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ORIGINAL ARTICLE

Renal biopsy criterion in idiopathic nephrotic syndrome with microscopic hematuria at onset

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Abstract
Background The criterion for performing a renal biopsy in children with idiopathic nephrotic syndrome (NS) showing microscopic hematuria at onset remains controversial.
Methods To determine an adequate renal biopsy criterion in children with NS showing hematuria, the optimal cutoff for the maximum red blood cell (RBC) range in urine sediment to separate minimal change disease (MCD) from other glomerular changes was obtained by receiver operating characteristic analysis. We studied 29 children with NS showing hematuria who were screened from 1,320 patients who underwent renal biopsies between January 2001 and September 2011. Patients were divided into two groups according to the cutoff value to verify its validity.
Results The optimal maximum RBC range was 30–49/high-power field (HPF). In group 1 (RBC ≤29/HPF, n=14), 3 patients showed nephritis and the other 11 patients showed MCD. In group 2 (RBC ≥30/HPF, n=15), 1 patient showed focal segmental glomerulosclerosis, 12 showed nephritis, and the other 2 showed MCD. These findings indicated that the ratio of non-MCD/MCD was significantly higher in group 2 than in group 1 (P<0.01).
Conclusions The use of maximum RBC range (30–49/HPF) for a criterion of renal biopsy in patients with NS showing hematuria may be reasonable for clinical practice.

Keywords Minimal change disease · Focal segmental glomerulosclerosis · IgA nephropathy · Membranoproliferative glomerulonephritis · Receiver operating characteristic analysis

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Introduction

Idiopathic nephrotic syndrome (NS) is defined as NS in the absence of systemic disease. This category is divided into two types of entities according to histopathological findings: minimal change disease (MCD) and other glomerular changes. Idiopathic NS is the most common type of childhood NS, representing more than 90 % of cases between 1 and 10 years of age and 50 % after 10 years of age [1]. Most NS patients have histological findings of MCD, and the vast majority of patients with MCD (>90 %) respond to steroid therapy [2]. Based on these observations, an initial trial of steroid therapy is generally administered to children who are likely to have MCD based on a clinical diagnosis, avoiding renal biopsy. Renal biopsies carried out before the initiation of therapy are recommended when children with idiopathic NS show continuous hematuria, hypertension, elevated serum creatinine levels, hypocomplementemia, or are less than 3 months of age, because these signs also suggest other diseases besides MCD [1, 3].

Although continuous hematuria is an indication for renal biopsy in children with idiopathic NS, the optimal cutoff of the red blood cell (RBC) count in urine sediment to separate

MCD from other glomerular changes remains unknown. Therefore, we attempted to determine an adequate maximum RBC count in urine sediment as a criterion for renal biopsy in children with idiopathic NS.

Materials and methods

Patients

The study protocol was approved by the regional research ethics vetting boards. A total of 1,320 children aged <20 years who had undergone a renal biopsy at one of four centers (Wakayama Medical University Hospital, Kobe University Hospital, Hyogo Prefectural Kobe Children's Hospital, and Himeji Red Cross Hospital) between January 2001 and September 2011 were screened retrospectively. Of the 1,320 patients, 754 underwent an initial renal biopsy, and of these, 237 underwent a renal biopsy because of NS. Patients with systemic diseases, such as Henoch–Schönlein purpura nephritis or systemic lupus erythematosus, hypertension, elevated serum creatinine levels, hypocomplementemia or treatment before a renal biopsy, were excluded from the analysis. All of the patients were at least 3 months of age or older. Finally, 29 patients underwent an initial renal biopsy only because of hematuria with NS at onset (Fig. 1). The criteria for NS were in accordance with the International Study of Kidney Disease in Children (heavy proteinuria, ≥40 mg/h/m^2 and hypoalbuminemia, ≤2.5 g/dl) [4]. Hematuria was defined as five or more RBCs in a properly collected and centrifuged urine specimen under high-power field (HPF, ×400 magnification) [5]. The RBC count in urine sediment was graded as 0, 1–4, 5–9, 10–19, 20–29, 30–49, 50–99, and 100–/HPF according to the method of the Japanese Committee for Clinical Laboratory Standards. According to clinical records, 4 patients underwent a renal biopsy because of continuous positive dipstick findings (≥2+) of occult blood, even with an RBC <5/HPF in the urine sediment. Data from these patients were included for analysis. Each patient's family gave written informed consent to a renal biopsy. Renal tissue was obtained by needle biopsy under ultrasound guidance. Renal biopsy specimens were investigated by routine light, immunofluorescence, and electron microscopy. All biopsy specimens were examined and diagnosed by one of the study investigators (N.Y.). Clinical data and information were obtained from medical records.

Determination of the optimal cutoff point of the maximum RBC count range

First, receiver operating characteristic (ROC) analysis was performed to examine the relationship between the maximum RBC range in the early morning urine sediment during the disease course before a renal biopsy and separation of MCD and other glomerular diseases [6]. The lowest count of each range of RBC in the urine sediment was used for analysis. The optimal cutoff point of the RBC count for a biopsy criterion was calculated using the Youden index [6].

Validity of the optimal cutoff point of the maximum RBC count range

Patients were then divided into two groups based on the optimal cutoff point of the maximum RBC count range in the early morning urine sediment during the disease course before a renal biopsy. Group 1 comprised patients who had a maximum RBC count range less than the optimal cutoff point before a renal biopsy. Group 2 comprised patients who had a maximum RBC count range equal to or greater than the optimal cutoff point at least once during the disease course before a renal biopsy. Histological diagnoses of the two groups at initial biopsies were compared. All of the patients who were analyzed had oral steroid treatment (including combination therapies for nephritis) after a renal biopsy. Responses to treatment at 4 weeks after initiation were investigated to assess the validity of the optimal cutoff point of the maximum RBC count range.

Statistical analysis

The results were analyzed using the JMP version 9 software package (SAS Institute Japan, Tokyo, Japan). The distribution of clinical and morphological attributes between the groups was examined using Fisher's exact test. Continuous characteristics of the groups were compared using the Mann–Whitney U test. A P value of <0.05 was taken as the level of significance.

Results

Determination of the optimal cutoff point of the maximum RBC count range

The number of patients for each maximum RBC count range is shown in Fig. 2. ROC analysis demonstrated that the optimal cutoff point of the maximum RBC count range to separate MCD from other glomerular diseases was 30–49/HPF. The area under the ROC curve was 0.91 (highly accurate; sensitivity was 0.81 and specificity was 0.85, Fig. 3). These findings suggested that the presence of more than 30–49 RBCs/HPF was valid as a renal biopsy criterion for NS with hematuria.

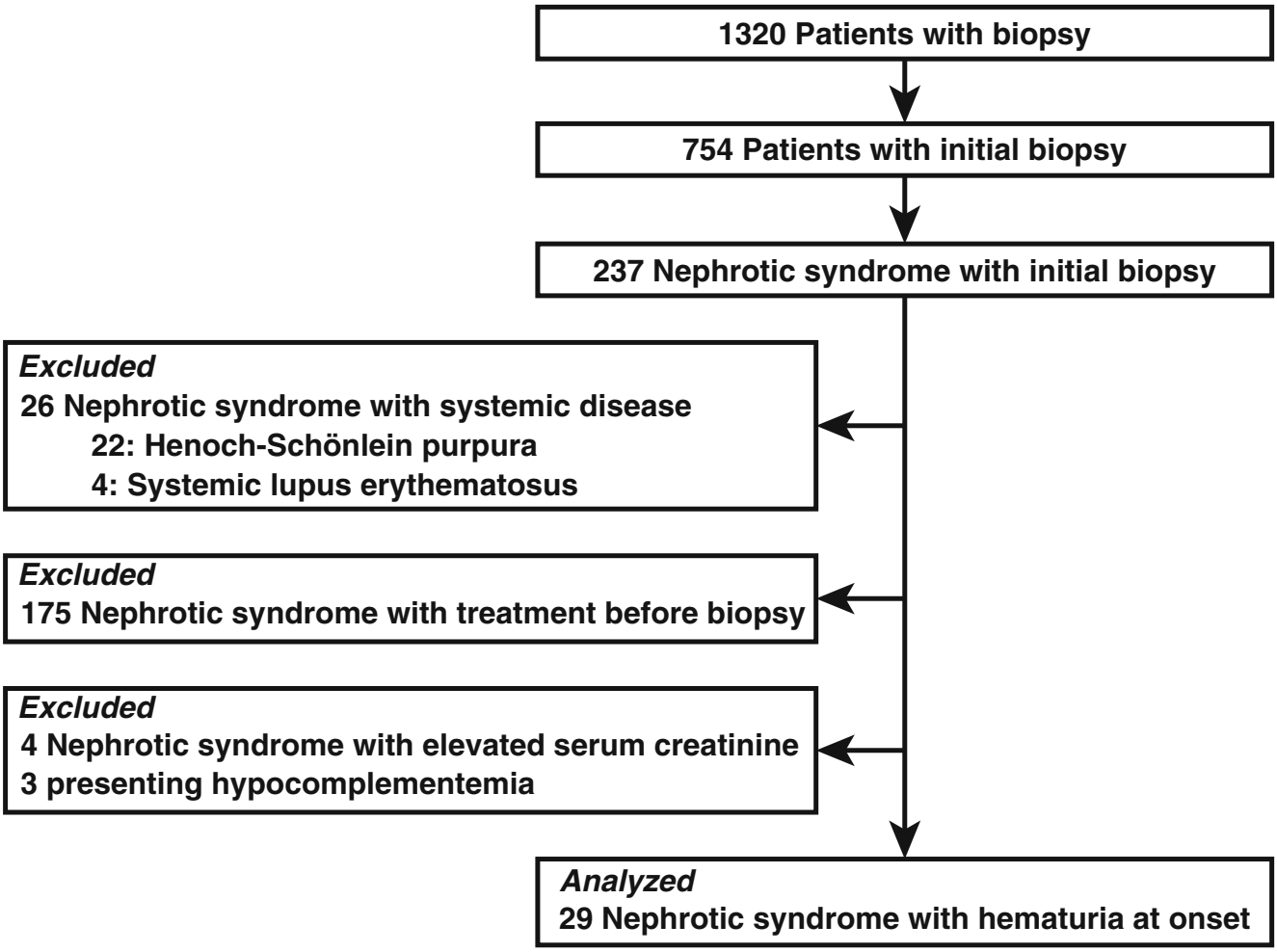

Fig. 1 Patients' profiles

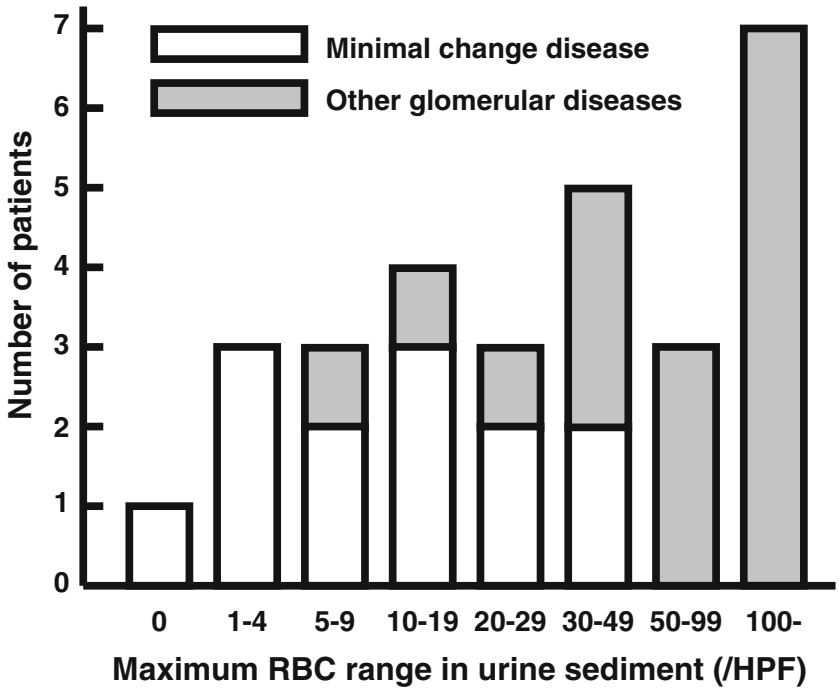

Fig. 2 Number of patients in each maximum red blood cell (RBC) count range

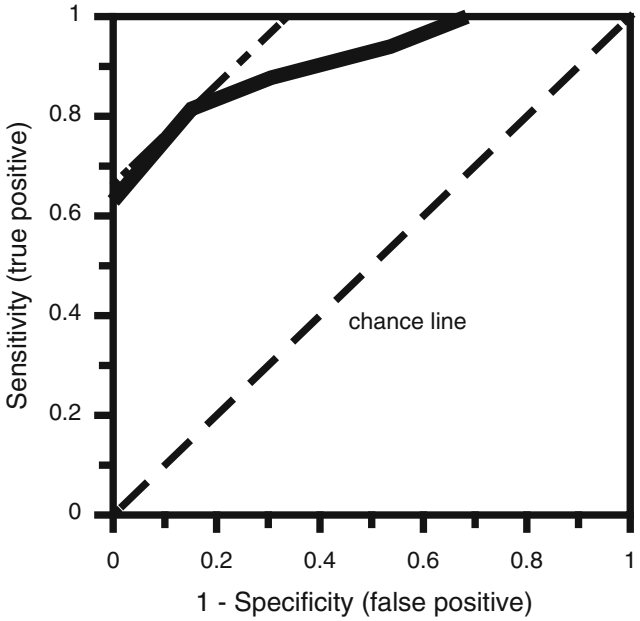

Fig. 3 Receiver operating characteristic (ROC) curve for separating minimal change disease (MCD) from other glomerular diseases. The area under the ROC curve was 0.906. The Youden index is defined as the maximum vertical distance between the ROC curve and the chance line (*dotted line*), and is calculated as maximum (sensitivity + specificity – 1). Using this measure, the cutoff point on the ROC curve, which corresponds to the Youden index, i.e., at which sensitivity + specificity – 1 is maximized, is taken as the optimal cutoff point. For an expedient, the intersection point of the chain line and the vertical axis indicates the Youden index

Validity of the presence of more than 30–49 RBCs/HPF

According to the results of the ROC analysis, 29 patients were divided into two groups based on the maximum RBC range. Group 1 comprised 14 patients who had ≤20–29 RBCs/HPF before a renal biopsy and group 2 comprised 15 patients who had ≥30–49 RBCs/HPF at least once before a renal biopsy. The baseline characteristics in both groups are shown in Table 1. There was no significant difference between the groups, except for the sex ratio (Table 1).

Each specimen in all of the 29 patients contained sufficient glomeruli for evaluation (≥10). The pathological diagnoses are shown in Table 2. In group 1, 11 patients (78.6 %) showed MCD. Two patients with non-IgA mesangial proliferative glomerulonephritis and 1 patient with IgA nephropathy were included in group 1. Their maximum RBC range before a renal biopsy was 5–9 (non-IgA), 10–19 (IgA), and 20–29/HPF (non-IgA). In Group 2, focal segmental glomerulosclerosis was detected in 1 patient and nephritis was detected in 12 patients (non-IgA mesangial proliferative glomerulonephritis, 6; IgA nephropathy, 4; membranoproliferative glomerulonephritis, 2). Two patients (13.3 %) had MCD. Their maximum RBC range before a renal biopsy was 30–49/HPF in both of the patients. The ratio of other glomerular changes versus MCD was significantly higher in group 2 than that in group 1 ($P<0.01$). The maximum RBC range in urine appeared to be a useful criterion for separating other glomerular changes from MCD. There was no significant difference in the ratio of patients with disappearance of proteinuria at 4 weeks after the initiation of treatment between the two groups, both in patients with MCD and those with other glomerular diseases ($P=0.99$, Table 2).

Discussion

Persistent hematuria even microscopic, is an indication for a renal biopsy in children with idiopathic NS. Microscopic hematuria is observed in as many as 20 % of cases in MCD [1]. However, currently, there is no clear criterion for RBC count in urine sediment for a renal biopsy in children with idiopathic NS. An unnecessary renal biopsy in MCD should be avoided. Therefore, our study results could be important for clinical practice.

In the present study, to avoid patient selection bias and to obtain accurate information, we first reviewed all initial renal biopsy cases in the study period. We found that 29 out of 754 (3.8 %) patients had initial renal biopsies only because of hematuria with NS at onset. Although the final number of patients available in the present study is not large (29), data from a 10-year study period should provide useful information.

Our study suggests that the presence of more than 30–49 RBCs/HPF in urine sediment is optimal as a renal biopsy criterion for NS showing microscopic hematuria by ROC analysis with the Youden index. The detection ratio of glomerular diseases of 13 out of 15 (86.7 %), including focal segmental glomerulosclerosis and nephritis, found in group 2 is considered to be appropriate in clinical practice. There were only 3 patients with glomerular diseases in group 1. Some of the patients with glomerular diseases may not undergo renal biopsies according to the criterion suggested in this study. Therefore, even after steroid therapy for NS has been initiated, when an abnormal RBC count range continues, performing a renal biopsy should be considered.

We did not observe a significant difference in the ratio of patients with disappearance of proteinuria at 4 weeks after the initiation of treatment between the two groups in patients with MCD and other glomerular diseases (Table 2). Therefore, the maximum RBC count range in urine sediment was not related to the response to treatments.

Some physicians may think that renal biopsies are unnecessary in patients with NS even showing hematuria if they are steroid-sensitive, because treatments for MCD and non-MCD are not different. However, if we can predict children with other diseases besides MCD by using the optimal cutoff point efficiently, it seems to be reasonable to diagnose by a biopsy and then consider a specific treatment for each disease.

Table 1 Baseline characteristics in the two groups

	Group 1 Maximum RBC range ≤20–29/HPF (n=14)	Group 2 Maximum RBC range ≥30–49/HPF (n=15)	P
Females/males	5/9	11/4	0.04
Age at initial biopsy (years)	7.3±3.9	8.7±4.9	0.38
Duration from first symptoms to renal biopsies (days)	27.2±23.3	24.7±16.6	0.97
Total protein (g/dl)	4.1±0.6	4.4±0.4	0.27
Albumin (g/dl)	1.7±0.6	1.9±0.5	0.17
Blood urea nitrogen (mg/dl)	13.6±6.5	18.2±11.7	0.32
Creatinine (mg/dl)	0.4±0.1	0.5±0.2	0.08
Body weight (kg)	27.8±14.7	26.5±12.2	0.95
Albumin intravenous infusion	7	6	0.72

Data are mean±SD where appropriate

RBC red blood cells, *HPF* high power field

Although microscopic sediment analysis is still used worldwide to examine cells in urine, and the method of urine sediment analysis for the detection of hematuria in this study is the Japanese standard, assessment of microscopic hematuria may vary around the globe depending on the method of collection, analysis or time until analysis [7]. Because of the nature of urinalysis used, we were afraid that we may estimate the optimal maximum RBC count range to be higher than the true value and miss non-MCD with a high frequency. Therefore, it may be reasonable for us to consider adjusting the criterion by one level for the maximum RBC count range so as not to miss glomerular diseases other than MCD. If this is the case, the maximum RBC count range of 20–29/HPF is the optimal cutoff point for a renal biopsy. This option may be reasonable for clinical practice and it appears to be widely accepted by physicians.

This study has several limitations. First, despite our effort to reduce patient selection bias, the retrospective nature of this study may result in such a bias. Second, the low number of patients analyzed is also a limitation. This means that the study may not have been powered, as such, to identify a correlation between number of RBCs and steroid responsiveness. Third, our data have not yet been validated in other cohorts. In order to make specific recommendations for an RBC cutoff for renal biopsy, a multinational, multicenter approach that prospectively examines the outcome of patients who present with nephrotic syndrome with a uniform method of evaluating microscopic hematuria would be ideal. Further evaluation of this criterion in a large-scale cohort is desirable to confirm its validity. The optimal maximum RBC range may need to be adjusted after further evaluation.

Table 2 Pathological diagnosis

Diseases	Group 1 Maximum RBC range ≤20–29/HPF (n=14)	Group 2 Maximum RBC range ≥30–49/HPF (n=15)
MCD	11	2
	8 (72.7 %)	2 (100 %)
Others	3	13
	1 (33.3 %)	5 (38.5 %)
Focal segmental glomerulosclerosis		1
		0 (0.0 %)
Mesangial proliferative glomerulonephritis (non-IgA)	2	6
	1 (50.0 %)	5 (83.3 %)
IgA nephropathy	1	4
	0 (0.0 %)	0 (0.0 %)
Membranoproliferative glomerulonephritis		2
		0 (0.0 %)

Number (ratio [%]) of patients with disappearance of proteinuria at 4 weeks after the initiation of treatment is shown beneath the total number of patients

In conclusion, the current study suggests that use of the maximum RBC range (30–49/HPF) as a criterion for performing a renal biopsy in patients with NS showing hematuria may be reasonable for clinical practice.

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