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# Relationship between Glomerular Number in Fresh Kidney Biopsy Samples and Light Microscopy Samples

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| **Abstract:** | Background: On-site evaluation of fresh kidney biopsy (FKB) samples at the time of biopsy is useful to verify that adequate specimens are acquired. However, some cases present poor correlation between glomerular number in FKB samples and light microscopy (LM) samples. We examined the usefulness of such on-site evaluation.<br>Methods: We conducted a retrospective cross-sectional observational study (n = 129) to assess the correlation between glomerular number in FKB samples and LM samples and the associated factors hindering the evaluation.<br>Results: There was a significant positive correlation between glomerular number in FKB samples and LM samples. The median ratio of glomerular number (LM samples/FKB samples) was 0.74. According to this ratio, cases were divided into three groups: reasonable estimation (65 cases), underestimation (32 cases), and overestimation (32 cases). Comparing the reasonable and underestimation groups, significant differences were detected in the extent of interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation. Logistic regression analysis demonstrated that IFTA and interstitial inflammation were significantly associated with the underestimation. Moreover, the cortex length of FKB samples correlated with glomerular number in LM samples regardless of tubulointerstitial lesions.<br>Conclusions: Glomerular number determined during on-site evaluation can be a reference for the actual number of glomeruli in LM samples. Since tubulointerstitial |

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| | lesions make it difficult to recognize glomeruli in FKB samples, the possibility of underestimation for cases with possibly severe tubulointerstitial lesions should be considered. In such cases, evaluation of cortex length of FKB samples may substitute for evaluating glomeruli on-site. |
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| **Author Comments:** | December 24, 2021<br><br>Shinya Kaname, M.D., Ph.D.<br>Editor-in-Chief<br>Clinical and Experimental Nephrology<br><br>Dear Dr. Kaname:<br><br>I wish to re-submit the manuscript titled “Relationship between Glomerular Number in Fresh Kidney Biopsy Samples and Light Microscopy Samples.” The manuscript ID is CENE-D-21-00430R1. |

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| | We thank you and the reviewers for your thoughtful suggestions and insights. The manuscript has benefited from these insightful suggestions. I look forward to working with you and the reviewers to move this manuscript closer to publication in Clinical and Experimental Nephrology.<br><br>The manuscript has been rechecked and the necessary changes have been made in accordance with the reviewers' suggestions. The responses to all comments have been prepared and attached herewith. The revisions are marked by single underlining with red font in the revised manuscript.<br><br>Thank you for your consideration. I look forward to hearing from you.<br><br>Sincerely,<br>Yuji Kamijo, M.D., Ph.D.<br>Department of Nephrology<br>Shinshu University Hospital<br>3-1-1, Asahi, Matsumoto, 390-8621, Japan<br>Tel.: +81-263-37-2634<br>Fax: +81-263-32-9412<br>yujibeat@shinshu-u.ac.jp |

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Responses to the reviewers' comments

We thank the reviewers for their valuable comments. We have revised the manuscript in accordance with the comments. The changes in the revised manuscript are marked by single underlining with red font.
Our responses to the reviewers' comments as given below.

Reviewer #1: Comment to the authors:
The manuscript is very nicely revised.
I would like to ask authors one more thing. The reviewer recommends to present Supplemental Figure 2 in the main text (not as supplemental material). I am sure that including the pictures of on-site evaluation greatly increase the impact to the readers. Additionally, please indicate glomeruli in the picture by arrows and add the explanations in the Figure legend.

Thank you for your valuable comment. We have moved Supplemental Figure 2 to Figure 2 shown in the main text. In addition, we have added arrows to the representative glomeruli among structures evaluated as glomeruli.

Reviewer #2: The manuscript has been modified to make it easier for the reader to understand.
It might be helpful to clarify that the authors evaluated ti (total inflammation) scores, not i (inflammation) scores for the Banff classification.
In supplemental figure 2, it is better to add arrows to the area evaluated as glomerulus.

Thank you for your valuable comment. We have added the explanation for the evaluation of the extent of interstitial inflammation, as described below.
"The extent of interstitial inflammation was evaluated as ti (total inflammation) scores rather than i (inflammation) scores, which was indicated by the Banff classification [15]."
We have added arrows to the representative glomeruli among structures evaluated as glomeruli in Figure 2 (modified Supplemental Figure 2).

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# Relationship between Glomerular Number in Fresh Kidney Biopsy Samples and Light Microscopy Samples

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## Abstract

**Background:** On-site evaluation of fresh kidney biopsy (FKB) samples at the time of biopsy is useful to verify that adequate specimens are acquired. However, some cases present poor correlation between glomerular number in FKB samples and light microscopy (LM) samples. We examined the usefulness of such on-site evaluation.

**Methods:** We conducted a retrospective cross-sectional observational study (n = 129) to assess the correlation between glomerular number in FKB samples and LM samples and the associated factors hindering the evaluation.

**Results:** There was a significant positive correlation between glomerular number in FKB samples and LM samples. The median ratio of glomerular number (LM samples/FKB samples) was 0.74. According to this ratio, cases were divided into three groups: reasonable estimation (65 cases), underestimation (32 cases), and overestimation (32 cases). Comparing the reasonable and underestimation groups, significant differences were detected in the extent of interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation. Logistic regression analysis demonstrated that IFTA and interstitial inflammation were significantly associated with the underestimation. Moreover, the cortex length of FKB samples correlated with glomerular number in LM samples regardless of tubulointerstitial lesions.

**Conclusions:** Glomerular number determined during on-site evaluation can be a reference for the actual number of glomeruli in LM samples. Since tubulointerstitial lesions make it difficult to recognize glomeruli in FKB samples, the possibility of underestimation for cases with possibly severe tubulointerstitial lesions should be considered. In such cases, evaluation of cortex length of FKB samples may substitute for evaluating glomeruli on-site.

**Keywords:** kidney biopsy, procedure, on-site evaluation, glomerular number, microscopy, tissue analysis

## Introduction

Evaluation of the pathological findings of kidney biopsy specimens is important for the diagnosis of and decisions on the management of kidney diseases [1]. Biopsied kidney tissue should contain the kidney cortex and an appropriate number of glomeruli. Kidney biopsy tissue is divided into three portions, for light microscopy (LM), immunofluorescence (IF), and electron microscopy (EM) analyses, and each portion should contain glomeruli [2]. Therefore, on-site evaluation at the time of kidney biopsy is useful for acquiring adequate specimens [1, 3–7]. However, renal pathological specimens may contain glomeruli even when glomeruli are not detected by on-site evaluation [8], and there have been cases with poor correlation between glomerular number in fresh kidney biopsy (FKB) samples and LM samples. In such cases, there is concern that the kidney needle pass number will unnecessarily increase, or inadequate number of glomeruli will be obtained because of underestimation or overestimation of results. Consequently, the following issues should be resolved: 1) the adequacy of the assessment of glomerular number in FKB samples and 2) the possible associated factors that affect the evaluation of glomerular number in FKB samples. Herein, we addressed these points to facilitate obtaining an adequate glomerular number from kidney biopsy.

## Materials and Methods

### Study Design

This was a single-center cross-sectional observational study. All cases (n = 151) who underwent ultrasonography-guided kidney biopsy at the Department of Nephrology, Shinshu University Hospital (Matsumoto, Japan) between November 2018 and May 2020 were enrolled. Patients who were younger than 20 years (6 cases) were excluded. Patients who received a 1-h biopsy after kidney transplantation (9 cases) were also excluded since this kidney biopsy procedure was different from that performed for other cases. Similarly, cases with insufficient data (7 cases) were excluded. Finally, 129 kidney samples from 122 patients were analyzed (Figure 1). In cases of multiple kidney biopsies of the same patient, each biopsy was treated as an independent kidney biopsy case. Five patients underwent kidney biopsy twice, and one patient underwent kidney biopsy three times. All procedures involving human participants were performed in accordance with the ethical standards of the Institutional Review Board of the Ethical Committee at Shinshu University School of Medicine (approval number: 4431) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The requirement of written informed consent was waived because of the retrospective nature of the study.

Patient background data [age, sex, height, body weight, body mass index (BMI), and medical history of diseases, such as hypertension, diabetes mellitus, and dyslipidemia] and laboratory data [serum

albumin, blood urea nitrogen, and creatinine levels; estimated glomerular filtration rate (eGFR) by creatinine; [9] eGFR by cystatin C; [10] β2-microglobulin, hemoglobin, and platelet counts; hemoglobin A1c (HbA1c) levels; and urinary findings, such as protein, β2-microglobulin, and *N*-acetyl-β-D-glucosaminidase levels] recorded at the time of kidney biopsy were obtained from medical records. History of smoking was also obtained. Hypertension was defined as the use of blood-pressure-lowering drugs and/or blood pressure ≥140/90 mmHg. Diabetes mellitus was defined as the use of insulin or anti-diabetic drugs or HbA1c level ≥6.5%. Dyslipidemia was defined as the use of statins and/or low-density lipoprotein cholesterol levels ≥140 mg/dl. Kidney biopsy information included needle pass number, core number, total core length, glomerular number, cortex percentage, cortex length, total division length for IF and EM, percentage of total division length to total core length, experience of the clinical physician who performed the on-site evaluation, and serious complications. The glomerular number and cortex percentage in FKB samples were evaluated prior to the division of sample for IF and EM. The cortex length of FKB samples was calculated by multiplying the cortex percentage by the total core length. Serious complications were defined as conditions that required unplanned treatment.

**Kidney Biopsy Procedure**

Kidney biopsy was performed in accordance with the methods described in previous reports [1, 3, 4]

and in a Japanese guidebook [11]. One nephrologist performed a percutaneous kidney biopsy with an automatic biopsy needle with ultrasound guidance. HI VISION Preirus (Hitachi Medical Corporation, Tokyo, Japan) was used as the ultrasonic device, and a BARD MONOPTY (C.R. Bard, Inc., New Jersey, USA) instrument with a gauge size of 16 and a needle length of 160 mm was used as the automatic biopsy needle. The collected FKB samples were placed on a glass slide and prevented from drying out by briefly immersing in a small amount of normal saline. Another nephrologist performed an on-site evaluation of the FKB samples as quickly as possible. The evaluation was performed with a standard light microscope adjusted to appropriate magnification and light source. The core length was measured by placing the glass slide with the core on a graph paper (Supplemental Figure 1). A representative picture of FKB samples visualized with a standard light microscope is presented in Figure 2. Glomeruli were recognized as circular structures with a color tone different from that of the surroundings (Figure 2a). The nephrologist performing the on-site evaluation apportioned the FKB samples for IF and EM according to the glomerular and cortex localization. Each length for the IF or EM portion was 1–2 mm. Both nephrologists came to a comprehensive decision on when to discontinue the needle passes based on the evaluation of FKB samples and the clinical situation.

**Pathological Analysis of Kidney Biopsy Specimens**

Total core length, glomerular number, cortex percentage, cortex length, glomerular sclerosis percentage, crescent formation percentage, presence of glomerular hypertrophy, extent of interstitial fibrosis and tubular atrophy (IFTA), extent of interstitial inflammation, presence of tubulitis, presence of arteriolar hyalinosis, and presence of arterial intimal fibrosis were evaluated by LM, with reference to previous reports [12, 13]. In addition, the presence of glomeruli in the IF and EM specimens was evaluated. The cortex length of LM samples was calculated by multiplying the cortex percentage by the total core length. Glomerular sclerosis percentage was defined as the total percentage of global sclerosis and segmental sclerosis of the glomeruli. Glomerular hypertrophy was defined as a glomerular diameter ≥250 μm. The extent of IFTA and interstitial inflammation was evaluated separately. These lesions were represented by the percentage of the lesion area in relation to all the cortical area, and these areas were measured using ImageJ [14]. The extent of interstitial inflammation was evaluated as ti (total inflammation) scores rather than i (inflammation) scores, which was indicated by the Banff classification [15]. Each extent was also classified into four categories as follows: none < 5%, 5% ≤ mild < 25%, 25% ≤ moderate < 50%, and 50% ≤ severe. Glomerular and vascular lesions were mainly evaluated by periodic acid–Schiff staining and periodic acid–methenamine silver staining; tubulointerstitial lesions were evaluated by hematoxylin and eosin staining and Masson trichrome staining. Besides the main renal pathologist, another renal pathologist evaluated the renal pathological findings.

**Statistical Analysis**

Continuous variables are presented as the median and interquartile range, and categorical variables are presented as numbers (n) and percentages (%). Continuous variables were compared using Mann–Whitney *U*-test, and categorical variables were compared using Fisher's exact test. Correlations were evaluated using Spearman's rank correlation coefficient.

Based on the interquartile range of the glomerular number ratio (LM samples/FKB samples), all cases were divided into three groups: reasonable estimation group, i.e., cases within the interquartile range; underestimation group, i.e., cases within the third quartile or higher; and overestimation group, i.e., cases within the first quartile or lower. Although $P < 0.05$ was generally considered to indicate statistical significance, in situations involving multiple comparisons (comparison of the underestimation and reasonable estimation groups and of the overestimation and reasonable estimation groups), $P < 0.025$ was the significance threshold. Associated factors hindering the evaluation of glomerular number were examined by univariate and multivariate logistic regression analyses adjusted for age and sex. All analyses were performed using IBM SPSS Statistics software package version 26 for Windows (IBM Co., Ltd., New York, NY, USA).

## Results

Patient's background, laboratory data and a part of renal pathological findings are presented in Supplemental Table 1. Of the 129 kidney biopsy cases, 29 (22.5%) underwent kidney allograft biopsies. Sixty-seven (51.9%) patients were male, and the median age of the patients was 49 years. The most common pathological diagnosis was IgA nephropathy (22.5%). Information of FKB samples and LM samples is shown in Table 1. The median needle pass number, core number, total core length, glomerular number, cortex percentage and cortex length in FKB samples were 2, 2, 25 mm, 29, 90%, and 21 mm, respectively. The median total division length for IF and EM was 2.0 mm, and the median percentage of total division length to total core length was 8.7%. Serious complications occurred in two patients (1.6%), with both requiring blood transfusion. Pathological findings evaluated by LM showed that the median glomerular number was 22. Glomeruli were observed in the IF and EM specimens in almost all cases (98.4%). The median ratio of the glomerular number in LM samples/FKB samples was 0.74 (0.48–0.97). The glomerular number in FKB and in LM samples showed a significant positive correlation ($r = 0.398$, $P < 0.001$) (Figure 3). Based on the interquartile range of the glomerular number ratio, 65 cases were assigned to the reasonable estimation group, 32 cases to the underestimation group, and 32 cases to the overestimation group (Table 1). Comparison of the reasonable estimation and underestimation groups revealed significant differences in the extent of IFTA (15% vs. 30%, respectively, $P = 0.003$)

and interstitial inflammation (10% vs. 20%, respectively, $P = 0.001$). This suggested that the IFTA and/or interstitial inflammation area were more pronounced in the underestimation group than those in the reasonable estimation group. The number of needle passes in the underestimation group tended to be higher than that in the reasonable estimation group ($P = 0.03$). Comparison of the reasonable estimation group with the overestimation group revealed significant differences in the cortex length of LM samples (16 mm vs. 12 mm, respectively, $P = 0.015$) (Table 1). Logistic regression analysis demonstrated that the extent of IFTA and interstitial inflammation was significantly associated with the underestimation of glomerular number (odds ratio, 1.031, $P = 0.002$, and odds ratio, 1.043, $P = 0.002$, respectively), while no factors were significantly associated with the overestimation of glomerular number (Table 2 and Supplemental Table 2). Multivariate logistic regression analysis adjusted for age and sex demonstrated that the extent of IFTA and interstitial inflammation was significantly associated with the underestimation of glomerular number (odds ratio, 1.035, $P = 0.001$, and odds ratio, 1.044, $P = 0.001$, respectively) (Table 3).

The cases were divided into two groups based on the extent of IFTA or interstitial inflammation: the none and mild group (n = 75 and n = 96, respectively) and the moderate and severe group (n = 54 and n = 33, respectively). As shown in Figure 4a, there was a significant positive correlation between the glomerular number in FKB and that in LM samples (r = 0.608, $P < 0.001$) when the extent of IFTA was <25% (none and mild group). No correlation was observed when the extent of

IFTA was ≥25% (moderate and severe group) (Figure 4b). Similarly, a significant positive correlation, with a correlation coefficient of 0.556 ($P < 0.001$), was observed when the extent of interstitial inflammation was <25%, but no correlation was observed when the extent of interstitial inflammation was ≥25% (Figure 4c, 4d).

In a previous study [5], the cortex length in LM samples was positively correlated with glomerular number in LM samples. We reconfirmed this relationship (r = 0.576, $P < 0.001$) and found a high correlation between cortex length of LM and FKB samples (r = 0.865, $P < 0.001$) (Supplemental Figure 2); therefore, we examined the possibility of cortex length of FKB samples as an alternative method for estimating the glomerular number in LM samples. Cortex length in FKB samples was positively correlated to glomerular number in LM samples (r = 0.485, $P < 0.001$) (Figure 5a), and this correlation was not affected by the degree of tubulointerstitial lesions (Figure 5b, 5c, 5d, 5e).

## Discussion

We investigated the adequacy of the assessment of glomerular number in FKB samples for estimation of glomerular number of LM samples, as well as the factors hindering this relationship. The findings clarify the usefulness and provide important points to note when on-site evaluation is performed.

We detected a significant positive correlation between glomerular number in FKB samples and LM samples. Ferrer et al. [5] and Sekulic and Crary [7] reported that more adequate kidney tissue samples could be obtained with on-site microscopic evaluation at the time of kidney biopsy than without it. However, the authors did not assess this finding or the specifics of the evaluation in FKB samples. Here, based on the comparison of glomerular number in FKB samples and LM samples, the rationale for the efficacy of on-site microscopic evaluation at the time of kidney biopsy was demonstrated. We report, for the first time, that the glomerular number determined in FKB samples is greater than that determined in LM samples. This finding is reasonable because the FKB sample is cut for IF and EM analyses and sliced during preparation of LM samples. Furthermore, we are the first to show that the median ratio of the glomerular number in LM samples/FKB samples was 0.74. With the multiplication of the glomerular number in FKB samples and this ratio, it is possible to estimate the glomerular number in LM samples, when kidney biopsy is performed with the procedure similar to that in this study. This information can be useful for obtaining the targeted

number of glomeruli in LM samples. Conversely, this ratio can be used to estimate the target number of glomeruli in FKB samples for obtaining desired number of glomeruli in LM samples. The target number of glomeruli in FKB samples is estimated to be 1.35 (1/0.74)-times the target number of glomeruli in LM samples. For observing 20 glomeruli under LM, observation of over 27 glomeruli in FKB samples would be recommended. However, we should note that this conversion ratio would be strongly affected by the tubulointerstitial situation.

Although the relationship between glomerular number in the FKB samples and LM samples was significant, the correlation coefficient was not very high. Some associated factors resulting in the underestimation or overestimation of the glomerular number impacted the value of the correlation coefficient. Factors associated with the underestimated value, namely, the extent of IFTA and interstitial inflammation, were statistically detected here for the first time. The light source of the light microscope makes FKB samples translucent and facilitates visual recognition of glomeruli [8]. We speculate that tubulointerstitial lesions may impair the translucency of FKB samples. According to a previous study, decreased glomerular blood flow makes it difficult to visually recognize the glomeruli in FKB samples [8]. However, a significant difference in glomerular blood flow-related factors, including glomerular disease type, glomerular sclerosis percentage, glomerular hypertrophy, arteriolar hyalinosis, and arterial intimal fibrosis, was not apparent. The current findings suggest that tubulointerstitial lesions, such as IFTA and/or interstitial inflammation, are the major factors

hindering the recognition of glomeruli in FKB samples. Since IFTA and interstitial inflammation lesions overlapped in most of the cases, we could hardly determine the major factor causing underestimation. There was only one case of tubulointerstitial nephritis in which interstitial inflammation was clearly predominant (the extent of interstitial inflammation being 80% and that of IFTA being 40%). This case had a low glomerular number ratio (LM samples/FKB samples) of 0.31 and belonged to the overestimation group, suggesting that a significant underestimation factor might be IFTA rather than interstitial inflammation.

A major clinical problem resulting from the underestimation results is an increase in unnecessary kidney biopsy needle passes. In fact, the number of needle passes in the underestimation group tended to be higher than that in the reasonable estimation group. When kidney biopsies are conducted in cases where tubulointerstitial lesions are strongly expected, we should consider the possibility that glomeruli may be present even if they are not confirmed in FKB samples, and avoid excessive unnecessary kidney biopsy needle passes.

Assessment prior to kidney biopsy for possibility of the underestimation would be useful for clinical physician. We demonstrate that tubulointerstitial lesions strongly influence the glomerular number estimate. Previous studies have indicated that tubulointerstitial lesions deteriorate upon eGFR decline and proteinuria [16, 17]. Therefore, serum and/or urinary markers reflecting kidney dysfunction and/or tubulointerstitial injuries may predict the underestimation. However, individual

serum or urinary markers, including serum creatinine levels, eGFR, proteinuria, urinary β2-microglobulin, or urinary *N*-acetyl-β-D-glucosaminidase, were not significantly associated with the underestimation. The possibility of severe tubulointerstitial lesions may need to be evaluated by total assessment, keeping in mind the clinical course and various laboratory data.

The current study also demonstrates a significant positive correlation between the cortex length in FKB samples and glomerular number in LM samples ($r = 0.485$, $P < 0.001$), which was not affected by the degree of tubulointerstitial lesions (Figure 5a). Ferrer et al. [5] reported that the cortex length in LM samples is positively correlated with glomerular number as shown in the current study ($r = 0.576$, $P < 0.001$) (Supplemental Figure 2a); however, they did not report the correlation between the cortex length of FKB samples and glomerular number in LM samples. We suggest that the cortex length of FKB samples could be used as an alternative method for estimating the glomerular number in LM samples, and this result appear to be useful when severe tubulointerstitial lesions are predicted before kidney biopsy, or when glomeruli are difficult to recognize in the cortical region of FKB samples. In the cases in which tubulointerstitial lesions were not severe, the cortex length of FKB samples weaker correlated to glomerular number in LM samples, compared to glomerular number in FKB samples, suggesting more clinical importance of on-site evaluation of glomerular number in FKB samples.

The logistic regression analyses detected no factors significantly associated with the overestimation

results; however, Table 1 indicates that the cortex length of LM samples in overestimation group was significantly shorter compared with that in reasonable estimation group. The cortex length of LM samples is thought to be affected by the cortex length of FKB samples, the total division length for IF and EM, and the percentage of total division length with respect to the total core length. These factors did not differ significantly among the estimation groups; however, the cortex length of FKB samples tended to be short and same length of division was performed in the overestimation group, which might result in the shorter cortex length of LM samples and lower glomerular number. These findings suggest that overestimation group consists of the cases with a shorter cortex in LM samples via the loss of large cortex area due to division for IF and EM.

We used a standard light microscope that is routinely used at the authors' institution. Currently, there is no consensus whether on-site evaluation microscope should be a standard light microscope or a dissecting microscope. Previous studies involved the use of a standard light microscope only [6], a dissecting microscope only [4, 7], or either [1, 3]. It is generally considered that these microscopes do not significantly affect the on-site evaluation because they are the same type of light microscope using visible light and lens system. Although it was reported that a standard light microscope can be used for a more facile and rapid visualization of glomeruli than a dissecting microscope [8], not enough evidence is available to support this notion. The efficacy of using a standard light microscope and that of a dissecting microscope should be compared to resolve this.

The current study has several limitations. First, the number of needle passes was determined not only by the need to obtain glomeruli, but also by the clinical situation, such as bleeding. It was, therefore, difficult to determine if the underestimation group tended to have more needle passes due to underestimation results. Second, this study was a single-center study; therefore, verification of the results, and their generalization, in other facilities is needed. Since the kidney biopsy procedure has been standardized according to previous reports and Japanese guidebooks, the results of the current study may be applicable to any facility performing kidney biopsy [1, 3, 4, 11]. Third, the sample size was small, and it is possible that the confounding factors were not fully adjusted for multivariate analysis. Fourth, the interobserver reproducibility of the on-site evaluation and the influence of the experience of the on-site evaluation physician were not evaluated in any of the cases in the current study. However, the data for eight cases in which two nephrologists performed on-site evaluation suggested that the results of on-site evaluation did not differ significantly between evaluation physicians and no particular tendency was observed depending on their experience (Supplementary Table 3). To reduce the possible effect of the evaluation physician's experience, the on-site evaluation in the current study was performed by different physicians with various years of experience. As a result, we did not detect a major influence of the evaluation physician's experience on underestimation or overestimation results.

In conclusion, a significant positive correlation between the glomerular number in FKB samples and

LM samples was detected. The glomerular number determined by on-site microscopic evaluation at the time of kidney biopsy can be used to estimate the actual glomerular number in LM samples, suggesting the clinical benefit of on-site microscopic evaluation. However, tubulointerstitial lesions, such as IFTA and/or interstitial inflammation, may make it difficult to recognize glomeruli in FKB samples. In cases with severe potential tubulointerstitial lesions, the possibility of glomerular number underestimation should be considered, and the appropriate timing of the discontinuation of kidney biopsy should be decided by other information. In such cases, evaluation of cortex length of FKB samples may substitute for evaluating glomeruli on-site.

## Acknowledgements

We would like to thank everyone who was involved in this study.

## Disclosure

All the authors have declared no competing interest.

## Author contribution

KS and MH designed the study. KS, DA, AY, YH, and YY collected the data. KS and YY performed statistical analyses. KS and MH drafted the manuscript. KH and YK revised the manuscript. All authors have approved the final version of the manuscript.

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## Figure Legends

**Fig. 1** Flow diagram of the inclusion and exclusion criteria in the current study

**Fig. 2** Fresh kidney biopsy (FKB) samples as seen with a standard light microscope. (a) Kidney cortex containing the glomeruli which are circular structures with a different color tone from the surroundings. The arrows indicate representative glomeruli among structures evaluated as glomeruli. (b) Kidney medulla showing reddish vasculature but no glomeruli.

**Fig. 3** Relationship between glomerular number in fresh kidney biopsy (FKB) samples and light microscopy (LM) samples. The Spearman's rank correlation coefficient is 0.398 ($P < 0.001$). The gray circles indicate cases in the reasonable estimation group, the blue inverted triangles represent the underestimation group, and the red triangles represent the overestimation group. The solid lines represent the regression line. The dashed line indicates the same values on the X- and Y-axes

**Fig. 4** Relationship between glomerular number in FKB samples and LM samples stratified by tubulointerstitial damage lesions. (a) Cases with the extent of interstitial fibrosis and tubular atrophy (IFTA) of less than 25%. The Spearman's rank correlation coefficient is 0.608 ($P < 0.001$). (b) Cases with the extent of IFTA of 25% or more. The Spearman's rank correlation coefficient is 0.159 ($P =$

0.25). (c) Cases with the extent of interstitial inflammation of less than 25%. The Spearman's rank correlation coefficient is 0.556 ($P < 0.001$). (d) Cases with the extent of interstitial inflammation of 25% or more. The Spearman's rank correlation coefficient is 0.008 ($P = 0.97$). The gray circles indicate cases in the reasonable estimation group; the blue inverted triangles represent the underestimation group; and the red triangles represent the overestimation group. The solid lines represent the regression line

**Fig. 5** Relationship between cortex length of FKB samples and glomerular number in LM samples. (a) All cases. The Spearman's rank correlation coefficient is 0.485 ($P < 0.001$). (b) Cases with the extent of interstitial fibrosis and tubular atrophy (IFTA) of less than 25%. The Spearman's rank correlation coefficient is 0.474 ($P < 0.001$). (c) Cases with the extent of IFTA of 25% or more. The Spearman's rank correlation coefficient is 0.462 ($P < 0.001$). (d) Cases with the extent of interstitial inflammation of less than 25%. The Spearman's rank correlation coefficient is 0.486 ($P < 0.001$). (e) Cases with the extent of interstitial inflammation of 25% or more. The Spearman's rank correlation coefficient is 0.435 ($P = 0.011$). The gray circles indicate cases in the reasonable estimation group, the blue inverted triangles represent the underestimation group, and the red triangles represent the overestimation group. The solid line represents the regression line

**Supplemental Figure Legends**

**Supplemental Fig. 1** Fresh kidney biopsy (FKB) samples on a glass slide placed on a graph paper.

**Supplemental Fig. 2** Analyses concerning cortex length of light microscopy (LM) samples. (a) Relationship between cortex length of LM samples and glomerular number in LM samples. The Spearman's rank correlation coefficient is 0.576 ($P < 0.001$). (b) Relationship between cortex length in fresh kidney biopsy (FKB) samples and that in LM samples. The Spearman's rank correlation coefficient is 0.865 ($P < 0.001$). The gray circles indicate cases in the reasonable estimation group, the blue inverted triangles represent the underestimation group, and the red triangles represent the overestimation group. The solid line represents the regression line.

**Table 1.** Information of FKB samples and LM samples classified by the ratio of the glomerular number in LM samples to that in FKB samples, and comparison between the reasonable estimation group and other groups

| | All (n = 129) | | Reasonable estimation group (n = 65) | | Underestimation group (n = 32) | | $P^1$ | Overestimation group (n = 32) | | $P^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Ratio of the glomerular number in LM samples to that in FKB samples | 0.74 | (0.48–0.97) | 0.74 | (0.59–0.83) | 1.28 | (1.05–1.70) | | 0.37 | (0.31–0.42) | |
| Information of FKB samples | | | | | | | | | | |
| Needle pass number | 2 | (2–2) | 2 | (2–2) | 2 | (2–3) | 0.03 | 2 | (2–2) | 0.08 |
| Core number | 2 | (2–2) | 2 | (2–2) | 2 | (2–2) | 0.67 | 2 | (2–2) | 0.09 |
| Total core length (mm) | 25 | (20–30) | 25 | (20–30) | 25 | (21–30) | 1.00 | 24 | (21–28) | 0.45 |
| Glomerular number | 29 | (22–38) | 31 | (23–41) | 23 | (17–30) | 0.003* | 31 | (25–41) | 0.72 |
| Cortex percentage (%) | 90 | (70–100) | 90 | (70–100) | 90 | (80–100) | 0.34 | 85 | (70–100) | 0.65 |
| Cortex length (mm) | 21 | (17–25) | 21 | (17–26) | 22 | (18–29) | 0.21 | 19 | (16–24) | 0.16 |
| Total division length for IF and EM (mm) | 2.0 | (2.0–2.0) | 2.0 | (2.0–2.0) | 2.0 | (2.0–2.0) | 0.93 | 2.0 | (2.0–3.0) | 0.12 |
| Percentage of total division length to total core length (%) | 8.7 | (6.9–11.1) | 8.3 | (6.9–10.7) | 9.1 | (7.3–10.5) | 0.74 | 8.7 | (7.3–13.4) | 0.22 |
| Experience of on-site evaluation physician (years) | 7 | (3–10) | 9 | (3–10) | 7 | (4–12) | 0.74 | 5 | (3–10) | 0.13 |
| Serious complication | 2 | (1.6) | 1 | (1.5) | 1 | (3.1) | 1.00 | 0 | (0) | 1.00 |
| Information of LM samples | | | | | | | | | | |
| Total core length (mm) | 22 | (13–31) | 23 | (12–34) | 23 | (13–33) | 0.88 | 22 | (13–31) | 0.25 |
| Glomerular number | 22 | (14–31) | 23 | (16–30) | 34 | (24–41) | < 0.001* | 11 | (8–16) | < 0.001* |
| Cortex percentage (%) | 80 | (60–100) | 80 | (65–100) | 90 | (75–100) | 0.08 | 70 | (50–100) | 0.10 |
| Cortex length (mm) | 17 | (13–22) | 16 | (14–22) | 19 | (16–25) | 0.03 | 12 | (9–21) | 0.015* |
| Glomerular sclerosis percentage (%) | 16.0 | (4.6–35.0) | 13.3 | (6.3–33.3) | 25.3 | (5.6–38.1) | 0.13 | 14.7 | (0–33.3) | 0.82 |
| Crescent formation percentage (%) | 0 | (0–0) | 0 | (0–0) | 0 | (0–0) | 0.86 | 0 | (0–0) | 0.11 |
| Presence of glomerular hypertrophy | 32 | (24.8) | 17 | (26.2) | 7 | (21.9) | 0.80 | 8 | (22.9) | 1.00 |
| Extent of IFTA (%) | 15 | (5–35) | 15 | (5–30) | 30 | (10–60) | 0.003* | 10 | (5–30) | 0.45 |
| Extent of interstitial inflammation (%) | 10 | (5–25) | 10 | (5–20) | 20 | (10–40) | 0.001* | 10 | (5–20) | 0.58 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Presence of tubulitis | 18 | (14.0) | 6 | (9.2) | 4 | (12.5) | 0.73 | 8 | (22.9) | 0.06 |
| Presence of arteriolar hyalinosis | 44 | (34.1) | 19 | (29.2) | 14 | (43.8) | 0.18 | 11 | (34.3) | 0.65 |
| Presence of arterial intimal fibrosis | 96 | (74.4) | 47 | (72.3) | 25 | (78.1) | 0.63 | 24 | (75.0) | 1.00 |
| Experience of renal pathologist (years) | 9 | (7–10) | 9 | (7–10) | 9 | (7–10) | 0.87 | 9 | (7–10) | 1.00 |

Continuous variables are presented as medians (interquartile range), and categorical variables are presented as numbers (percentages).

FKB, fresh kidney biopsy; LM, light microscopy; IFTA, interstitial fibrosis and tubular atrophy.

* $P < 0.025$; [1], *P*-value for comparison between the reasonable estimation group and underestimation group; [2], *P*-value for comparison between the reasonable estimation group and overestimation group.

**Table 2.** Analysis of the association between wrong estimation of the glomerular number in LM samples and background clinical factors

| | Underestimation | | | Overestimation | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P* | Odds ratio | 95% CI | *P* |
| Background | | | | | | |
| Age (years) | 1.000 | 0.975–1.024 | 0.97 | 0.999 | 0.973–1.024 | 0.91 |
| Male | 0.856 | 0.367–1.997 | 0.72 | 1.417 | 0.602–3.339 | 0.43 |
| Information of FKB samples | | | | | | |
| Needle pass number | 1.939 | 0.957–3.929 | 0.07 | 0.346 | 0.098–1.229 | 0.10 |
| Core number | 1.290 | 0.488–3.410 | 0.61 | 0.315 | 0.080–1.249 | 0.10 |
| Total core length (mm) | 1.004 | 0.950–1.062 | 0.88 | 0.982 | 0.929–1.039 | 0.52 |
| Glomerular number | 0.936 | 0.895–0.979 | 0.004* | 1.001 | 0.969–1.035 | 0.94 |
| Cortex percentage (%) | 1.020 | 0.990–1.050 | 0.19 | 0.997 | 0.972–1.022 | 0.79 |
| Cortex length (mm) | 1.045 | 0.975–1.119 | 0.21 | 0.968 | 0.903–1.039 | 0.37 |
| Total division length for IF and EM (mm) | 1.059 | 0.393–2.850 | 0.91 | 1.908 | 0.829–4.390 | 0.13 |
| Percentage of total division length to total core length (%) | 0.983 | 0.874–1.105 | 0.77 | 1.077 | 0.981–1.183 | 0.12 |
| Experience of on-site evaluation physician (years) | 1.011 | 0.918–1.113 | 0.82 | 0.906 | 0.810–1.013 | 0.08 |
| Information of LM samples | | | | | | |
| Total core length (mm) | 1.004 | 0.950–1.062 | 0.88 | 0.980 | 0.927–1.036 | 0.48 |
| Glomerular number | 1.107 | 1.053–1.163 | < 0.001* | 0.789 | 0.711–0.874 | < 0.001* |
| Cortex percentage (%) | 1.025 | 1.000–1.051 | 0.05 | 0.982 | 0.963–1.001 | 0.07 |
| Cortex length (mm) | 1.070 | 0.993–1.153 | 0.08 | 0.926 | 0.860–0.997 | 0.04 |
| Glomerular sclerosis percentage (%) | 0.018 | 0.998–1.039 | 0.07 | 0.999 | 0.978–1.021 | 0.95 |
| Crescent formation percentage (%) | 0.996 | 0.948–1.046 | 0.87 | 1.002 | 0.966–1.039 | 0.93 |
| Presence of glomerular hypertrophy | 0.791 | 0.290–2.158 | 0.65 | 0.941 | 0.356–2.490 | 0.90 |
| Extent of IFTA (%) | 1.031 | 1.011–1.052 | 0.002* | 0.987 | 0.962–1.012 | 0.29 |
| Extent of interstitial inflammation (%) | 1.043 | 1.016–1.070 | 0.002* | 1.002 | 0.973–1.032 | 0.88 |
| Presence of tubulitis | 1.405 | 0.367–5.379 | 0.62 | 3.278 | 1.028–10.456 | 0.05 |
| Presence of arteriolar hyalinosis | 1.883 | 0.781–4.537 | 0.16 | 1.268 | 0.513–3.133 | 0.61 |
| Presence of arterial intimal fibrosis | 1.231 | 0.816–1.856 | 0.32 | 1.064 | 0.709–1.596 | 0.77 |
| Experience of renal pathologist (years) | 0.944 | 0.753–1.184 | 0.62 | 1.026 | 0.832–1.266 | 0.81 |

Univariate logistic regression analysis was performed.

LM, light microscopy; CI, confidence interval; FKB, fresh kidney biopsy; IFTA, interstitial fibrosis and tubular atrophy; IF, immunofluorescence; EM, electron microscopy. * $P < 0.025$.

**Table 3.** Multivariate analysis of the association of IFTA or interstitial inflammation with the underestimation or overestimation results

| | | Underestimation | | | Overestimation | | |
|---|---|---|---|---|---|---|---|
| Model | | Odds ratio | 95% CI | *P* | Odds ratio | 95% CI | *P* |
| 1 | Extent of IFTA (%) | 1.031 | 1.011–1.052 | 0.002* | 0.987 | 0.962–1.012 | 0.29 |
| 2 | Extent of IFTA (%) | 1.035 | 1.013–1.056 | 0.001* | 0.983 | 0.957–1.010 | 0.23 |
| 3 | Extent of interstitial inflammation (%) | 1.043 | 1.016–1.070 | 0.002* | 1.002 | 0.973–1.032 | 0.88 |
| 4 | Extent of interstitial inflammation (%) | 1.044 | 1.017–1.073 | 0.001* | 1.002 | 0.972–1.032 | 0.92 |

Multivariate logistic regression analysis was performed. Model 1,3 is an unadjusted model. Model 2,4 is adjusted for age and sex.

CI, confidence interval; IFTA, interstitial fibrosis and tubular atrophy. * $P < 0.025$.

Figure1

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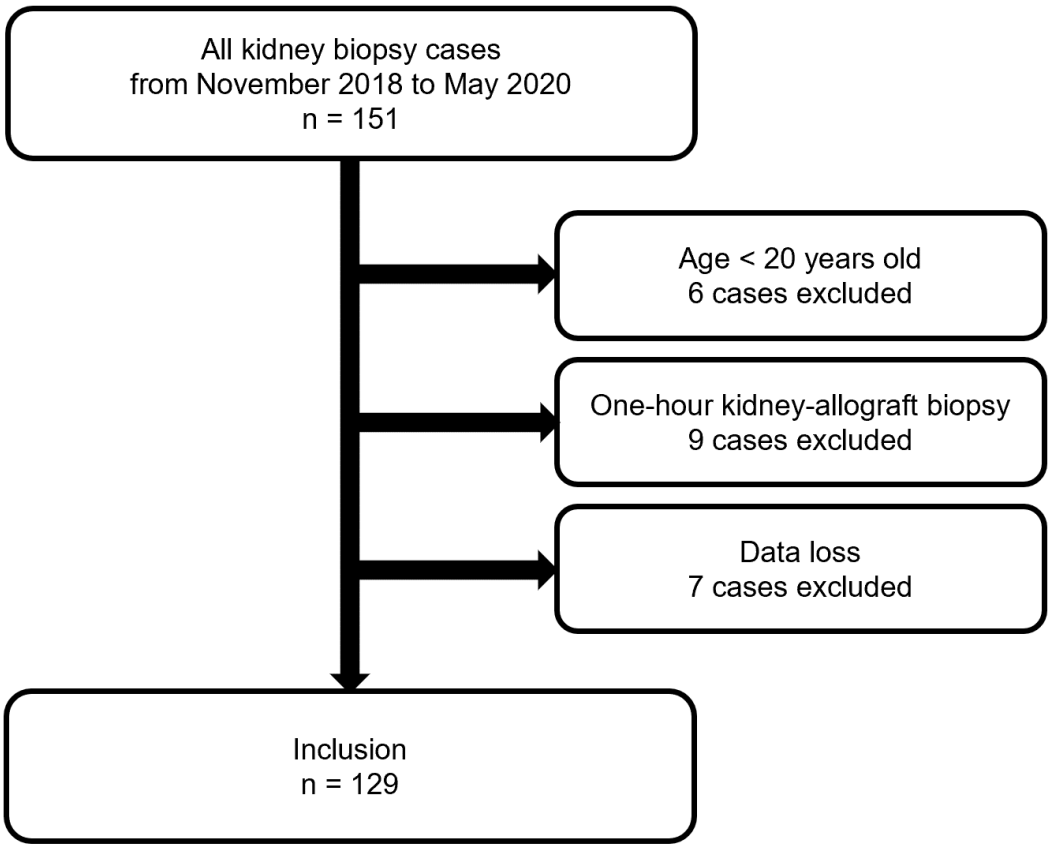

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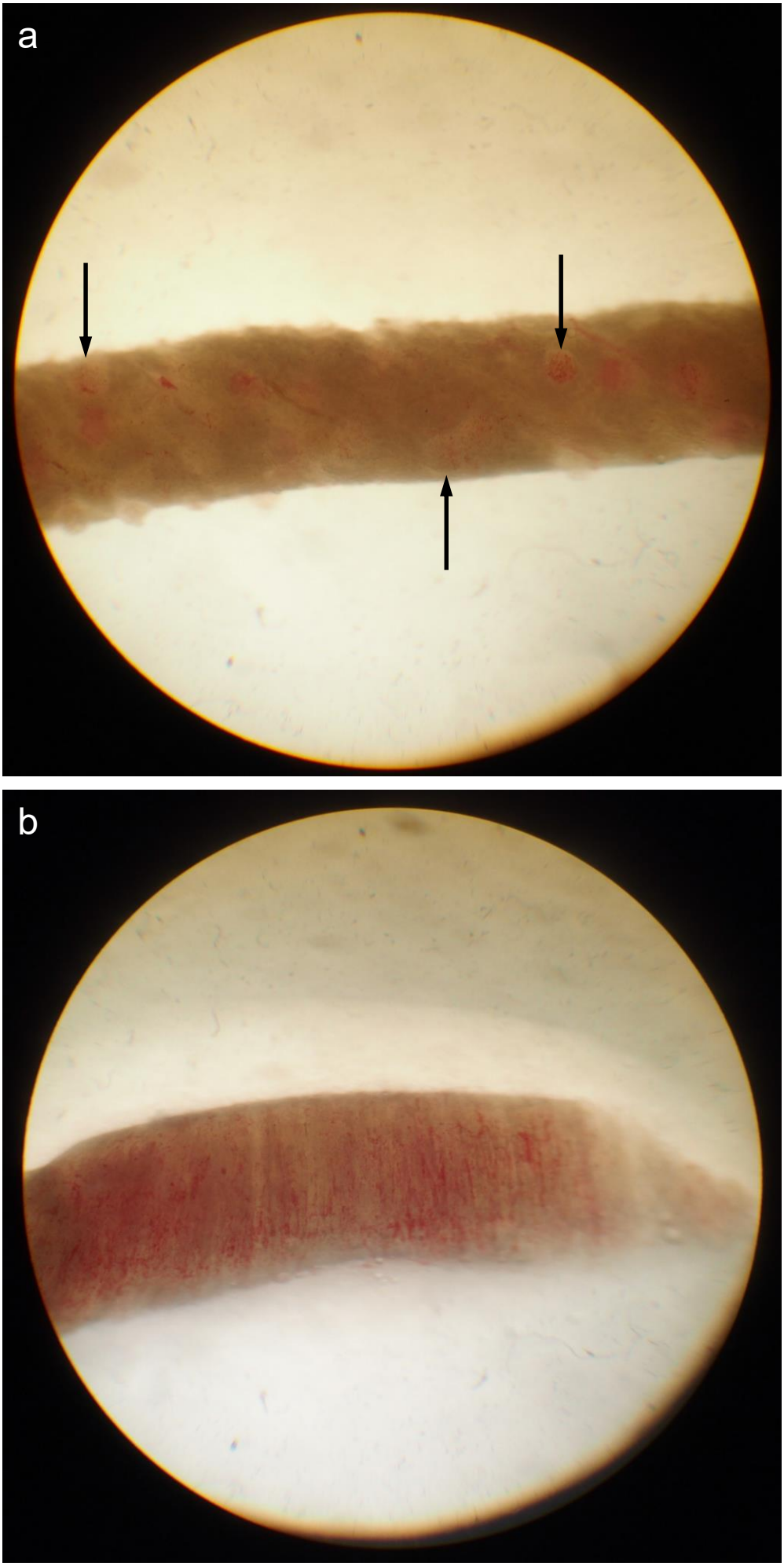

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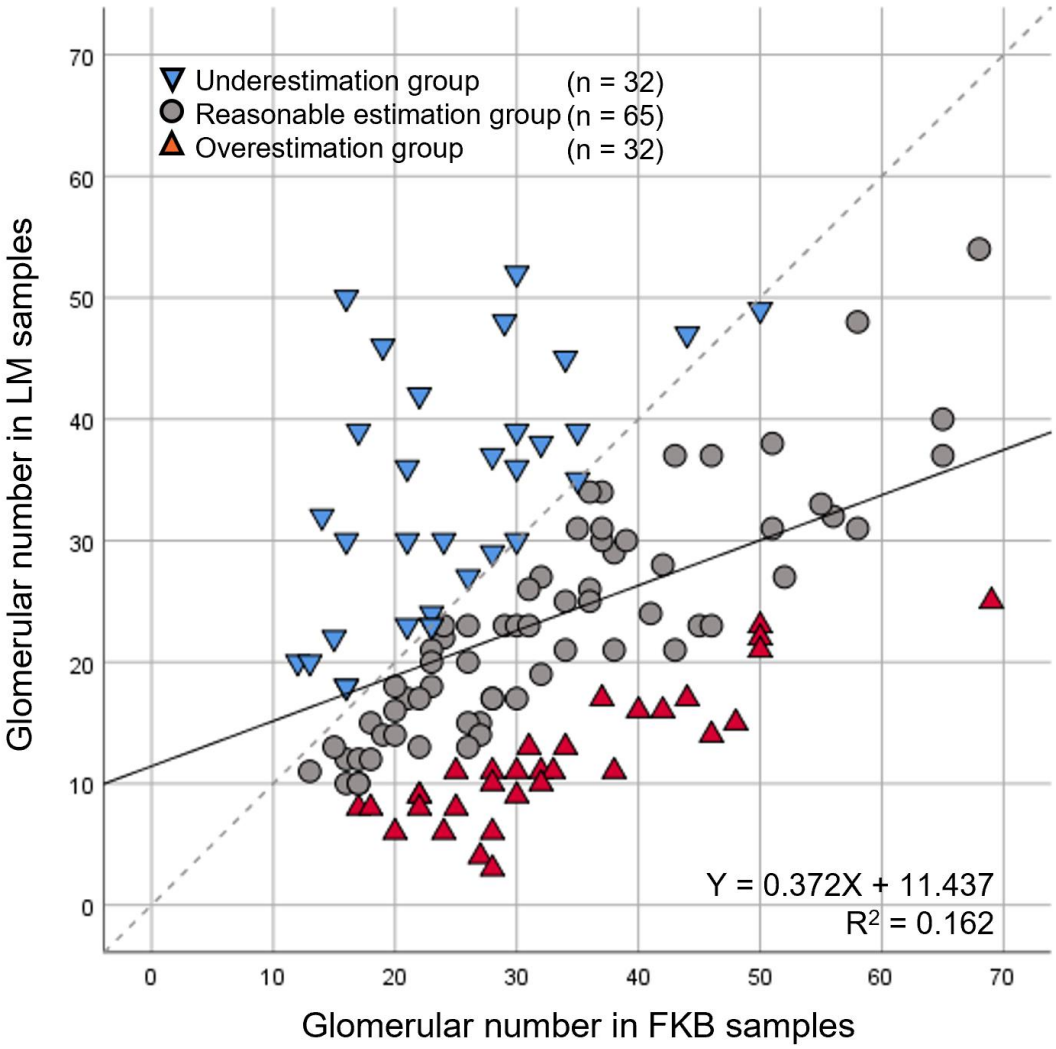

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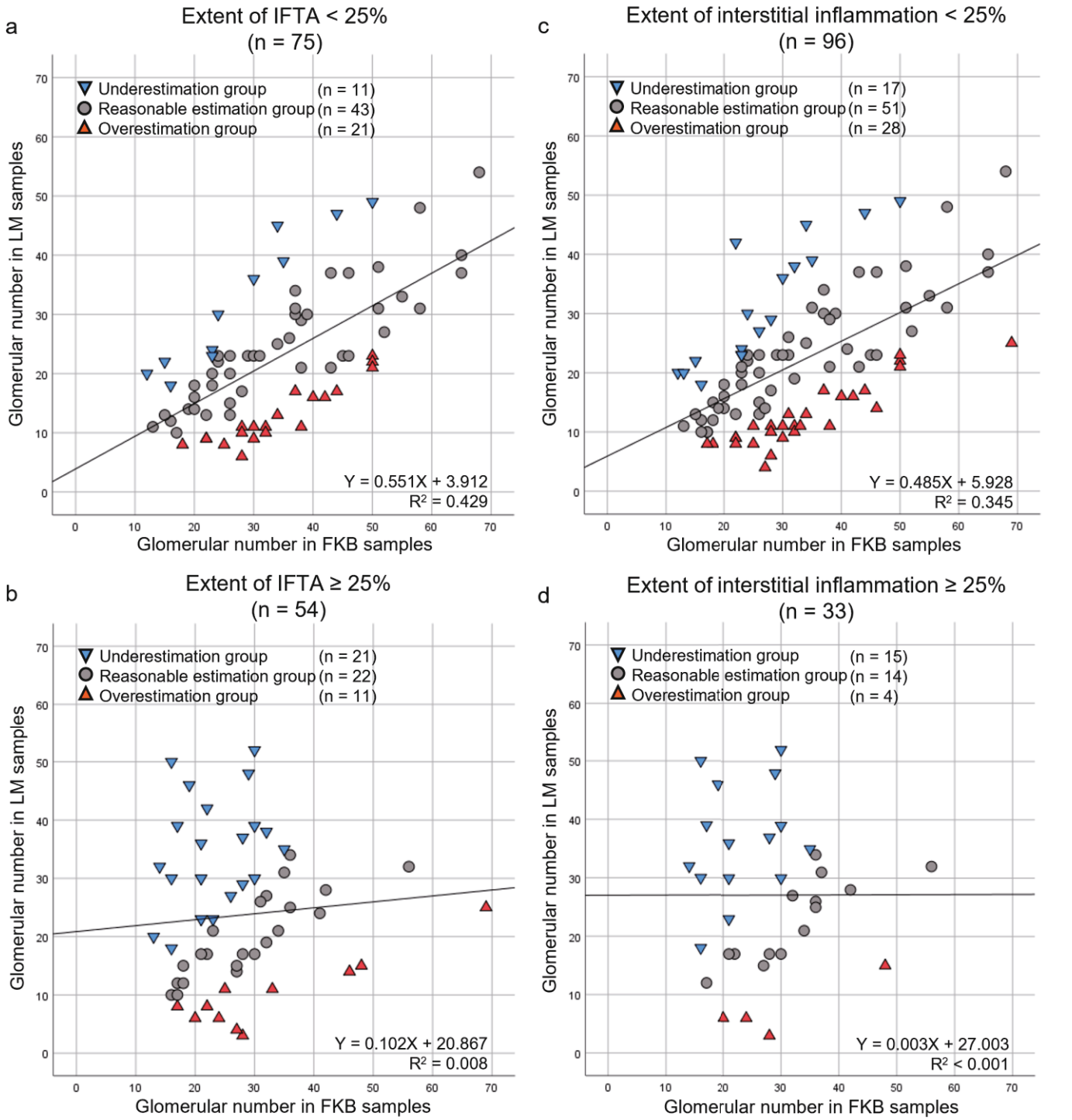

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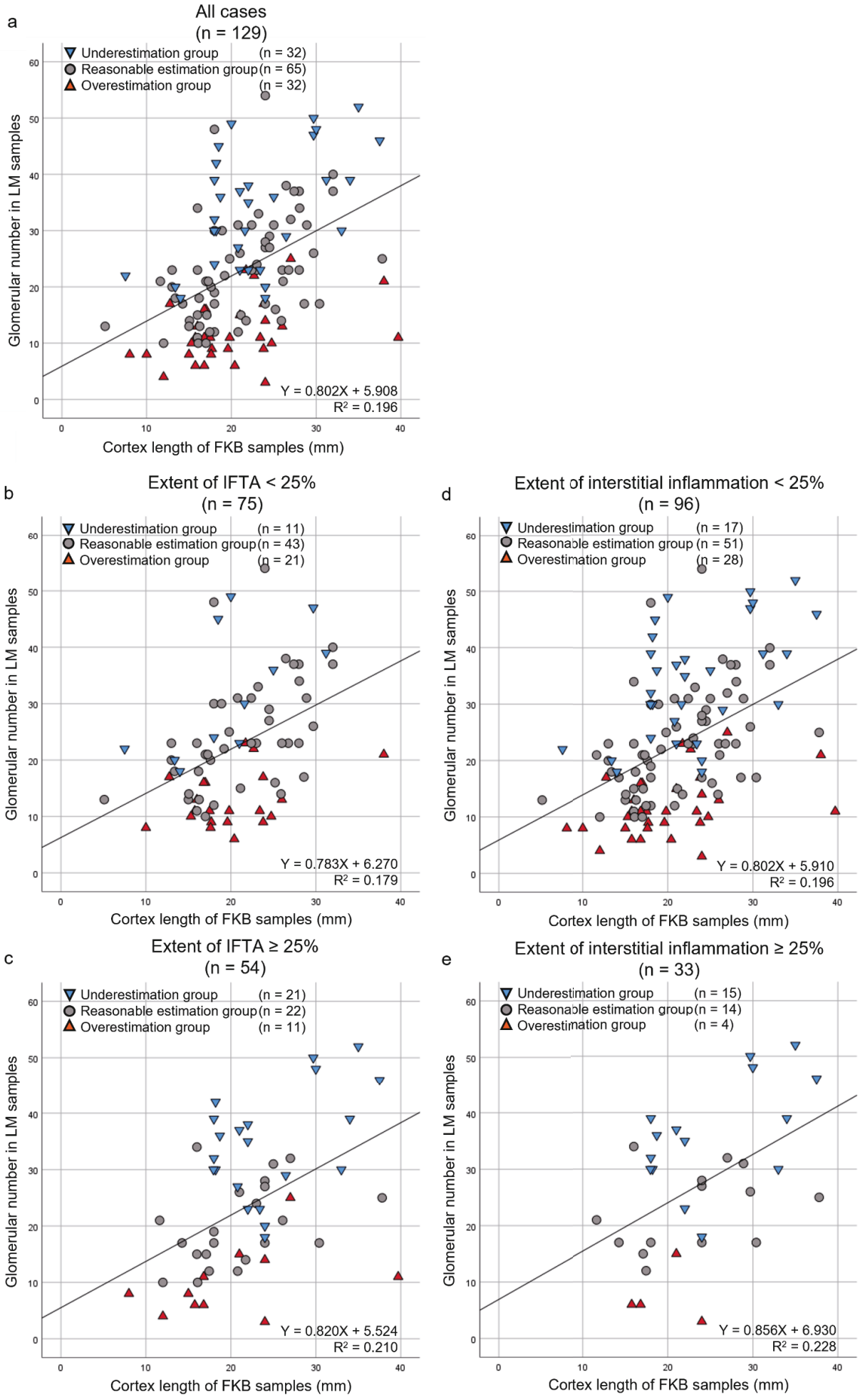

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