ratio was a diagnostic indicator of prostate cancer, for patients with a serum PSA level <10 ng/ml, and correlated with the Gleason score and with the presence of metastases (stage) of prostate cancer.

MeSH Keywords: Diagnosis • Prostate-Specific Antigen • Prostatic Neoplasms • Sarcosine Oxidase

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909949
Background

Worldwide, prostate cancer is a common cancer in men, with an incidence that increases with age, and now ranks as the second most frequent cause of death from male cancer [1]. Although the prevalence of prostate cancer in men in China is lower than that in the United States (US) and other western countries, the incidence has been shown to increase during the recent years [2]. According to the US National Cancer Institute (NCI), the five-year survival rate of localized, non-metastatic prostate cancer is 100%, is 89% when prostate cancer invades beyond the prostate gland and is 37% when the patient has metastatic prostate cancer (www.cancer.gov/types/prostate/). Therefore, screening for prostate cancer and early diagnosis are the key to reduce the mortality rate from prostate cancer. However, early diagnosis is difficult because early-stage prostate cancer lacks specific clinical symptoms and diagnostic imaging markers. Current clinical diagnosis depends on the analysis of serum levels of prostate-specific antigen (PSA) and the histology of the prostate biopsy of suspected cases.

Measurement of serum PSA levels was introduced into clinical use in the 1980s and has significantly improved the early diagnosis of prostate cancer [3]. However, the sensitivity and specificity of the serum PSA test are poor, for several reasons. An elevated serum PSA level is not specific for the presence of prostate cancer, serum PSA levels can be normal in some patients with prostate cancer, and the serum PSA is not an indicator of tumor stage or of prognosis. Therefore, more sensitive and specific markers for the presence of prostate cancer, particularly early-stage prostate cancer, are still required.

In 2009, a metabolomic study conducted by Sreekumar et al. [4] showed that 176 metabolites from 1,126 candidates associated with prostate cancer, among which, sarcosine had the strongest association with the presence of prostate cancer. Sarcosine (N-methylglycine), which is derived from the metabolism of glycine, can be detected in urine. Sarcosine is expressed at very low levels in normal patients or patients with benign prostate hyperplasia (BPH), levels of urinary sarcosine are increased in men with localized prostate cancer, and have been shown to be present at the highest level in the urine of men with metastatic prostate cancer [5]. Since this study was published, several further studies have been undertaken to evaluate the application of measurement of urinary sarcosine in the diagnosis of prostate cancer, but a consensus has been reached, and the study findings have been controversial [6–8].

The aim of this study was to evaluate the role of the urinary sarcosine/creatinine ratio in the diagnosis and prognosis of prostate cancer, using a sarcosine oxidase assay, to evaluate the potential of this assay in the diagnosis of prostate cancer, and as a prognostic marker, using the known Gleason scores and clinical stage.

Material and Methods

Subjects

This study was approved by the Ethics Committee of Beijing Hospital (Ref: LLKYP: 2013030512). The study participants provided written informed consent to participate in this study, which included 209 patients with prostate cancer and 203 patients with benign prostate hyperplasia (BPH). The diagnosis in all participating patients was confirmed histologically by biopsy or from surgical resection specimens. Patients were enrolled in this study between February 2012 and September 2016. Fresh urine and blood samples were collected before the biopsy procedure or surgery. The patients were assigned into groups according to their serum prostate-specific antigen (PSA) test results, as follows: PSA $<10$ ng/ml, PSA $\geq 10$ ng/ml, and PSA at any other level.

Equipment and reagents

Equipment and reagents included a Beckman AU5400 automatic biochemical analyzer (Beckman, USA), Eppendorf research pipettes (Eppendorf, Germany), an Abbott i4000 automatic immune-analyzer (Abbott Labs, USA), Tris(hydroxymethyl)aminomethane (BDI Pharma, USA), peroxidase (Sangon Biotech, Shanghai, China), 4-amino antipyrine (East China Normal University Chemical Factory), sarcosine oxidase (synthesized in-house), creatinine (Bio Basic, NY, USA), 6N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl aniline salt (TOOS) (Sigma-Aldrich, St Louis, MI, USA), and a creatinine assay kit (G Cell Technologies).

Specimen collection and processing

Whole blood samples were centrifuged at 2,280×g for 5 min within 2 hours of collection, the serum was added to freezer tubes and stored at $–20^\circ$C. The urine samples (10–15 ml) were centrifuged at 700×g for 10 min within 2 hours of collection, and the supernatants were added to freezer tubes and stored at $–20^\circ$C. All specimens were collected before the patients were treated. Gleason scores were assigned by histopathological examination to the specimens of prostate cancer acquired from prostatectomies or prostate biopsies.

Determination of sarcosine, creatinine, and other analytes

The algorithms for the sarcosine oxidase method in the detection of urinary sarcosine were as follows:

1. Sarcosine + H$_2$O + O$_2$ → glycine + methanol + H$_2$O$_2$ (catalyzed by sarcosine oxidase).
2. H$_2$O$_2$ + 4-amino-antipyrine + chromogen → Quinone imine colored substance + H$_2$O (catalyzed by peroxidase).

Sarcosine was measured using a sarcosine oxidase assay. The target concentration was 2.9 μmol/L for fresh urine specimens,
with a total error of <14%. At sarcosine concentrations of 6.35 µmol/L and 9.8 µmol/L, the total error levels were <10%. In the sarcosine oxidase assay, the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were the smallest concentrations that could be reliably measured by the analytical method. The LoB was approximately 0.35 µmol/L, the LoD was 1.18 µmol/L, and the LoQ was 2.05 µmol/L. The linear range of the sarcosine oxidase assay was 2.05–125.65 µmol/L. LoD was 1.18 µmol/L, and the LoQ was 2.05 µmol/L. The linear range concentrations that could be reliably measured by the analytical method. The LoB was approximately 0.35 µmol/L, the LoD was 1.18 µmol/L, and the LoQ was 2.05 µmol/L. The linear range of the sarcosine oxidase assay was 2.05–125.65 µmol/L.

Urinary creatinine concentrations were measured using an enzymatic assay and a Beckman AU 5400 analyzer (Beckman, Brea, CA, USA). Serum PSA and free PSA concentrations were measured using a chemiluminescence immunoassay and an Abbott i4000 automatic immune analyzer (Abbott Labs, USA). Detection of serum PSA and the urinary sarcosine/creatinine ratio was conducted in a double-blind manner, and the concentrations of sarcosine and creatinine of each sample were randomly recorded along with the corresponding levels of PSA and the free PSA concentrations.

### Patient characteristics

The clinical data of the patients in the two groups in this study, patients with prostate cancer and patients with benign prostate...
hyperplasia (BPH), are presented in Table 1. There was no significant difference in age between the two group of patients. However, the urinary sarcosine/creatinine ratio, serum level of prostate-specific antigen (PSA), and the free/total (F/T) PSA ratio were significantly different between the two groups (P<0.05). For further analysis, the patients with prostate cancer were grouped according to tumor grades (Gleason score ≤ 6, 7, or >8) and metastatic tumor status, or stage (metastatic prostate cancer or non-metastatic prostate cancer) based on clinical and pathological data.

Receiver-operator characteristic (ROC) curves, area under the curve (AUC), and the differential diagnosis

The diagnostic performances of the variables studied are summarized in Table 2. In the PSA <10 ng/ml group, the AUC$_{sar/cr}$ was 0.855 (95% CI, 0.802–0.908) was the highest. The AUC$_{PSA}$ and AUC$_{F/T-PSA}$ were 0.743 and 0.745, respectively. When 0.063 was used as the cutoff value of the urinary sarcosine/creatinine ratio, the sensitivity and specificity for the diagnosis of prostate cancer were 79.4% and 88.5%, respectively (Figure 1A). In the PSA ≥10 ng/ml group, the AUC$_{sar/cr}$ was 0.816 (95% CI, 0.753–0.879) was the highest. The AUC$_{PSA}$ and AUC$_{F/T-PSA}$ were 0.740 and 0.805 respectively. When 0.069 was used as the cut-off value of the urinary sarcosine/creatinine ratio, the sensitivity and specificity were 77.7% and 76.7%, respectively (Figure 1B). In the PSA at any concentration group, the AUC$_{sar/cr}$ was 0.841 (95% CI, 0.802–0.881) was the highest. The AUC$_{PSA}$ and AUC$_{F/T-PSA}$ were 0.728 and 0.797, respectively. When 0.062 was used as the cut-off value of the urinary sarcosine/creatinine ratio, the sensitivity and specificity were 81.3% and 75.9%, respectively (Figure 1C). The AUC of the urinary sarcosine/creatinine ratio was higher when compared with the serum PSA and the F/T PSA ratio, suggesting that the urinary sarcosine/creatinine ratio might be a good diagnostic metabolomics biomarker of prostate cancer.

The relationship between the urinary sarcosine/creatinine ratio and the Gleason score in prostate cancer

When the patients with prostate cancer were divided into three groups according to the Gleason scores (Gleason score ≤6, 7, or >8), statistical analysis showed a significant difference between the urinary sarcosine/creatinine ratio between the groups (P<0.05) (Table 3). As shown in Figure 2A, in the prostate cancer group not distinguished by the histopathological tumor

### Table 2. ROC curves for differential diagnosis.

| Items                | PSA <10 ng/ml | PSA ≥10 ng/ml | PSA not limited |
|----------------------|---------------|---------------|-----------------|
|                      | AUC 95%CI P-value | AUC 95%CI P-value | AUC 95%CI P-value |
| Sarcosine/creatinine | 0.855 0.802–0.908 0.000 | 0.740 0.669–0.811 0.000 | 0.728 0.680–0.776 0.000 |
| PSA                  | 0.743 0.678–0.808 0.000 | 0.740 0.669–0.811 0.000 | 0.728 0.680–0.776 0.000 |
| F/T PSA ratio        | 0.745 0.678–0.813 0.000 | 0.805 0.738–0.873 0.000 | 0.797 0.755–0.840 0.000 |

Figure 1. Receiver-operating characteristic (ROC) curves of the urinary sarcosine/creatinine ratio, prostate-specific antigen (PSA), and the free/total (F/T) PSA ratio in the diagnosis of prostate cancer. (A) Prostate-specific antigen (PSA) <10 ng/ml. (B) PSA ≥10 ng/ml. (C) PSA, any concentration.
Table 3. The sarcosine/creatinine ratio of PCa with the pathological types and the Gleason scores.

| Specimens from surgical removal | Specimens from prostate biopsy | No distinguishing specimens |
|--------------------------------|-------------------------------|-----------------------------|
| n Sarcosine/creatinine ratio | n Sarcosine/creatinine ratio | n Sarcosine/creatinine ratio |
| ≤6 22 | 0.050 (0.018–0.105) | 15 | 0.049 (0.014–0.094) | 37 | 0.050 (0.017–0.102) |
| 7 34 | 0.088 (0.031–0.570) | 57 | 0.099 (0.035–0.667) | 91 | 0.096 (0.037–0.573) |
| ≥8 25 | 0.130 (0.069–0.919) | 56 | 0.128 (0.072–0.700) | 81 | 0.129 (0.073–0.693) |
| P-value* | – 0.000 | – 0.000 | – 0.000 |

* Kruskal-Wallis test

Figure 2. The urinary sarcosine/creatinine ratios and the Gleason scores and the stages of prostate cancer. (A–C) The urinary sarcosine/creatinine ratio and Gleason scores. (D) The urinary sarcosine/creatinine ratio according to clinical stages (non-metastatic and metastatic prostate cancer).
Table 4. The test value of metastatic and non-metastatic PCAs.

|                         | Metastatic PCa | Non-metastatic PCa | P-value* |
|-------------------------|----------------|-------------------|----------|
| n                       | 50             | 159               |          |
| Sarcosine/creatinine ratio Median (range) | 0.133 (0.052–0.814) | 0.097 (0.020–0.513) | 0.004    |
| PSA (ng ml⁻¹) Median (range) | 23.83 (1.78–253.08) | 10.78 (2.12–214.48) | 0.177    |
| F/T PSA ratio Median (range)  | 0.126 (0.043–0.326) | 0.126 (0.052–0.328) | 0.891    |

* Mann-Whitney U-test.

There have been several recently published basic research studies and clinical studies to evaluate the role of sarcosine in the diagnosis of prostate cancer [12,13]. However, currently, studies have shown no significant differences in sarcosine levels between the prostate cancer samples and control samples. Struys et al. [14] compared sarcosine concentrations, measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS), between three groups of men, one group were prostate cancer patients, the second group were men who had increased levels of prostate-specific antigen (PSA) without a confirmed diagnosis of prostate cancer, and the third group were men who had serum samples evaluated for vitamin B12 status. The results obtained by Struys and colleagues demonstrate that the concentrations of sarcosine in serum did not discriminate between the three groups of men [14]. Jentzmik et al. [15] showed that the urinary sarcosine/creatinine ratio was 13% lower in men with prostate cancer when compared with a control group, but receiver-operating characteristic (ROC) analysis showed that the discrimination between patients with prostate cancer and controls was not improved by measurement of sarcosine levels when compared with serum PSA measurement [15]. However, these investigators did not divide the patients into different groups by PSA level and did not perform an independent evaluation [15].

Therefore, the specificity of the urinary sarcosine/creatinine ratio in the diagnosis of patients with low PSA levels requires further investigation. This analysis was not included in the present study and can be considered as a study limitation. However, previous studies had confirmed sarcosine as a potential biomarker for early detection of prostate cancer, and have shown that differences in sarcosine levels were significantly different when patients with prostate cancer were compared with healthy control individuals [16]. Narwal et al. [17] found that sarcosine levels in patients with prostate cancer patients were significantly increased compared with healthy individuals. Heger et al. [18] suggested that measurement of urinary sarcosine might be a non-invasive, rapid, screening method for the diagnosis of prostate cancer. Therefore, the findings of the present study are supported by some previously published studies and showed that the urinary sarcosine/creatinine ratio was significantly different between the group of patients with prostate cancer and the group of patients with benign prostate hyperplasia (BPH). In the present study, the area under the curve (AUC) of the urinary sarcosine/creatinine ratio was found to be significantly increased when compared with the AUC of serum PSA in the patients with PSA less than 10 ng/ml. This finding suggests that in patients with prostate cancer who have low serum PSA levels, the urinary sarcosine/creatinine ratio could be a more reliable biomarker for the detection of prostate cancer.

Sreekumar et al. [4] used RNA interference techniques to inhibit the expression of the enzymes, glycine N-methyltransferase
(GNMT), dimethylglycine dehydrogenase (DMGDH), and sarcosine dehydrogenase (SARDH) by prostate cells, which affect the intracellular levels of sarcosine and contribute to the invasive properties of certain cancers. Khan et al. [19] validated sarcosine as an important tumor-related metabolite in preclinical models, both in vivo and in vitro. Also, these investigators confirmed that the overexpression of the GNMT gene in cells also elevated the levels of sarcosine, but had no effects on cell proliferation [19]. Elevated intracellular levels of sarcosine have been shown to be positively correlated with the invasive properties of prostate cancer and decreased intracellular sarcosine levels have been shown to be correlated with reduced tumor cell invasion [19]. Also, testosterone and members of the erythroblast transformation-specific (ETS) transcription factor family genes (ERG, and ETV1) have been shown to be key factors in the development of prostate cancer [4]. Sreekumar et al. [4] treated the androgen-responsive VCaP prostate cancer cell line (ERG-positive) and the LNCaP prostate cancer cell line (ETV1-positive) for 48 hours and found that sarcosine metabolic pathways were affected by androgens and the expression levels of the ERG and ETV1 genes. These findings might indicate that the sarcosine level is associated with more aggressive forms of prostate cancer.

The histological Gleason score is the main histopathological approach to evaluate the grade and degree of aggression of prostate cancer [20]. The findings of the present study showed a positive correlation between the Gleason score and the urinary sarcosine/creatinine ratio. The urinary sarcosine/creatinine ratio in patients with prostate cancer and a Gleason score ≥8 was significantly increased compared to patients with a Gleason score ≤6. These findings are supported by those of Lucarelli et al. [21] who found that increased sarcosine levels were significantly associated with low-grade and intermediate-grade tumors in men with a serum PSA <4 ng/mL. However, at this time, it would be important to combine measurements of sarcosine with other tumor markers, including serum levels of PSA.

In the present study, the role of the urinary sarcosine/creatinine ratio and its association with the clinical stage of prostate cancer was evaluated. There was a significant difference in the urinary sarcosine/creatinine ratio between patients with metastatic and non-metastatic prostate cancer, which suggests that the urinary sarcosine/creatinine ratio in patients might be used as a potential indicator of metastatic prostate cancer. The role of the measurement of the urinary sarcosine/creatinine ratio as a metabolomic diagnostic and prognostic biomarker for prostate cancer requires support from large-scale, multicenter, controlled clinical studies.

Conclusions

The findings from this study showed that the urinary sarcosine/creatinine ratio might be used as a diagnostic indicator of prostate cancer. When the prostate-specific antigen (PSA) concentration was less than 10 ng/ml, its diagnostic value of the urinary sarcosine/creatinine ratio was better than for the measurement of serum PSA and the free/total (F/T) PSA ratio. In this study, the urinary sarcosine/creatinine ratio was significantly correlated with the Gleason score of prostate cancer, and with the presence of metastatic prostate cancer, or tumor stage, which is a clinical indicator of prognosis.

Competing interests

None.

References:

1. Abidi SH, Bilwani F, Ghias K, Abbas F: Viral etiology of prostate cancer: Genetic alterations and immune response. A literature review. Int J Surg, 2018; 52: 156–40
2. Wang X, Fang G, Pang Y: Chinese medicines in the treatment of prostate cancer: from formulas to extracts and compounds. Nutrients, 2018; 10(3): pii: E283
3. Logozzi M, Angelini DF, Iessi E et al: Increased PSA expression on prostate cancer cells. Eur Urol, 2016; 9: 357–70
4. Wang M. et al.: Edible protein serum markers in the primary diagnostics of prostate cancer. Future Oncol, 2015; 95: 429–35
5. Lima AR, Bastos Mde L, Carvalho M, Guedes de Pinho P: Biomarker discovery in human prostate cancer: an update in metabolomics studies. Transl Oncol, 2016; 9: 357–70
6. Sroka WD, Boughton BA, Reddy P et al: Determination of amino acids in urine of patients with prostate cancer and benign prostate growth. Eur J Cancer Prev, 2017; 26: 131–34
7. Sroka WD, Boughton BA, Reddy P et al: Determination of amino acids in urine of patients with prostate cancer and benign prostate growth. Eur J Cancer Prev, 2017; 26: 131–34
8. Ankerst DP, Liss M, Zapata D et al: A case control study of sarcosine as an early prostate cancer detection biomarker. BMC Urol, 2015; 15: 99
9. Tedeschi PM, Markert EK, Gounder M et al: Contribution of serine, folate and glycine metabolism to the ATP, NADPH and purine requirements of cancer cells. Cell Death Dis, 2013; 4: e877
10. Heger Z, Merlos Rodrigo MA, Michalek P et al: Sarcosine upregulates expression of genes involved in cell cycle progression of metastatic models of prostate cancer. PLoS One, 2016; 11: e0165830
11. Putluri N, Shoji A, Vasu VT et al: Metabolomic profiling reveals a role for androgen in activating amino acid metabolism and methylation in prostate cancer cells. PLoS One, 2011;6: e21417
12. Gkotsos G, Virgiliou C, Lagoudaki I et al: The role of sarcosine, uracil, and kynurenic acid metabolism in urine for diagnosis and progression monitoring of prostate cancer. Metabolites, 2017; 7: pii: E9
13. Capoun O, Sokup V, Kalousová M et al: Diagnostic importance of selected protein serum markers in the primary diagnostics of prostate cancer. Urol Int, 2015; 95: 429–35
14. Struys EA, Heijboer AC, van Mooijerse J et al: Serum sarcosine is not a marker for prostate cancer. Ann Clin Biochem, 2010; 47: 282
15. Jentzmik F, Stephan C, Miller K et al: Sarcosine in urine after digital rectal examination fails as a marker in prostate cancer detection and identification of aggressive tumours. Eur Urol, 2010; 58: 12–18

16. Cernei N, Heger Z, Gumulec J et al: Sarcosine as a potential prostate cancer biomarker – a review. Int J Mol Sci, 2013; 14: 13893–908

17. Narwal V, Kumar P, Joon P, Pundir CS: Fabrication of an amperometric sarcosine biosensor based on sarcosine oxidase/chitosan/CuNPs/c-MWCNT/Au electrode for detection of prostate cancer. Enzyme Microb Technol, 2018; 113: 44–51

18. Heger Z, Cernei N, Gumulec J et al: Determination of common urine substances as an assay for improving prostate carcinoma diagnostics. Oncol Rep, 2014; 31: 1846–54

19. Khan AP, Rajendiran TM, Ateeq B et al: The role of sarcosine metabolism in prostate cancer progression. Neoplasia, 2013; 15: 491–501

20. De Nunzio C, Pastore AL, Lombardo R et al: The new Epstein Gleason score classification significantly reduces upgrading in prostate cancer patients. Eur J Surg Oncol, 2018 [Epub ahead of print]

21. Lucarelli G, Fanelli M, Larocca AM et al: Serum sarcosine increases the accuracy of prostate cancer detection in patients with total serum PSA less than 4.0 ng/ml. Prostate, 2012; 72: 1611–21