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Diagnostic Value of Urinary Dysmorphic Erythrocytes in Clinical Practice

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Key Words
Diagnostic value · Urinary dysmorphic erythrocytes · Hematuria · Erythrocyturia · Red blood cells

Abstract
Background: In clinical practice, discriminating between glomerular and nonglomerular causes of hematuria is often difficult. Dysmorphic red blood cells (dRBC) in the urinary sediment are claimed to be effective, but the cutoff points in the literature vary. This follow-up study aimed to determine the diagnostic value of dRBC. ***Methods:*** We investigated 134 hematuria patients in the departments of nephrology and urology. To diagnose the origin of hematuria, urological and/or nephrological examination was performed and the %dRBC identified by microscopy. Follow-up was performed after 3.5 years. ***Results:*** The cause of hematuria was proven in 68 patients (35% glomerular; 65% nonglomerular). Patients with glomerular disease had significantly more albuminuria and dRBC than patients with nonglomerular disease, but the %dRBC ranged from 1 to 50% and no optimal cutoff could be identified. Logistic regression analysis showed that %dRBC had a predicted probability to diagnose glomerular disease of 77.9% (area under the curve, AUC, 0.85). When %dRBC was combined with other risk factors such as serum creatinine, sex, age, dipstick erythrocyte or proteinuria score and number of casts, the predictive probability increased to 90.6% (AUC 0.97). Follow-up of the included patients showed no benefit of dRBC to identify patients at risk for glomerular disease. ***Conclusions:*** The diagnostic value of routinely collected urinary dRBC to diagnose glomerular disease in patients presenting with hematuria is modest. However, including dRBC with other variables, such as age and erythrocyte score on dipstick testing may increase the sensitivity, but needs to be confirmed in another, preferably larger, population.

Introduction

Hematuria is a common problem in clinical practice, with a reported prevalence ranging from 0.2 to 16.1%, depending on population screened [1–2]. Transient hematuria can be caused by vigorous exercise, sexual intercourse, mild trauma or menstrual contamination. Persistent hematuria can generally be divided into hematuria of glomerular and nonglomerular sources. Common causes of glomerular hematuria are glomerulonephritis, IgA nephropathy, thin membrane disease and Alport's disease [3]. Hematuria of nonglomerular origin usually results from urological abnormalities,

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such as urinary tract infections, urological malignancy or urolithiasis.

As it is important to exclude the presence of a urological malignancy as the source of bleeding, patients presenting with hematuria primarily undergo urological analysis [4–5]. According to the guidelines of the American Urological Association, the urological evaluation of hematuria should include radiological imaging of the upper urinary tract followed by cystoscopic evaluation of the urinary bladder or additional examinations, depending on additional risk factors for urological disease [5]. Patients with persistent hematuria in whom the urological analysis is negative and having additional symptoms such as hypertension, proteinuria or renal impairment, should be referred to the nephrologist to be evaluated for glomerular disease.

Urinalysis is still considered an important diagnostic tool for nephrologists. Although dipstick testing is easy to perform in general practice, the morphology of urinary red blood cells (RBC) has been advocated as a fine diagnostic tool to differentiate between glomerular and nonglomerular causes of hematuria [6–8]. In glomerular hematuria, the variation in size and shape of the urinary RBC is increased, and such RBC are generally called dysmorphic RBC (dRBC). The exact pathophysiological mechanism of the formation of dRBC is unknown. Nevertheless, it is hypothesized, based on in vitro evidence, that dRBC are RBC which leaked through the diseased glomerulus and were damaged by mechanical and osmotic influences during their passage through the tubular system of the kidney, especially the collecting duct [9–13]. In contrast, in nonglomerular hematuria urinary RBC have a more uniform morphology and are therefore called isomorphic RBC.

In theory, a low percentage of dRBC (%dRBC) excludes a glomerular cause of hematuria and it is therefore not indicated to refer these patients to a nephrologist, whereas a higher %dRBC may demand further examination for glomerular disease. However, in the literature there is widespread controversy regarding the diagnostic value of urinary dRBC to identify the hematuria as glomerular in origin, because the criteria for dysmorphism of the urinary erythrocytes are vague and not standardized. Various dRBC have been identified based on their morphology such as acanthocytes or G1 cells [14–17]. Moreover, reported cutoff points of the %dRBC range from 10 to 90%, depending on screened population and study design [6, 18–20].

The present study was undertaken to determine the diagnostic value of urinary dRBC in 134 patients presenting with hematuria in both urological and nephrological outpatient departments. After inclusion, the percentage of urinary dRBC was determined and was correlated with the clinical diagnosis of the source of the hematuria.

Patients and Methods

Study Protocol

Clinicians (both nephrologists and urologists) included patients referred for hematuria to the outpatient departments of nephrology and urology in the Erasmus Medical Center (2002–2004). Hematuria was defined as 1 + (≥20 RBC/μl) after dipstick. During the study period, the treating physicians remained responsible for the care of the patients included in this study. Treating physicians were not aware of the results of urinalysis and the investigators did not intervene at any point. After a follow-up of 3.8 (0–6.7) years, it was investigated whether the included hematuria patients had developed glomerular disease. Follow-up information was retrieved from the hospital electronic information system.

Study Groups

Per patient, the final diagnosis was collected by reviewing clinical charts, the electronic hospital information system and discharge letters. The level of certainty that the definitive diagnosis was indeed the cause of hematuria was scored in five diagnostic groups (numbered –2 to +2; table 1). Patients in whom a glomerular source of hematuria was proven by renal biopsy (e.g. glomerulonephritis or IgA nephropathy) were classified as +2, whereas those in whom urological examination identified a definitive urological source of bleeding (e.g. urinary tract infection, malignancy of the urinary tract or urolithiasis) were classified as –2. Cases in which the cause of hematuria was totally unknown were coded as 0. In diagnostic groups +1 and –1, there were findings indicating either a glomerular or a urological source, but no final diagnosis for the hematuria was made. In our analyses of the predictive value of urinary dRBC, only patients with a proven cause (i.e. diagnostic groups –2 and +2) were included and the percentage of urinary dRBC correlated with clinical findings and definitive diagnosis.

Analytical Methods

Fresh urine samples were analyzed within 2 h after voiding or fixated with CellFIX™ (Becton Dickinson, San Jose, Calif., USA). Firstly, before microscopic examination of the urine sediment, the urine specimens were routinely examined with an automated semiquantitative urinalysis using Combur^{10} Test M strips on a Miditron® M (Roche Diagnostics, Mannheim, Germany). Hematuria was scored in +, ++ and +++ corresponding to 3–20, 20–100 and >100 erythrocytes/μl, respectively. Using the same test system, albuminuria was also measured semiquantitatively (+ = 200–500 mg/l; ++ = 500–1,000 mg/l; +++ = >1,000 mg/l) [21]. Urinary pH was measured on a semiquantitative scale with increments of 0.5. To estimate renal function of each patient, serum creatinine was measured.

Next, urine samples (6–12 ml, depending on concentration of erythrocytes) were centrifuged in a Kova tube (Instruchemie, Delfzijl, The Netherlands) at 1,600 rpm for 5 min. Supernatant was

Table 1. Diagnostic groups

Diagnostic group	Diagnostic cause of hematuria	Final diagnoses
–2	Proven urological (44)	Urinary tract infection (21) Obstructive uropathy (10), including: urolithiasis (5) benign prostate hypertrophy (1) malignancy of the urinary tract (4) Other (10): e.g. trauma, (congenital) anatomic abnormalities, anticoagulant drugs Non-glomerular renal disease (3): e.g. fibrosis
–1	Possible urological (14)	
0	Uncertain (35)	
+1	Possible glomerular (16)	
+2	Proven glomerular (24)	Glomerulonephritis (18): primary/secondary IgA nephropathy (6)

Figures in parentheses indicate number of cases.

discarded, and a drop of sediment suspension was placed on a slide with a covering glass. The presence of erythrocyte casts was examined using a bright-field microscope (Olympus, PAES, The Netherlands), while the presence of dRBC was examined using phase-contrast microscopy under high-power magnification (×400). In each case, 100 erythrocytes were counted to determine the percentage of dysmorphic erythrocytes. Dysmorphic erythrocytes were defined using the criteria as reported previously [6, 22–23]. In brief, dysmorphic erythrocytes exhibited irregular membranes or small surface blebs and showed an annular or vesicular structure. Hematuria of a glomerular source shows a polymorphic aspect and erythrocyte casts can be present. In this study, we did not discriminate between the different types of dysmorphic erythrocytes, although the presence of erythrocyte casts was scored separately.

Examination was carried out by two well-trained technicians. If trained technicians were not present at the time the urine sample was delivered to the laboratory, a CellFIX fixation method was used as described by Huussen et al. [24]. The technicians were blinded to their colleagues' results and the patients' clinical information.

Statistical Analysis

The level of albuminuria and the %dRBC were compared using the Fisher exact test. The mean %dRBC in the five diagnostic groups were compared using a one-way ANOVA. Serum creatinine levels in these groups were compared using Friedman's ANOVA by ranks. If either yielded a significant F, multiple posthoc comparisons were performed using the Student-Newman-Keuls test. Interobserver variation of counted %dRBC was assessed by means of a Bland-Altman plot and the calculation of the Wilcoxon signed ranks test. Sensitivity was calculated as the proportion of all patients with the disease (true positives + false negatives) who indeed have a positive test result (true positives), as defined previously [25]. Specificity was calculated as proportion of all patients without the disease and a negative test result (true negatives) of all those without the disease (true negatives + false positives). Binary logistic regression analysis was performed to make a prediction model for the variables: (1) dipstick erythrocytes score, (2) %dRBC; (3) dipstick proteinuria score; (4) sex; (5) age; (6) number of erythrocyte casts, and (7) serum creatinine. ROC curves of the predicted probabilities were made. Data were analyzed using SPSS (version 15.0; Chicago, Ill., USA). Data are expressed as mean ± standard deviation, or as median (range), depending on the distribution of the data. Area under the curve (AUC) is shown with 95% confidence interval. Statistical significance was defined as $p < 0.05$.

Results

Patients

In this study, 134 patients presenting with hematuria in the outpatient departments of nephrology and urology were included. In total, 93 adults and 41 children (<18 years) were included, including 68 females (50.7%). The median age of this study population was 43 (1–86) years. The median creatinine concentration in serum was 71.5 μM (11–454).

Semiquantitative Urinalysis

Hematuria in the included patients had the following distribution: + in 24 (17.9%), ++ in 19 (14.2%) and +++ in 91 (67.9%; table 2). Albuminuria was present in 26.9% (36/134) of all cases, and higher levels of albuminuria were significantly associated with higher levels of hematuria ($p < 0.05$). Interestingly, in the group with proven uro-

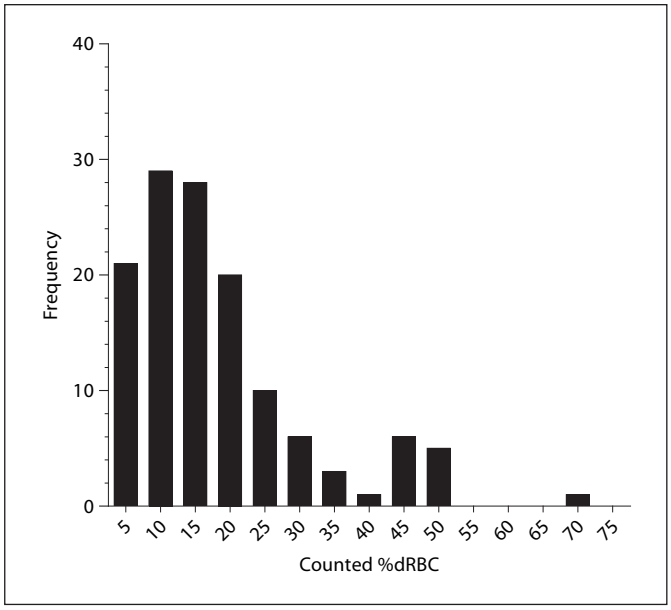


Fig. 1. Frequency distribution of microscopically counted %dRBC in 134 hematuria patients. Percentages are shown as medians of two measurements.

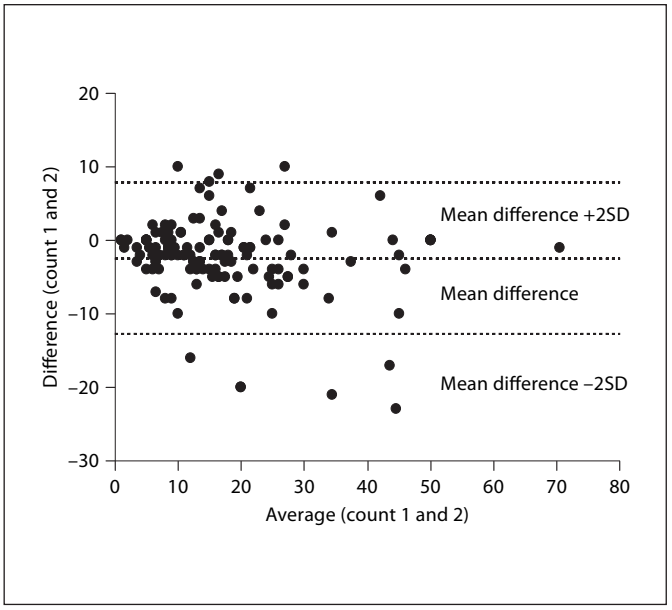


Fig. 2. Interobserver variability of counted %dRBC (Bland-Altman plot). The mean differences between the counted percentages of urinary dRBC by 2 technicians (count 1 and count 2) were plotted.

logical disease, i.e. diagnostic group –2, there were still 6 patients who had 3+ albuminuria scored on the test strip. However, the mean counted percentages of urinary dRBC in these patients ranged from 1 to 18. The median urinary pH in the general study population was 6.0 (5.0–8.0).

Microscopic Analysis of the Urine Sediment

The %dRBC detected in the urine samples ranged from 1 to 71%. Figure 1 shows the distribution of the percentages of urinary dRBC of all 134 hematuria patients included in this study population. There was no significant difference in %dRBC counted by the two technicians. Figure 2 shows a Bland-Altman plot of the mean differences of the dRBC counts by observer 1 and 2. The %dRBC was significantly higher in the ++ than in the + and +++ hematuria groups ($p < 0.05$; table 2). Erythrocyte casts were found in 6 of the 134 samples.

Table 2. Results of urine semiquantitative test strip and microscopic analysis of 134 hematuria patients

	Hematuria			
	overall (n = 134)	+ (n = 24)	++ (n = 19)	+++ (n = 91)
Albuminuria				
+		0	2	10
++		0	2	9
+++		1	1	11
Total	36	1	5	30[a]
%dRBC		15 (10)	26 (12)[a, b]	17 (13)
Erythrocyte casts	6	1	2	3

[a] $p < 0.05$ vs. + ; [b] $p < 0.05$ vs. +++. Values for %dRBC are expressed as mean (SD).

Diagnostic Performance

Dysmorphic RBC

In total, we found 68 (51%) patients with a proven cause of hematuria, either urological or glomerular. Table 1 shows the definitive diagnoses of the patients with a proven origin of the hematuria after urological and/or nephrological examination. In 66% (27/41) of the pediatric patients, a proven cause of hematuria was found: 48% (13/27) nonglomerular and 52% (14/27) glomerular. In adult patients, 79% (33/42) had a urological and 21% (9/42) had glomerular pathology as the cause of hematuria.

The percentage of urinary dRBC differed significantly between the five diagnostic groups and was highest in the group with proven glomerular disease ($p < 0.05$; table 3).

Table 3. Characteristics and results of urinalysis of diagnostic groups

Diagnostic group	–2 Proven urological	–1 Possible urological	0 Uncertain	1 Possible glomerular	2 Proven glomerular
Patients	44	15	35	16	24
Female sex	20 (45.5%)	11 (73.3%)	19 (54.3%)	5 (31.3%)	9 (37.5%)
Age	41.9 ± 24.4	45.9 ± 27.8	48.5 ± 17.4	31.6 ± 20.1[c]	21.8 ± 15.4[a–c]
Children	13 (28.3%)	4 (28.6%)	3 (8.6%)	7 (43.8%)	14 (60.9%)
Serum creatinine, μM	79 (12–454)	59 (32–273)	74 (27–297)	71 (11–110)	64 (29–211)
Semiquantitative urinalysis					
Albuminuria					
1+	4	1	0	0	7
2+	5	0	1	2	3
3+	6	1	0	1	5
Total	15/46	2/14	1/35	3/16	15/23[a–d]
Erythrocyturia					
1+	9	5	9	0	1
2+	7	2	5	4	1
3+	30	7	21	12	21
Total	46	14	35	16	23
pH	6.0 (5.0–8.0)	6.0 (5.0–8.0)	6.0 (5.0–8.0)	6.0 (5.0–7.0)	6.0 (5.0–8.0)
Microscopic analysis					
%dRBC	12 ± 7	21 ± 13	18 ± 15	22 ± 13[a]	25 ± 13[a, c]
Erythrocyte casts	0	1	2	0	3

[a] $p < 0.05$ vs. group –2; [b] $p < 0.05$ vs. group –1; [c] $p < 0.05$ vs. group 0; [d] $p < 0.05$ vs. group 1. Values for serum creatinine and pH are expressed as median (range), and values for %dRBC as mean ± SD.

Table 4. Sensitivity and specificity of %dRBC for diagnosing glomerular disease at various cutoff points in 68 patients with proven disease

Cutoff points	Positive test for proven urological disease, n	Positive test for proven glomerular disease, n	Sensitivity of %dRBC for glomerular disease, %	Specificity of %dRBC for glomerular disease, %
0%	44	24	100 (24/24)	0 (44/44)
10%	24	23	96 (23/24)	45 (20/44)
20%	4	13	54 (13/24)	91 (40/44)
30%	1	6	25 (6/24)	98 (43/44)
40%	0	5	21 (5/24)	100 (44/44)
50%	0	2	8 (2/24)	100 (44/44)

Albuminuria was also significantly higher in the group in which the hematuria resulted from a glomerular disease ($p < 0.05$). Serum creatinine concentrations were not significantly different in the various diagnostic groups. Patients with proven glomerular hematuria were significantly younger than patients in the other diagnostic groups ($p < 0.05$).

Cutoff Value

As various cutoff points have been reported in the literature, we were interested what the effect was of different cutoff values on the sensitivity and specificity of urinary dRBC for glomerular disease. In table 4, the results of these calculations are shown. Using a cutoff point of 40% dRBC showed that none of the patients with a

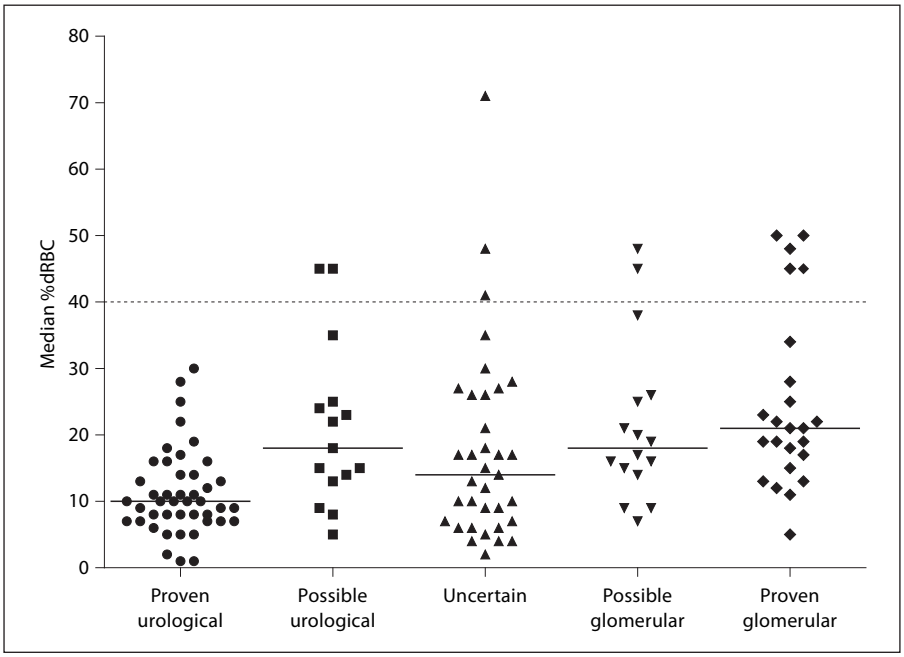


Fig. 3. %dRBC per diagnostic group defined on certainty of the cause of hematuria. At a percentage of 40% dRBC, a horizontal line is drawn showing the diagnostic value of dRBC at this cutoff value for each diagnostic group: all patients with proven urological pathology had less than 40% dRBC, whereas 5 patients with proven glomerular pathology had more than 40%. In 3 diagnostic groups (possible urological, uncertain and possible glomerular), no final cause of hematuria could be made.

proven urological cause of hematuria had an increased %dRBC in their urine (fig. 3), while only 5 of the 23 patients with a histological proven glomerular cause of hematuria had an elevated percentage (>40%) of urinary dRBC. Thus, at a 40% cutoff point the sensitivity of urinary dRBC for excluding glomerular disease in patients with urological diseases was 100% (46/46 dRBC <40%), while still 78% of the patients with a glomerular cause of hematuria had less than 40% dRBC. None of the patients with proven urological disease showed dRBC above the cutoff of 40%.

Binary logistic regression analysis of all patients with proven causes of hematuria showed that the predicted probability of %dRBC for glomerular disease was 77.9%. The ROC curve of the predicted probability of %dRBC is shown in figure 4a with an estimated AUC of 0.84 (0.76–0.95). Addition of proteinuria to %dRBC hardly increased the predicted probability to 79.4%, AUC 0.86 (0.77–0.95; fig. 4a). The predicted probability for glomerular disease improved to 90.6%, AUC 0.97 (0.94–1.00) when the following variables were added to the prediction model: (1) dipstick erythrocyte score, (2) %dRBC, (3) dipstick proteinuria score, (4) sex, (5) age, (6) erythrocyte casts and (7) serum creatinine (fig. 4b). The regression formula of this full model was: (glomerular disease) = –14.9 –3.93 · (erythrocyte score) +0.48 · (%dRBC) +0.35 · (proteinuria score) –2.29 · (sex) –0.10 · (age) +21.54 · (erythrocyte casts) –0.004 · (serum creatinine).

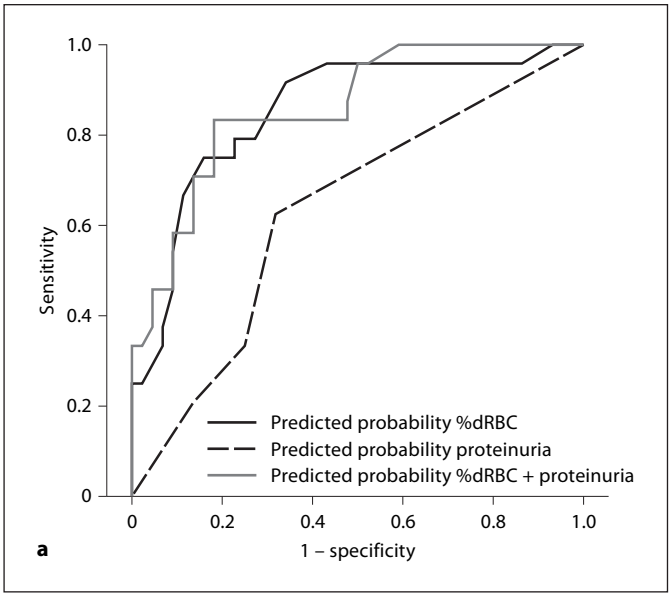
Based on our data, only %dRBC, dipstick erythrocyte score and age significantly contributed to the prediction model. The regression formula of this reduced model was: (glomerular disease) = –8.7 +0.25 · (%dRBC) +2.13 · (erythrocyte score) –0.058 · (age). Figure 4b shows the ROC curve of the predicted probabilities of this reduced model. The predictive probability of this model was 85.3% with an AUC of 0.95 (0.89–1.00).

Erythrocyte Casts

In our study, erythrocyte casts were seen in 6 out of 134 cases (table 2, 3). Five of those patients had ++ or more erythrocytes on the test strip. In 3 out of the 6, the origin of hematuria (all glomerular) was found. One patient had IgA glomerulopathy and 2 patients suffered from glomerulonephritis. The percentages of urinary dRBC were 5, 21 and 34, respectively.

Follow-Up

To determine whether dysmorphic erythrocytes had a predictive value for the development of glomerular disease in the long-term, we reevaluated several years after inclusion whether the hematuria patients developed glomerular disease. The median follow-up time was approximately 3.8 years, with a maximum of 6.7 years. One year after inclusion, 29 patients were lost to follow-up, which was likely due to discharge or referral of patients to other hospitals. The follow-up of the rest of the patients was 1–4



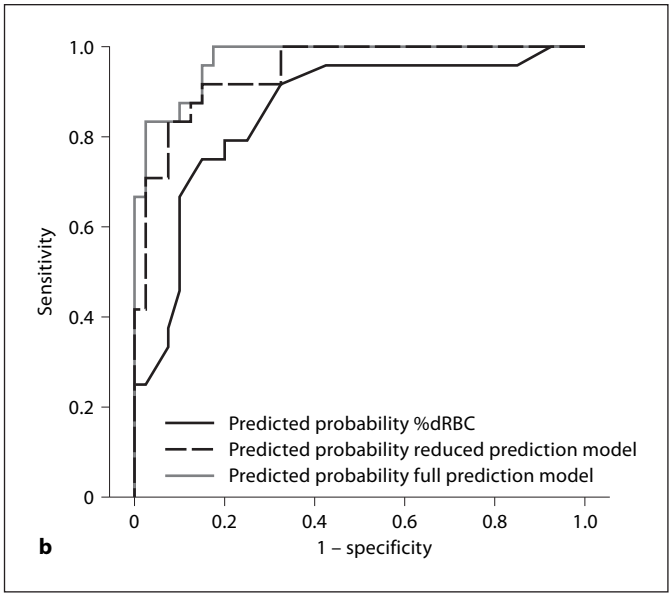


Fig. 4. ROC curves of predicted probabilities for glomerular disease in patients with proven disease. **a** ROC curves of the predicted probability of %dRBC only, proteinuria only and a combination of %dRBC and proteinuria to identify glomerular disease. **b** ROC curves of the predicted probability of %dRBC only, all risk factors (full prediction model) and the reduced prediction model. The full prediction model consisted of %dRBC, erythrocyte score on dipstick, proteinuria score on dipstick, sex, age, serum creatinine and number of erythrocyte casts. The reduced prediction model consisted of %dRBC, erythrocyte score on dipstick and age.

years (46 patients), while 59 patients had been followed for at least 4 years. It can be hypothesized that patients with a high percentage of urinary dRBC might be at risk to have or develop glomerular disease. From the 7 patients with more than 40% dRBC and no previously diagnosed glomerular disease, one patient developed IgA nephropathy after a follow-up of 2.0 years. In contrast, there were 2 patients with a proven urological cause who both developed IgA nephropathy, while the %dRBC was only 10 and 11 in their urine sediments.

Discussion

This study, performed in 134 patients presenting with hematuria in both urological and nephrological outpatient departments, shows that the measurement of urinary dRBC has a moderate diagnostic value to identify glomerular disease. Therefore, it may be useful in the urological department to identify patients with glomerular diseases who may benefit from referral to a nephrologist.

In only 51% of the patients in our study could a definite diagnosis explaining the hematuria be made after a full urological and/or histopathological evaluation: 18% with glomerular pathology versus 34% with urological pathology. Although this percentage appears low, previous studies reported similar percentages of identified causes of hematuria (ranging from 32 to 90% even after full examination) [4, 5, 26]. We failed to determine optimal diagnostic cutoff values, which was due to the wide frequency distribution of urinary dRBC in these patients.

The Value of dRBC in Urological Disease

In our study, 34% of all patients had proven urological pathology. We found that these patients were significantly older than the patients in the other diagnostic groups. This most likely resulted from the fact that urinary tract infections, malignancies of the urinary tract, prostate hypertrophy and stones are more frequent in elderly [27, 28]. All patients with a urological cause of hematuria had less than the arbitrary chosen level of 40% dRBC in their urine. This would suggest that identifying ≥40% dRBC in the urinary sediment excludes urological pathology. Using this argument, it has been suggested that all patients with more than 40% urinary dRBC should be referred to a nephrologist as they have a low risk of uro-

logical disease and may be spared a full urological work-up [26]. However, patients with a high %dRBC due to subclinical glomerular abnormality (such as thin membrane disease) may also develop a urological malignancy. Indeed, the question remains how such high %dRBC can occur in patients with a urological source of hematuria. There is no clear pathophysiological explanation for this, as dRBC are supposed to be formed in the tubular system of the kidney due to mechanical and osmotic changes and not in the lower urinary tract [29]. A possible explanation for the relatively high %dRBC in these patients with an established urological cause could be the coexistence of (subclinical) glomerular disease pathology, such as thin-membrane disease. Some reports estimate the frequency of this disorder to be as high as 1–10% in the general population [30]. However, for obvious reasons, renal biopsy was not performed in these patients. Six patients with proven urological disease and low urinary dRBC (<40%) had a significant amount of albuminuria on the test strip. These patients would not have been identified by the use of only urinary dRBC, but should nevertheless be referred to a nephrologist for the investigation of coexisting renal disease.

Follow-up showed that 2 patients with proven urological disease developed glomerular disease, although the %dRBC in their urine sediment was only 10 and 11%, respectively. This suggests that the determination of urinary dRBC is not useful to detect glomerular disease in the urological department, but may be useful to exclude glomerular disease in patients with urological diseases.

The Value of dRBC in Glomerular Disease

In total, 18% of the patients enrolled in our study had biopsy-proven glomerular disease. When investigating the diagnostic value of urinary dRBC for glomerular disease, we found that the percentage of urinary dRBC was significantly higher in patients with proven glomerular disease than in patients with urological pathology. However, a low %dRBC did not rule out glomerular pathology as the %dRBC ranged from 1 to 50%. Albuminuria is an important indicator of glomerular damage and was significantly more frequent in patients with proven glomerular hematuria. Two of these patients had no albuminuria at all, leaving dRBC as the only indicator of a glomerular abnormality. Conversely, 8 patients with proven glomerular disease had marked albuminuria but less than 40% urinary dRBC. These patients would not have been identified by the use of urinary dRBC alone, and this finding challenges the validity of dRBC as a diagnostic tool for glomerular disease. Serum creatinine, as an estimate of glomerular filtration rate, could also be of value for diagnosing glomerular disease. In our study, however, there was no significant difference in serum creatinine between the diagnostic groups. In the 5 patients with proven glomerular disease and dRBC >40%, the median serum creatinine concentration was 61 (range 40–71) μM and did not add to the diagnosis. Another indication that glomerular pathology is the cause of hematuria is the presence of urinary erythrocyte casts which is virtually pathognomonic of glomerular bleeding. Unfortunately, they are a relatively insensitive marker and are frequently not present in urine of patients with glomerular pathology [18, 19]. In our study, erythrocyte casts were observed in only 6 urine specimens, which were in 3 out of the 6 cases derived from patients with proven glomerular disease.

Furthermore, it can be hypothesized that using a combination of variables/risk factors such as %dRBC, erythrocyte score on dipstick, proteinuria score on dipstick, sex, age, serum creatinine and number of erythrocyte casts, can increase the sensitivity for glomerular disease. In our hands, especially %dRBC, age and erythrocyte score on dipstick showed a significant contribution to predict glomerular disease. Although these findings should first be confirmed in another, preferably larger, study population, we can speculate that the composition of a scoring system based on these risk factors may improve the identification of patients with glomerular disease. This can be of special interest in the department of urology to identify patients who need referral to a nephrologist.

Study Limitations

In our study, we did not routinely acidify hematuria patients before obtaining urine samples, and our analysis showed that the median pH of the urinary specimens was 6.0. This may have affected our results, as both the generation of dRBC and the formation of erythrocyte casts is enhanced by acidic urine [9, 29]. Urinary osmolality has also been reported to be important for the formation of dRBC and erythrocyte casts [9]. However, in our study, urine osmolality was not measured and no specific effort was made to ensure that concentrated urine samples were obtained, although all samples were collected in the morning. All urine specimens were analyzed within 2 h after voiding or fixated with CellFIX. Moreover, we found that fixation did not affect the percentages of dRBC in the urine (data not shown), which is in line with the previous literature [24]. Possibly, the %dRBC in patients with renal disease can be increased, thus increasing the sensitivity,

by a more rigorous study protocol, controlling both pH and osmolality in the urine samples. However, in none of the previously reported studies was such a protocol applied. Moreover, preconditioning of the patient would severely hamper the application of this test in clinical practice.

To standardize measurements and to reduce the workload of conventional microscopic examination, automated systems using computer-assisted light microscopy [31, 32], immunocytochemical staining, flow cytometric analysis of erythrocytes [33] or measurement of size or mean corpuscular volume of erythrocytes [34] have been used in the laboratories. These methods, however, are still not qualified to detect and quantify urinary dRBC. The sensitivity and specificity of these methods to diagnose pathology are still under investigation, and manual microscopic examination is still required to identify some cell types [35]. The present study evaluated whether there is still reason to perform manual microscopic examination of urinary specimens in clinical practice.

An unanswered, yet important, question is: should all patients with isolated hematuria and high percentages of urinary dRBC be referred to a nephrologist? Follow-up of patients with more than 40% dRBC and no previously proven glomerular disease showed that only one of the 7 developed glomerular disease during this period, suggesting that a high dysmorphism of urinary RBC does not predict glomerular disease in the short-term.

Furthermore, the definitive diagnosis of glomerular disease can only be made by renal biopsy. This is often not indicated, as the likelihood of finding a treatable disease in isolated glomerular hematuria is very low and these patients appear to have a low risk of progressive renal disease [36]. In these patients, a conservative policy is justified and regular monitoring of these patients for the development of hypertension, renal insufficiency and albuminuria appears sufficient.

In conclusion, this follow-up study in patients presenting with hematuria shows that the diagnostic value of routinely collected urinary dRBC to diagnose glomerular disease is modest, as the presence of a low %dRBC failed to exclude glomerular disease. Although there is an urgent need for both urologists and nephrologists to discriminate between hematuria of glomerular and nonglomerular origin, we feel that currently the manual determination of urinary dRBC is not recommended in clinical practice. Making a scoring system based on a combination of several risk factors, especially %dRBC, age and erythrocyturia, may increase the probability to identify glomerular disease. This needs to be further investigated in another, preferably larger, population.

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