Use of Glomerular CD68+ Cells as a Surrogate Marker for Endocapillary Hypercellularity in Lupus Nephritis

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PII: S2468-0249(21)01620-X
Reference: EKIR 1729

To appear in: Kidney International Reports

Received Date: 1 December 2021
Accepted Date: 27 December 2021


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Use of Glomerular CD68+ Cells as a Surrogate Marker for Endocapillary Hypercellularity in Lupus Nephritis

Lupus nephritis (Kidney Biopsy) n=92

CD68 staining
Endocapillary hypercellularity
Activity index

New activity index was calculated in which CD68+ cells replaced endocapillary hypercellularity

A cut-off value of 7 for the maximum number of CD68+ cells within one glomerulus in a biopsy

88% 67%
Sensitivity Specificity
For endocapillary hypercellularity

Number of glomerular CD68+ cells significantly correlated with endocapillary hypercellularity

Endocapillary hypercellularity and CD68+ cells both correlated with renal function during follow-up

The current and the new activity index correlated equally well with clinical outcome

Conclusion In lupus nephritis, CD68+ cells can be used as a surrogate marker for endocapillary hypercellularity.
Title: Use of Glomerular CD68+ Cells as a Surrogate Marker for Endocapillary Hypercellularity in Lupus Nephritis

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Running head: CD68+ cells in lupus nephritis
Abstract

Introduction

Lupus Nephritis class III or IV is strongly related to patient mortality and morbidity. The interobserver agreement of endocapillary hypercellularity by routine light microscopy, one of the most important lesions determining whether class III or IV is present, is moderate. In IgAN, the presence of glomerular CD68+ cells was shown to be a good surrogate marker for endocapillary hypercellularity. We investigated whether in lupus nephritis, the presence of glomerular CD68+ cells could serve as a surrogate marker for endocapillary hypercellularity as well.

Methods

92 lupus nephritis biopsies were scored for the number of glomerular CD68+ cells using CD68 staining, as well endocapillary hypercellularity and the activity index. A new activity index was calculated in which CD68+ cells replaced endocapillary hypercellularity. Clinical parameters were obtained from time of biopsy, one and two years later.

Results

Number of glomerular CD68+ cells significantly correlated with endocapillary hypercellularity. A cut-off value of 7 for the maximum number of CD68+ cells within one glomerulus in a biopsy, yielded a sensitivity of 88% and a specificity of 67% for the presence of endocapillary hypercellularity. Endocapillary hypercellularity and CD68+ cells both correlated with renal function during follow-up. The current and the new activity index correlated equally well with clinical outcome.

Conclusion
In lupus nephritis, CD68+ cells can be used as a surrogate marker for endocapillary hypercellularity.
Keywords:

- Lupus nephritis
- Endocapillary hypercellularity
- Glomerular CD68+ cells
- Activity index
Introduction

Systemic Lupus Erythematosus (SLE) is a highly heterogeneous autoimmune multisystem disease. Lupus nephritis (LN), a manifestation that occurs in up to 60% of SLE patients, is associated with increased mortality and morbidity. Patients with class III/IV LN have higher mortality and morbidity rates than those with other classes, and they receive more aggressive immunosuppressants which can have a huge impact on their quality of life. In class III and IV LN, subendothelial immune deposits elicit influx of inflammatory cells and consequently, lead to lesions of which ‘endocapillary hypercellularity’ is the most important. Endocapillary hypercellularity in a single glomerulus is enough to classify the biopsy as class III, which emphasizes the importance of recognizing this lesion. Unfortunately, the interobserver agreement in recognizing endocapillary hypercellularity is moderate.

It was suggested previously that problems with the definition of endocapillary hypercellularity may be critical to interobserver variation. Therefore, the definition was recently modified in the revised ISN/RPS classification in which it was stated that influx of inflammatory cells is the most important component of endocapillary hypercellularity, and that if an intracapillary lesion has no inflammatory cells but only consists of endothelial swelling, this lesion should not be regarded as endocapillary hypercellularity. Of all inflammatory cells contributing to endocapillary hypercellularity, monocytes are the most interesting, in particular in relation to the development of the lesion. Monocytes were demonstrated to constitute the majority of inflammatory cells in glomeruli of LN patients affected by class III/IV. Moreover, monocytes were shown to be present in similar hypercellular lesions in other renal diseases such as IgA nephropathy.
In various renal diseases the role of monocytes as part of the spectrum of CD68 positive cells was investigated in detail. In IgA nephropathy (IgAN), the presence of CD68+ cells correlated with the occurrence of endocapillary hypercellularity. In IgAN there was a similar issue with interobserver variation in relation to this lesion as in lupus nephritis. Therefore, a study was performed which hypothesized that endocapillary hypercellularity in IgAN mainly reflects glomerular inflammation and that the presence of CD68+ cells would be a more robust marker to determine the E-score. CD68+ cells as a surrogate marker for endocapillary hypercellularity was shown to be helpful in decreasing interobserver variation: in IgAN it was shown that if in any glomerulus in a biopsy seven or more CD68+ cells were present as determined by immunohistochemistry, endocapillary hypercellularity was most likely present, with a sensitivity of 94.1% and a specificity of 71%. Therefore, in this study, we investigated whether a similar approach could be helpful to recognize endocapillary hypercellularity in LN. We investigated whether CD68 positivity could be used as a surrogate marker for endocapillary hypercellularity in lupus nephritis.

Methods

Study population

From July 2010 to January 2012, 164 patients with LN were recruited from two designated tertiary referral clinical centres. Inclusion criteria were as follows: a definite diagnosis of SLE in accordance with the American College of Rheumatology (ACR) revised classification criteria, biopsy proven LN and written informed consent. All study work was conducted in accordance with the requirements of the Helsinki Declaration and the study
was approved by the Outer South East London and the London City Road and Hampstead Research Ethics Committees.

Renal histology

Paraffin-embedded renal biopsy tissue was available from 126 LN patients recruited for this study. Biopsies were traced back to the time of the patients’ original diagnosis (n=107) or if this was not possible, biopsies taken at the onset of a new nephritis flare before induction immunosuppression was commenced were obtained (n=19). In total, 92 cases were included in the study. Twenty-four cases were excluded because the biopsy contained ≤5 glomeruli, 2 cases had inadequate tissue, in 1 case all glomeruli were globally sclerosed (class VI) and for 7 patients insufficient clinical data was available. The classification of a biopsy was recorded from the original pathology report. If a biopsy was classified as either class III+V or class IV+V, it was regarded as class III and IV for analysis.

Endocapillary hypercellularity was re-evaluated blindly by a nephropathologist who was unaware of the original classification and CD68+ cell score, using one slide per patient. The presence of endocapillary hypercellularity was scored for each glomerulus. The definition of endocapillary hypercellularity used was: ‘Endocapillary hypercellularity due to increased number of endothelial cells and infiltrating inflammatory cells, and causing narrowing of the glomerular capillary lumina,’ according to the definition used in the recent recommendations for LN.\(^6\) \(E \ continuous\) represents the number of glomeruli in a biopsy with endocapillary hypercellularity in relation to the total number of viable glomeruli.

Immunohistochemistry
Immunohistochemical staining was performed for CD68. Slides were deparaffinised and subjected to antigen retrieval (Tris/EDTA buffer). After blocking endogenous peroxidase, the sections were incubated with mouse anti-human CD68 (KP-1; Dako, Glostrup, Denmark) for 1 hour. Sections were then counterstained with haematoxylin. Once mounted and dried, the slides were scanned and the number of CD68+ cells in each glomerulus was counted (viewer software: 3DHISTECH Panoramic viewer or Philips Digital Pathology Solution). A maximum CD68+ score was determined for each biopsy, which consisted of the number of CD68+ cells present in a single glomerulus with the highest number of CD68+ cells of that biopsy. The fraction of glomeruli with ≥ 7 CD68+ cells in a biopsy was determined as based on a ROC-analysis (see below); this variable was called 

$CD68_{continuous}$.

**Activity index**

All biopsies were scored using the modified NIH activity index (AI). To determine whether CD68+ cells are a robust surrogate marker for endocapillary hypercellularity in the context of the AI, a new AI was calculated in which CD68+ cells replace endocapillary hypercellularity. This was done by determining the percentage of glomeruli in which 7 or more CD68+ cells were found. In accordance with the scoring system for parameters of the current AI, a score between 0-3 based on the percentage of glomeruli in which ≥7 CD68+ cells were found was given: 0 if none of the glomeruli had ≥7 CD68+ cells, 1 if ≥7 CD68+ cells were present in up to 25% of glomeruli, 2 if ≥7 CD68+ cells were present in 25-50% of glomeruli and 3 if ≥7 CD68+ cells were present in >50% of glomeruli. Next, scores obtained for CD68 positive cells were replaced by scores originally obtained for endocapillary hypercellularity. This new score was called $AI_{CD68}$. Both the modified AI and the AI $CD68$
were correlated with clinical data, to establish whether replacing endocapillary hypercellularity by CD68+ cells in the AI would not lead to a loss of correlation with clinical parameters.

**Clinical variables**

We retrospectively collected the clinical data at time of renal biopsy and one and two years after the biopsy was taken. We obtained data on sex, age of diagnosis, ancestry, eGFR, urine protein creatinine ratio (uPCR), therapy and whether a renal flare occurred during these two years.

**Statistical analysis**

Statistical analysis was performed using SPSS statistics 23.0 (IBM, Armonk, NY). The Kolmogorov-Smirnov test was used to determine whether data were normally distributed. Normally distributed data were presented as mean and standard deviation (SD) and analysed using one-way ANOVA for categorical data. Continuous variables were correlated using Pearson’s correlation. To test whether correlated Pearson’s rs differed significantly from each other, Hotelling’s T was used. Non-normally distributed data were presented as median and inter-quartile range (IQR), associations with categorical variables were determined using a Kruskal-Wallis test and Mann-Whitney U test, for continuous variables, this was done using a Spearman correlation test. For non-normally distributed paired data, Wilcoxon’s test and Friedman’s test were used. A cut-off value of the maximum CD68+ score was established using ROC curve analysis. Statistical significance level was set at 0.05.

**Results**
Baseline characteristics are described in Table 1. According to protocol, the treatment was based on the findings of the kidney biopsy. Therefore, no LN treatment was given prior to the biopsy. Examples of histology and immunohistochemistry findings are shown in Figure 1.

**Renal histology**

The median number of glomeruli per biopsy was 22 (IQR 13-33). Endocapillary hypercellularity was found in 74 (80.4%) biopsies. The median E continuous in the total cohort was 0.31 (IQR 0.07-0.50). The median of the maximum CD68+ score in the whole cohort was 17 (IQR 7-33).

E continuous and CD68 continuous correlated in a statistically significant way (Rho=0.784; p<0.001) (Figure 2). Both E continuous and CD68 continuous differed significantly between classes (p<0.001 for both) (Figure 3). E continuous as well as CD68 continuous were highest in class IV patients. The median of the maximum CD68+ cell score was significantly higher (p<0.001) in biopsies with endocapillary hypercellularity than without. Using ROC curve analysis (AUC=0.877), a cut-off value of 7 or more of the maximum CD68 score was determined (Supplementary Figure 1). This generated a sensitivity of 88% and a specificity of 67% (Table 2).

In nine patients, endocapillary hypercellularity was scored as present, but the maximum CD68 score was less than 7. The original classes as stated in the biopsy reports of these patients were class III (7 patients), class I (1 patient) and class II (1 patient). In the detailed scoring process, which was blinded for the original classes, biopsies of the latter 2 patients were scored to have a single glomerulus with an endocapillary lesion. Biopsies
originally scored as class III also had only one glomerulus with an endocapillary lesion in 4 cases, whereas the other 3 cases had endocapillary hypercellularity lesions in 5/29, 3/10 and 4/23 glomeruli. There were three cases in which sampling error accounted for the inconsistency between endocapillary hypercellularity lesion at light microscopy in the absence of a CD68 score of 7 or more: in these cases, the glomeruli with the endocapillary hypercellularity lesions were absent in the immunohistochemical staining.

In six patients, endocapillary hypercellularity was scored to be absent, but maximum CD68 score was 7 or more. Of these patients, one was class I, one was class II and one was class V. The other 3 patients were classified as class III, likely due to other inflammatory lesions. Two patients originally classified as class I and II who had no endocapillary hypercellularity but did score positive on the presence of neutrophils and karyorrhexis: these two biopsies showed more than 7 CD68 positive cells in 5/29 and 10/17 glomeruli. There was 1 patient originally classified as class V with more than 7 CD68 positive cells in 1/19 glomeruli.

**Clinical parameters**

eGFR was significantly lower in patients with endocapillary hypercellularity at time of biopsy, one year and two years later (p<0.001, p=0.003 and p=0.027, respectively). This was also true for patients with a maximum CD68 score of 7 or more (p=0.005, p=0.028 and p=0.016). Both E continuous and CD68 continuous were correlated with eGFR at time of biopsy (rho=-0.405; p<0.001 and rho=-0.295; p=0.008, respectively) and one year after biopsy (rho=-0.259, p=0.022 and rho=-0.219, p=0.048, respectively). Neither E continuous nor CD68 continuous were significantly correlated to eGFR two years after biopsy.
Proteinuria measured by uPCR did not correlate with the presence of endocapillary hypercellularity, the maximum CD68 score, E continuous and CD68 continuous at any of the time points.

**Activity index**

The Pearson’s correlation co-efficients of the current AI and the AI CD68 correlated with eGFR were completely comparable, since Hotelling’s T was not significant. This was true for the correlation with eGFR at all three timepoints. Moreover, almost all correlations between both the current AI and the AI CD68 with eGFR were statistically significant. (Table 3) Neither the modified AI nor the AI CD68 significantly correlated with uPCR at any time point.

**Discussion**

This study demonstrates that in lupus nephritis, the presence of glomerular CD68+ cells correlates with the presence of endocapillary hypercellularity. A cut-off value for the maximum CD68+ cell count in a biopsy, that can be helpful in determining whether endocapillary hypercellularity is present in a biopsy, was identified as 7 CD68+ cells, which is comparable to findings in IgAN. We propose that in cases where the pathologist cannot be certain using routine light microscopy whether endocapillary hypercellularity is present, quantifying CD68+ cells can be useful in making this decision. If 7 or more CD68+ cells are found within a glomerulus, it is likely that in that biopsy, endocapillary hypercellularity is present. As a guideline, if less than 7 cells are present, it could be argued that if an endocapillary hypercellularity lesion is recognized with a relatively high amount of certainty by light microscopy this overrules the findings by immunohistochemistry.
Neither the sensitivity nor specificity of the cut-off value and the correlation between E continuous and CD68 continuous were perfect. To assess this, patients who had endocapillary hypercellularity but a maximum CD68 score of <7 and patients without endocapillary hypercellularity but a maximum CD68 score of ≥7 were studied in more detail. It seems that in biopsies where there is endocapillary hypercellularity in combination with a maximum CD68 score lower than 7, there is often a very mild class III with, in most cases, only 1 glomerulus showing endocapillary hypercellularity. The discordance between endocapillary hypercellularity and CD68+ cells was caused by sampling error in some cases. To minimize the risk of sampling error we recommend the use of consecutive slides to assess endocapillary hypercellularity and CD68+ cells and to keep in mind that glomeruli on the edge of the slide may not be present on the other slide.

In our study, the absence of endocapillary hypercellularity in combination with a maximum CD68 score of 7 or more, could be due to a class III biopsy based on lesions other than endocapillary hypercellularity, or class I, II or V with a minority of glomeruli showing active lesions like the presence of neutrophils/karyorrhexis or hyaline deposits. Alternatively, CD68 positivity in some patients may be a sign of inflammation that has not progressed to inflammatory lesions such as endocapillary hypercellularity yet. Therefore, our findings lead to the question whether an overall inflammation score, possibly incorporating immunohistochemistry markers such as CD68, adds to the classification of LN. The international working group of nephropathologists who presented the revised ISN/RPS classification of LN in 2018 addressed this as well. They stated that it should be investigated further if such an overall inflammation score is valuable. Such an inflammation score could be useful in demonstrating a continuum of the disease, which may be more precise in
describing active inflammation than the current six classes of the classification. Moreover, an overall inflammation score may also be helpful as a prognostic tool in subsequent biopsies following treatment, to indicate whether active lesions are present. In the current study, it was shown that by replacing endocapillary hypercellularity in the current AI with a score for glomerular CD68+ cells, virtually no loss of correlation with renal function occurs as compared to the current AI. This indicates that CD68+ cells can be used as a surrogate marker for endocapillary hypercellularity in the context of the AI.

Both the presence of and the fraction of glomeruli with endocapillary hypercellularity and CD68+ cells are associated with worse eGFR at time of biopsy, one and two years later. This implies that inflammation, indicated by either the inflammatory lesion endocapillary hypercellularity or the inflammatory CD68+ cells, leads to lower renal function. This inflammation was not correlated with proteinuria. In previous studies, clinical data and outcomes were primarily associated with tubulointerstitial CD68+ cells in LN patients.

The study by Soares et al. about CD68+ cells in IgAN and this study set out to try to solve the problem of low interobserver agreement of endocapillary hypercellularity, a lesion important in the classification of IgAN and LN, using CD68+ cells as a surrogate marker. In both studies, the maximum CD68 score was used and the cut-off value was identical: seven or more cells. The similarity of the cut-off value demonstrates that although these are two different diseases, endocapillary hypercellularity lesions may develop from similar mechanism. Sensitivity and specificity were higher in IgAN and in both studies, specificity was lower than sensitivity. Clinical data was not assessed by Soares et al. In LN and IgAN,
CD68+ cells can be utilized as a surrogate marker for endocapillary hypercellularity using a similar approach.

The use of CD68+ cells as a surrogate marker for endocapillary hypercellularity has limitations. The specificity is relatively low and sampling errors are possible, for instance if the area of the glomerulus in which endocapillary hypercellularity is present is not included in the immunohistochemistry slide or vice versa. In addition, an extra staining would need to be performed, leading to higher costs and workload. To validate CD68 as a surrogate marker, the findings of this study should be verified using a validation cohort and more nephropathologists to score endocapillary hypercellularity and CD68+ cells in order to determine reproducibility. Moreover, in this study it was not conclusively demonstrated that quantifying CD68+ cells is more objective and has a higher interobserver agreement compared to endocapillary hypercellularity, although it is likely that this is the case since Soares et al. established a good interobserver agreement for the presence of seven or more CD68+ cells in a glomerulus.10

The results of our study show that the presence of CD68 positive cells does not equal the presence of endocapillary hypercellularity, however, there is a strong correlation between the two lesions. Using the maximum CD68 score with a cut-off value of 7, CD68+ cells can function as a surrogate marker for endocapillary hypercellularity in LN. Naturally, the presence of CD68 positive cells can reflect lesions other than endocapillary hypercellularity as well. CD68+ cells may be an indicator of overall inflammation within the kidney of LN patients and could be incorporated into an inflammation score, either in addition to or incorporated in the current AI. A new AI in which CD68+ cells were included,
correlates with clinical outcome equally well as the current AI, further demonstrating the importance of CD68+ cells as a marker of inflammation in LN.

Disclosure

Elisabeth Bos, Shirish Sangle, Suzanne Wilhelmus, Ron Wolterbeek, Natasha Jordan, David Isenberg, Terence Cook and Jan Bruijn declare that they have no conflict of interest. David D’Cruz has received consulting fees and speaker honorarium from GlaxoSmithKline. Ingeborg Bajema has received consulting fees from Aurinia Pharmaceuticals, Boehringer-Ingelheim and SDE Research.

Supplementary material

Supplementary Figure 1. ROC curve. ROC curve of maximum CD68 score to determine whether endocapillary hypercellularity is present. (PDF)

STROBE statement (PDF)

Supplementary information is available at KI Report’s website.
References

### Tables

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n=92)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female, n (%)</strong></td>
<td>76 (82.6%)</td>
</tr>
<tr>
<td><strong>Age at diagnosis, mean (SD)</strong></td>
<td>26.2 (10.7)</td>
</tr>
<tr>
<td><strong>Ethnicity, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>7 (7.6%)</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>28 (30.4%)</td>
</tr>
<tr>
<td>Asian</td>
<td>21 (22.8%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>35 (38.0%)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td><strong>Class, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>Class II</td>
<td>8 (8.7%)</td>
</tr>
<tr>
<td>Class III</td>
<td>28 (30.4%)</td>
</tr>
<tr>
<td>Class IV</td>
<td>45 (48.9%)</td>
</tr>
<tr>
<td>Class V</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²), n=69, median (IQR)</td>
<td>80 (58-106)</td>
</tr>
<tr>
<td>uPCR (mg/mmol), n=72, median (IQR)</td>
<td>189 (95-437)</td>
</tr>
<tr>
<td><strong>Medication, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>74 (80.4%)</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>56 (60.9%)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>18 (19.6%)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>24 (26.1%)</td>
</tr>
</tbody>
</table>

**Table 1 Baseline characteristics** Treatment for LN was initiated after the renal biopsy.
<table>
<thead>
<tr>
<th>Endocapillary hypercellularity</th>
<th>Endocapillary hypercellularity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>absent</td>
<td>present</td>
<td></td>
</tr>
<tr>
<td>Maximum CD68 score &lt;7</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Maximum CD68 score ≥7</td>
<td>6</td>
<td>65</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>74</td>
</tr>
</tbody>
</table>

**Table 2 Endocapillary hypercellularity and maximum CD68 score.** Number of patients in which endocapillary hypercellularity is either absent or present and in which the maximum CD68 score is <7 or ≥7. Sensitivity = 88%; specificity = 67%.

<table>
<thead>
<tr>
<th></th>
<th>Modified AI</th>
<th>AI CD68</th>
<th>Hotelling’s T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>eGFR at time of biopsy</strong></td>
<td>-0.453 (p&lt;0.001)</td>
<td>-0.442 (p&lt;0.001)</td>
<td>-0.508 (p=0.613)</td>
</tr>
<tr>
<td><strong>eGFR one year after biopsy</strong></td>
<td>-0.218 (p=0.049)</td>
<td>-0.225 (p=0.042)</td>
<td>0.304 (p=0.762)</td>
</tr>
<tr>
<td><strong>eGFR two years after biopsy</strong></td>
<td>-0.250 (p=0.025)</td>
<td>-0.269 (p=0.015)</td>
<td>0.832 (p=0.408)</td>
</tr>
</tbody>
</table>

**Table 3 Pearson’s correlation between eGFR and modified AI and AI CD68.** The correlation between the currently used Modified AI and AI CD68, in which the score for endocapillary hypercellularity is replaced by CD68+ cells. Hotelling’s T describes whether the correlated Pearson’s correlations are significantly different, which is not true for any of the time points.
Figure legends

**Figure 1** CD68 positive cells in lupus nephritis in relation to histological findings. A: LN class IV, glomerulus with abundance of CD68 positive cells. B: same glomerulus as shown in A; CD68 positive cells are present in areas with endocapillary hypercellularity (big arrows in A and B) but also in extracapillary proliferation (small arrows in A and B). C: absence of glomerular CD68 positive cells whereas a small lesion with endocapillary hypercellularity (arrow) is present (D), showing that sampling error may give rise to inconsistent results. E: virtual absence of CD68 positive cells in a glomerulus with many wire loops, consistent with class IV but in the absence of endocapillary hypercellularity.

**Figure 2** E continuous and CD68 continuous. Rho=0.784; p<0.001. The fraction of glomeruli with endocapillary hypercellularity and ≥7 CD68+ cells in a biopsy, each dot represents a biopsy.

**Figure 3** E continuous and CD68 continuous per class. The fraction of glomeruli with endocapillary hypercellularity and ≥7 CD68+ cells in a biopsy compared between classes. Bars represent the median of the group, whiskers the inter quartile range. ***p≤0.001; ****p≤0.0001