Pathology Section

Utility of a Flowcytometry Based Urine Analyser in Screening of Urothelial Malignancies

MONA BARGOTYA[1], LALIT KUMAR[2], PAYEL DAS[3], PINKEY KACHHAP[4], RAHUL YADAV[5]

(cc) BY-NC-ND

ABSTRACT

Introduction: Urothelial malignancies are one of the most prevalent carcinomas. In clinical practice, all patients undergo routine urine examination. If urine examination is used as a screening test to detect malignancies, it will indeed be of great help in early detection and initiation of treatment, leading to better patient care and disease outcome. There is a paucity of literature on detection of urothelial carcinomas based on flowcytometric urine analysers.

Aim: To establish flowcytometry based screening criteria which could assist in detecting urothelial carcinomas on routine urine examination.

Materials and Methods: The prospective study was conducted in the Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Tahirpur, Delhi, India. All the samples which were sent for urine cytology from July 2017 to June 2018 were included in the study. The samples underwent cytological examination using Giemsa and Papanicolaou stain after being analysed in Sysmex UX 2000. The statistical analysis was performed using Microsoft Excel 2010 and SPSS statistical program (Version 24).

Results: Total of 160 patients were included in the study. The male to female ratio in the present study was 5.67:1. Around 22% (35) cases turned out malignant, with 31 (88.6%) men and 4 (11.4%) women. The mean age for malignant cases was 60.2±5.2 years compared to 48.9±33.4 years for non-malignant cases. Multiple parameters were evaluated; only Red Blood Cell (RBC), White Blood Cells (WBC), Small Round Cells (SRC), and Epithelial Cells (EC) were statistically significant. Cut-off value for RBC was established at 52.9 cells/µL with high sensitivity, specificity and Negative Predictive Value (NPV); whereas WBC, SRC and epithelial cells showed increased NPV only.

Conclusion: Automated urine analysers, with established cut-off values for detection of urothelial malignancies, could help in screening of the routine samples for early detection of urothelial malignancies.

INTRODUCTION

Carcinoma of the urinary bladder is one of the most common malignancies around the world. It ranks seventh among cancers in men while 17th in females [1]. In 2012, bladder carcinoma accounts for an estimated 0.43 million new cases with a strong male predominance. Less developed regions of the world account for 60% of all bladder cancer cases and half of all bladder cancer related deaths [2,3]. Urothelial carcinoma accounted for 0.16 million cancer related deaths in 2012 [3]. The incidence of urothelial carcinoma has increased to 0.54 million in 2018 and accounted for approximately 0.2 million deaths worldwide [4]. In India, carcinomas of urinary bladder were reported as the fourth most common carcinoma affecting males. Urinary tract cancers account for 4.89% of all cancers reported (6.96% in males and 1.89% in females); urinary bladder cancers formed 5.31% of all cancer cases in men while only 0.95% in women [5].

Screening for malignancies of the urinary tract is most commonly done using voided urine cytology. It is a non-invasive test that aids in detection as well as surveillance of atypical or malignant urothelial cells [6-9]. Urine cytology has shown to be highly sensitive (94.5%) for detection of high grade urothelial tumours [10].

Urine analysis is one of the most common tests conducted in a clinical laboratory. Flow cytometry based analysers can detect particles in urine like RBC, WBC, SRC, EC, Yeast Like Cells (YLC) and crystals [11]. The test is done for almost all the patients attending urology clinic and can be used as a screening test to detect early urothelial carcinoma.

There is a paucity of literature on the topic of the study of urine cytology. To best of our knowledge, no study has been conducted which evaluates the flow cytometry based parameters with voided urine cytology to detect urothelial carcinomas. The objective of the study was to establish benchmarks using parameters from UX2000 urine analyser which could be used as an advantage for isolating cases with suspicious atypical cells or with malignant urothelial cells during routine urine examination.

MATERIALS AND METHODS

The prospective study was conducted in the Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Tahirpur, Delhi, India. All the samples received for urine cytology for malignant cells from July 2017 to June 2018 were included in the study. All the tests were performed on the samples left after the routine procedure. No fresh sample was requested for the current study. Hence, no ethical clearance was obtained for this study. An informed consent was obtained at the time of sample collection stating that the remained of sample and test data in de-identified condition may be used for quality improvement, research studies, presentations and publications. The samples were collected in a sterile container. Samples with inadequately filled requisition forms, samples with an inadequate amount, samples collected two hours ago or samples collected outside the hospital were excluded from the study.

Each sample underwent flowcytometric examination using the Sysmex UX 2000 (Sysmex, Japan), a fully automated urine analyser. The UX 2000 aspirates 1.2 mL for Flowcytometric (FCM) analysis. The aspirated sample is segregated in two channels, namely WBC and bacterial channels, and is stained with polymethine dye. The stained sample passes through a fluidic system which arranges the cells in a linear file. Thereafter, a laser beam strikes each particle individually and produces forward scattered light signal, laterally scattered light signal and lateral fluorescent light signal which is read by arrays of detectors and electrical signals are generated which are represented as scattergrams. The particles in urine are

segregated into RBC, WBC, EC, crystals, SRC, YLC and bacteria using a classification algorithm [11,12].

All the samples were also centrifuged at 1000 rpm for 10 minutes, smears prepared from the sediment were stained with Giemsa stain (Merck, Mumbai, India) and Papanicolaou stain (Biolab Diagnostics Pvt., Ltd., MS, India). Each slide was analysed by two cytopathologists before coming to a final diagnosis. The samples were reported as per the Paris system of reporting urinary cytology [13].

STATISTICAL ANALYSIS

Sensitivity, specificity, Positive Predictive Value (PPV), NPV, Odd's ratio and Area Under the Curve (AUC) were calculated using Microsoft Excel 2010 (Microsoft Corporation, Washington, USA) along with SPSS statistical program, Version 24 (IBM, Chicago, USA).

RESULTS

Total of 160 patients were included in the study. Majority of the patients registered in the study were males, with male to female ratio 5.67:1. Male and female patients registered in the study were 136 (85%) and 24 (15%), respectively. The age of the study group ranged from 20 years to 85-year-old.

Out of 160 cases, 35 (21.9%) cases turned out to be positive for malignant cells, while 125 (78.1%) were negative. Of the 35 positive cases, 31 (88.6%) were men and 4 (11.4%) were women. The mean age for men showing positive results was 59.8±35.2 years, while the mean age for men with negative results was 47.9±26.9 years. Similarly, the mean age for women showing positive for malignant cells was 62.3±22.6 years, while for negative was 55.3±20.4 years. Of the 35 positive cases, only 22 cases were confirmed histopathologically. Rest were lost to follow-up for histopathological examination. The histopathological diagnosis of 21 cases matched with the cytological report. Just a single case didn't correlate with the cytological diagnosis.

The positive specimens were reported as per the Paris System of reporting urine cytology [13]. Of the positive 35 samples, 27 (77.1%) cases were reported as category 5-High Grade Urothelial Carcinoma (HGUC), 3 (8.6%) as Category 6-Low Grade Urothelial Carcinoma (LGUC), 1 (2.9%) as Category 4-Suspicious of HGUC and remaining 4 (10.5%) were reported as Category 7-Other: primary and secondary malignancies and miscellaneous lesions [Table/Fig-1,2a-d].

	Paris system for reporting urine cytology	No. of cases
Category 1	Nondiagnostic/unsatisfactory	7
Category 2	Negative for High Grade Urothelial Carcinoma (NHGUC)	112
Category 3	Atypical Urothelial Cells (AUC)	6
Category 4	Suspicious for High-Grade Urothelial Carcinoma (SHGUC)	1
Category 5	High-Grade Urothelial Carcinoma (HGUC)	27
Category 6	Low-Grade Urothelial Neoplasm (LGUN)	3
Category 7	Other: primary and secondary malignancies and miscellaneous lesions	4

[Table/Fig-1]: Case distribution as per Paris system of reporting urine cytology.

Multiple parameters, namely RBC, WBC, EC, SRC, YLC and crystals, were evaluated with results of urine cytology for malignant cells. Receiver Operating Curves (ROC) were plotted for all the parameters. Of all the parameters evaluated, RBC, EC, WBC and SRC showed statistical significance. AUC of RBC was 0.857 (95% CI, 0.80-0.92; p<0.0001), of EC was 0.809 (95% CI, 0.73-0.89; p<0.0001), of WBC was 0.798 (95% CI, 0.72-0.88; p<0.0001) and of SRC was 0.750 (95% CI, 0.66-0.84; p<0.0001) [Table/Fig-3,4]. Using ROC, cut-offs were established for all the statistically significant parameters. Only RBC, at cut-off of 52.9 cells/µL, showed high sensitivity (92.1%), specificity (72.5%), NPV (96.7%) and Odd's Ratio of 31.5, whereas, the PPV was 51.5%. Rest of the studied parameters showed high NPV only.
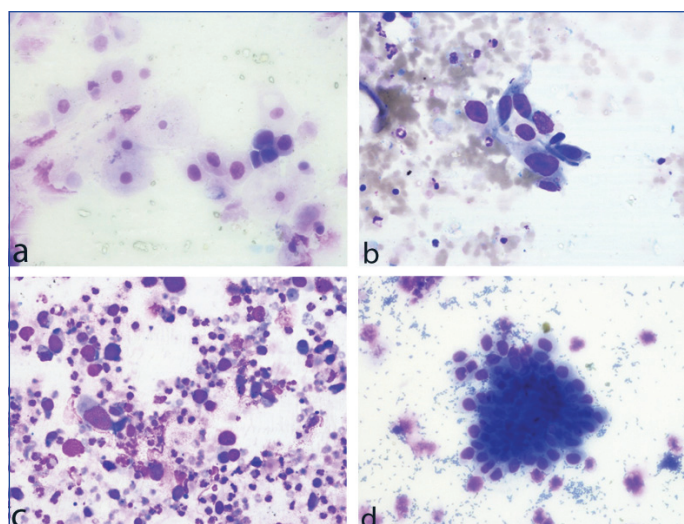
[Table/Fig-2]: a: Category 3-Atypical Urothelial Cells (AUC). b: Category 4-Suspicious for High-Grade Urothelial Carcinoma (SHGUC).c: Category 5-High Grade Urothelial Carcinoma (HGUC). d: Category 6-Low Grade Urothelial Neoplasm (LGUN).

	AUC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV	Odd's ratio
RBC	0.857	0.80-0.92	52.9	92.1	72.5	51.5	96.7	31.5
WBC	0.798	0.72-0.88	44.5	76.4	72.5	40.6	90.6	8.5
EC	0.809	0.73-0.89	11.5	76.4	73.3	46.7	89.8	8.5
SRC	0.750	0.66-0.84	4.4	68.4	69.2	41.3	87.4	5.0

[Table/Fig-3]: Analysis of multiple parameters.
AUC: Area under the curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; RBC: Red blood cell; WBC: White blood cells; SBC: Small round cells; EC: Epithelial cells
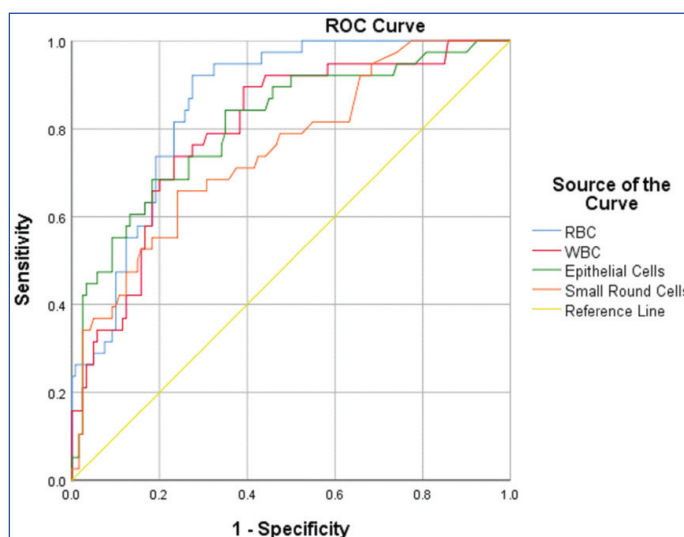
[Table/Fig-4]: Receiver operating curves (ROC) of WBC, RBC, EC and SRC.

DISCUSSION

Urinary bladder carcinomas are among the most common cancers. It accounts for 3% of new cancers worldwide and shows an increasing trend across the globe [14]. In 2018, 549,393 new cases of urinary bladder carcinoma cases were recorded worldwide and accounted for 199,922 bladder carcinoma related deaths. The highest incidence was reported in Southern Europe while the lowest was recorded in East Africa. Higher incidence and mortality was noted in more developed world as compared to lesser developed countries [4]. The age standardised incidence is 9 per 100,000 for men and 2 per 100,000 women worldwide. As per European Union, the same is 27 per 100,000 among men and 6 per 100,000 among women [1]. In India, genitourinary malignancies formed 17.48% of all cancers among men with bladder carcinomas accounting for 30.4% of all genitourinary malignancies [15].

Sex ratio of urothelial carcinomas varies around the world with majority showing a male preponderance. According to a study

conducted in Lucknow, India by Gupta P et al., the mean age was reported as 60.2±4.4 years with male to female ratio was 8.6:1 [16]. Another Indian paper showed that marked male preponderance in Indian population (8.9:1) [15]. The sex ratio for urothelial carcinomas varies around the world. A study conducted at Marburg University Hospital, German showed a sex ratio of 3.6:1 wherein 479 patients were diagnosed with urothelial carcinomas [17]. A different observation was noted in a study conducted in Taiwan which showed a female preponderance where 239 female patients were diagnosed with urothelial carcinomas as compared with 182 males [18]. The sex ratio in the present study correlates with the Indian data with male to female ratio is 7.8:1. It was also observed that the age of patients with malignancy was significantly higher than as seen in negative cases. The youngest positive case in the study was 35 years as compared to 20 years for non-malignant cases.

The risk factors for bladder carcinoma include tobacco smoking, occupational exposure to paints, dyes, metal and petroleum products, exposure to ionising radiation, Schistosomiasis and genetic predisposition [1]. Quantity and duration of tobacco smoking showed the most significant association with bladder carcinoma [15].

Of the many parameters assessed by UX2000 urine analyser, we evaluated four parameters for the current study in correlation with urothelial malignancies; namely RBC, WBC, EC and SRC. Out of these RBC was found to be most significant and had the highest AUC of 0.857.

Haematuria is one of the common findings on routine urine examination [19]. Carcinomas of the urinary tract most commonly present with painless haematuria [14-16]. Significant microscopic haematuria is defined by the American Urological Association as more than 2 RBCs/hpf on two microscopic urinalysis without recent menses, exercise, sexual activity or instrumentation [20]. Causes of microscopic haematuria could be genitourinary malignancy, stone in the urinary tract, urinary infection, prolonged bleeding or medications like anticoagulants, aspirin, non-steroidal anti-inflammatory drugs, etc., [21]. Loo RK et al., reported that high grade haematuria (>50 RBC/hpf) was a definite risk factor with 6.36 times higher risk for genitourinary tract malignancy [22]. The mean RBC counts were 2309.3 RBC/hpf in positive cases in present study as compared to 181.3 RBC/hpf seen in the negative cases. Majority of the specimen positive for malignant cells showed high grade haematuria as compared with the negative specimens. Of all the parameters studied, only RBC counts proved to be the most sensitive, specific and with highest NPV. The other statistically significant parameters were WBC counts, EC and SRC. A mean of 2131 WBC/µL was noted in specimens positive for urothelial carcinomas as compared to 251 WBC/µL in negative cases. Elevated WBC levels in urothelial cancers could signify that urothelial carcinomas are associated with pyuria. Few studies have proven that pyuria is associated with higher pathological tumour stage, higher risk of recurrence of bladder carcinoma and a poorer prognosis [23-25]. We have noted similar results; elevated WBC counts correlated with higher histological grade.

SRC is another parameter which was evaluated in the present study for its relation with urothelial carcinomas. SRC are generally detected in advanced renal failure and could be used as a marker for the same in diabetic as well as non-diabetic kidney diseases [26]. SRC in the urine could be used as an early indicator of diabetic renal disease [27]. SRC detected by the UX2000 was also statistically significant. SRC proved to have a high NPV whereas low sensitivity and specificity for urothelial cancers.

EC showed high NPV. All the specimens with elevated EC counts should be repeated following proper collection protocol, as it could be due to contamination. If EC were still high on a repeat sample, cytological examination should be done to exclude malignant cause.

Limitation(s)

One of the limitations noted is that our institute is one of the referral centers in Delhi and patients that are referred have high suspicion of urothelial malignancies. This leads to a skewed male to female ratio in present study as there is a higher prevalence of urothelial malignancies in men as compared to women. We also noted that at times UX2000 urine analyser is unable to distinguish RBC from YLC and crystals. A warning flag is generated in such samples and microscopy becomes essential in distinguishing between the three parameters. This is also noted in other models of urine analysers from Sysmex (Japan) [28].

CONCLUSION(S)

To summarise, automated urine analysers could be used as a screening tool to detect urothelial malignancies; this would aid in isolating malignancies which would have been overlooked otherwise on a routine urine examination. Cases with an isolated increase in RBC count or in association with increased WBC count should be taken up for cytological examination to rule out urothelial malignancies; especially if the sample belongs to an elderly male patient. Early detection of malignancy could significantly alter the clinical outcome for the patient. In the end, multiple large studies are required to convert the hypothesis into practice.

REFERENCES

[1]   Redondo-Gonzalez E, de Castro LN, Moreno-Sierra J, Maestro de las Casas ML, Vera-Gonzalez V, Ferrari DG, et al. Bladder carcinoma data with clinical risk factors and molecular markers: A cluster analysis. Biomed Res Int. 2015;2015:168682.

[2]   Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: A global overview and recent trends. Eur Urol. 2017;71(1):96-108.

[3]   Wong MCS, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng CF. The global epidemiology of bladder cancer: A joinpoint regression analysis of its incidence and mortality trends and projection. Sci Rep. 2018;8:1129.

[4]   Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68(6):394-424.

[5]   Sharma R, Kumar R, Jain S, Jhajhria S, Gupta N, Gupta S, et al. Distribution of malignant neoplasms reported at different pathology centers and hospitals in Jaipur, Rajasthan. Indian J Cancer. 2009;46(4):323-30.

[6]   Bhatia A, Dey P, Kakkar N, Srinivasan R, Nijhawan R. Malignant atypical cell in urine cytology: A diagnostic dilemma. Cytojournal. 2006;3(1):28.

[7]   Manna AK, Sarkar M, Bandyopadhyay U, Chakrabarti S, Pathak S, Sarkar DK. Cytological and morphometric study of urinary epithelial cells with histopathological correlation. Indian J Surg. 2014;76(1):26-30.

[8]   Fogazzi GB, Pallotti F, Garigali G. Atypical/malignant urothelial cells in routine urinary sediment: Worth knowing and reporting. Clin Chim Acta. 2015;439:107-11.

[9]   Deshpande V, McKee GT. Analysis of atypical urine cytology in a tertiary care center. Cancer Cytopathol. 2005;105(6):468-75.

[10] Koss LG, Deitch D, Ramanathan R, Sherman AB. Diagnostic value of cytology of voided urine. Acta Cytol. 1985;29(5):810-16.

[11] Fully automated integrated urine analyser UX-2000. Instructions for use. Kobe, Japan: Sysmex Corporation; 2015.

[12] Khejonnit V, Pratumvinit B, Reesukumal K, Meepanya S, Pattanavin C, Wongkrajang P. Optimal criteria for microscopic review of urinalysis following use of automated urine analyser. Clin Chim Acta. 2015;439:01-04.

[13] Barkan GA, Wojcik EM, Nayar R, Savic-Prince S, Quek ML, Kurtycz DFI, et al. The Paris system for reporting urinary cytology: The Quest to develop a standardized terminology. Acta Cytol. 2016;60(3):185-97.

[14] Yaxley JP. Urinary tract cancers: An overview for general practice. J Family Med Prim Care. 2016;5(3):533-38.

[15] Yuvaraja TB, Waigankar S, Bakshi G, Prakash G. Genitourinary cancers: Summary of Indian data. South Asian J Cancer. 2016;5(3):122-24.

[16] Gupta P, Jain M, Kapoor R, Muruganandham K, Srivastava A, Mandhani A. Impact of age and gender on the clinicopathological characteristics of bladder cancer. Indian J Urol. 2009;25(2):207-10.

[17] Scheller T, Hofmann R, Hegele A. Sex-related differences in urothelial cell carcinoma of the bladder in Germany. Cancer Manag Res. 2018;11:309-16.

[18] Huang CC, Su YL, Luo HL, Chen YT, Sio TT, Hsu HC, et al. Gender is a Significant significant prognostic factor for upper tract urothelial carcinoma: A large hospital-based cancer registry study in an endemic area. Front Oncol. 2019;9:157.

[19] Bruyninckx R, Buntinx F, Aertgeerts B, Van Casteren V. The diagnostic value of macroscopic haematuria for the diagnosis of urological cancer in general practice. Br J Gen Pract. 2003;53(486):31-35.

[20] Wollin T, Laroche B, Psooy K. Canadian guidelines for the management of asymptomatic microscopic hematuria in adults. Can Urol Assoc J. 2009;3(1):77-80.

[21] Khadra MH, Pickard RS, Charlton M, Powell PH, Neal DE. A prospective analysis of 1930 patients with hematuria to evaluate current diagnostic practice. J Urol. 2000;163(2):524-27.

[22] Loo RK, Lieberman SF, Slezak JM, Landa HM, Mariani AJ, Nicolaisen G, et al. Stratifying risk of urinary tract malignant tumours in patients with asymptomatic microscopic hematuria. Mayo Clin Proc. 2013;88(2):129-38.

[23] Azuma T, Nagase Y, Oshi M. Pyuria predicts poor prognosis in patients with non-muscle-invasive bladder cancer treated with bacillus calmette-guérin. Mol Clin Oncol. 2015;3(5):1113-16.

[24] Satake N, Ohno Y, Nakashima J, Ohori M, Tachibana M. Prognostic value of preoperative pyuria in patients with non-muscle-invasive bladder cancer. Int J Urol. 2015;22(7):645-49.

[25] Liang C, Wang J, Liu H, Huang L, Xu D, Qian S, et al. Preoperative pyuria predicts advanced pathologic tumour stage and worse survival in patients with urothelial carcinoma of the upper urinary tract treated by radical nephroureterectomy. Urol Oncol. 2016;34(9):418.e1-7.

[26] Shukuya K, Ogura S, Tokuhara Y, Okubo S, Yatomi Y, Tozuka M, et al. Novel round cells in urine sediment and their clinical implications. Clin Chim Acta. 2016;457:142-49.

[27] Detrisac CJ, Mayfield RK, Colwell JA, Garvin AJ, Sens DA. In vitro culture of cells exfoliated in the urine by patients with diabetes mellitus. The Journal of Clinical Investigation. 1983;71(1):170-73.

[28] Previtali G, Ravasio R, Seghezzi M, Buoro S, Alessio MG. Performance evaluation of the new fully automated urine particle analyser UF-5000 compared to the reference method of the Fuchs-Rosenthal chamber. Clinica Chimica Acta. 2017;472:123-30.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
2. Senior Resident, Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
3. Senior Resident, Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
4. Senior Resident, Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.
5. Assistant Professor, Department of Urology, Rajiv Gandhi Super Speciality Hospital, Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Lalit Kumar,
Department of Pathology, Rajiv Gandhi Super Speciality Hospital,
Tahirpur, Delhi, India.
E-mail: drlkumar@gmail.com

AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was Ethics Committee Approval obtained for this study? No
• Was informed consent obtained from the subjects involved in the study? Yes
• For any images presented appropriate consent has been obtained from the subjects. NA