TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

# Three-year outcome of isolated glomerulitis on 3-month protocol biopsies of donor HLA antibody negative patients

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**Keywords**
antibody-mediated rejection, glomerulitis, HLA antibody, protocol biopsies.

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**Conflicts of Interest**
The authors have declared no conflicts of interest.

Received: 24 September 2011
Revision requested: 17 October 2011
Accepted: 6 March 2012
Published online: 5 April 2012

doi:10.1111/j.1432-2277.2012.01473.x

## Summary

Transplant glomerulitis (TG) can lead to the diagnosis of acute humoral rejection when associated with C4d. Recent data have shown that, in patients with donor-specific antibodies, TG is a sign of humoral rejection, even in the absence of C4d. However, the clinical significance of isolated TG, i.e. TG without C4d deposition or morphological evidence of rejection, has not been specifically studied in protocol biopsies of recipients without donor-specific antibodies. We compared 20 isolated TG-patients with 44 selected recipients without TG or any rejection-associated change. The two groups had similar baseline characteristics. After a 3 year follow-up, renal function, acute rejection rate, and development of HLA antibodies were not significantly different between the two groups. Isolated TG had no deleterious consequences on the 3 year graft outcome. Eleven patients of the glomerulitis-group had another allograft biopsy during follow-up: glomerular lesions returned to normal in six patients whereas the persistence of glomerulitis or features consistent with chronic transplant glomerulopathy were noticed in the remaining five patients. Four of these five patients had pretransplant non-donor specific HLA antibodies. In conclusion, although isolated TG had no impact on allograft function at 3 year, histological outcome could be related to patient sensitization.

## Introduction

During the last decade, transplant centers have increasingly used protocol biopsies of stable renal allografts. Histological examination of such biopsies is likely to provide information concerning asymptomatic immunological injuries of renal parenchyma, i.e. subclinical rejection. Although the exact significance of subclinical rejection remains unclear, its identification usually prompts clinicians to intensify immunosuppressive therapy [1,2].

Transplant glomerulitis (TG) is defined in the Banff 97 classification scheme as 'mononuclear cell infiltrate and endothelial cell enlargement' of glomerular capillaries [3]. TG belongs to the pathological spectrum of allograft antibody-mediated rejection (AMR) [4,5]. Indeed, the association of TG and C4d deposition in peritubular capillaries (PTC) in the presence of donor specific antibodies (DSA) indicates type 2 AMR in the updated Banff 01 classification [6]. TG typically occurs in the context of AMR with acute loss of graft function [7]. However, more recent

studies report subclinical AMR in protocol biopsies of patients with DSA and a positive crossmatch, or with ABO-incompatible kidney transplantations [8,9]. In this context, AMR-related lesions, including TG, contribute to the development of interstitial fibrosis and tubular atrophy and are associated with late renal failure [8]. Furthermore, data from protocol biopsies in DSA-positive recipients indicate that microcirculatory changes such as TG and peritubular capillaritis may occur in the absence of C4d deposition [10–12], and are also associated with deleterious effects on renal function [11,13].

On the other hand, the precise significance of subclinical isolated TG (ITG), defined as TG without C4d deposition and any other morphological evidence of rejection, on protocol biopsies of non-DSA renal transplant recipients remains unclear. To address this question, we retrospectively analyzed the 3 year outcome of 20 *de novo* renal transplant recipients who showed subclinical ITG on a 3 month protocol biopsy.

## Materials and methods

### Patients

At our center, 3 month protocol graft biopsies were performed in 476 consecutive *de novo* renal transplant recipients between January 2005 and December 2008. Twenty of these (4.2%) presented subclinical ITG, defined as the presence of TG without any other rejection lesion and C4d deposits, in the absence of renal failure. These patients were compared to a control group of 44 recipients whose 3 month protocol biopsies did not display significant inflammation, especially TG, and matched for age, gender, rank of the graft, HLA matching, sensitization and year of transplant. Patients previously identified as having donor-specific anti-HLA antibodies prior to transplant (positive crossmatch) were excluded from the study. All patients had a negative T-cell crossmatch at transplantation as assessed by an anti-human globulin enhanced complement-dependent cytotoxicity (CDC) assay.

This cohort of patients with subclinical ITG has otherwise been included in a transcriptional study comparing glomerulitis occurring in different settings [14].

### Histological analyses and definition of TG

Two core biopsies were obtained for each patient using 15-gauge spring-loaded needles (Boston Scientific®, Nanterre, France) under real-time ultrasound guidance. For light microscopy, one needle core biopsy was fixed in AFA (alcohol, formalin, acetic acid) and embedded in paraffin. Sections were cut at 2 μm and stained with Masson's trichrome, periodic acid-Schiff, Jones silver methenamine, and hematoxylin-eosin-saffron. A total of 24 sections were analyzed at optic microscopy for each biopsy. Grading was performed according to the updated Banff 07 classification [15], without knowledge of patients' antibody status.

The diagnosis of TG was based on the presence of inflammatory cells occluding glomerular capillary lumens as shown in Fig. 1. Endothelial cell enlargement was an inconstant feature. We defined ITG as TG occurring in the absence of T-cell mediated or AMR. Thus, the following were considered as exclusion criteria: tubulointerstitial and/or vascular inflammation consistent with borderline changes or T-cell mediated rejection (both acute and chronic), duplications of glomerular basement membranes consistent with chronic transplant glomerulopathy, and C4d deposits in PTC.

### Immunohistochemical analyses

One needle core biopsy was snap frozen in embedding resin (Shandon Cryomatrix, Thermo Scientific®, Cergy Pontoise, France) for immunofluorescence studies. Acetone-fixed cryostat sections were stained with commercially available mouse monoclonal antibody specific for complement split factor C4d (Quidel®, San Diego, CA, USA) at 1:20 dilution, followed by fluorescein isothiocyanate-conjugated goat antimouse IgG (DAKO®, Trappes, France). To increase C4d detection sensitivity, additional immunohistochemical C4d staining was performed on paraffin sections using human polyclonal antibody (Biomedica®, Vienna, Austria). The intensity for C4d staining in PTC was staged according to the updated Banff 07 classification [15].

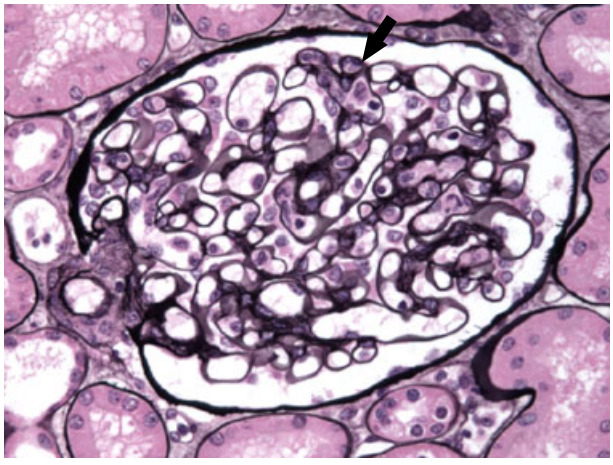

**Figure 1** Mononuclear cell infiltrate occluding the capillary lumen in a segmental manner (arrow). Jones methenamine silver, magnification ×40.

Alcohol, formalin, acetic acid-fixed sections embedded in paraffin were stained with commercially available mouse monoclonal antibodies. CD3 staining (LN10, Novocastra®, Rungis, France) was systematically performed in both groups. CD68 staining (KP1, DAKO®), a marker for macrophages, was performed when ITG was suspected at light microscopy in 15 of the 20 biopsies with TG to finally confirm the diagnosis. Antibody localization was performed using the avidin–biotin peroxidase complex procedure.

### Clinical follow-up and monitoring

The following clinical parameters were retrospectively assessed at baseline: recipient gender, donor type (living or deceased donor), current and historical panel-reactive antibody (PRA) levels, number of previous transplants, HLA matching.

During the follow-up period, the following data were recorded: death, graft loss, histological results of clinically indicated allograft biopsies, serum creatinine (at 3, 12, 24 and 36 months), proteinuria/creatininuria ratio (at 3, 12, 24 and 36 months), cytomegalovirus (CMV) disease episodes, *de novo* anti-HLA antibodies (assessed at month 3, 12, then yearly), and changes to immunosuppressive treatments.

### Immunosuppression

All of the patients received an induction treatment (Thymoglobulin: 1.25 mg/kg per day during 4 days or Basiliximab: 20 mg at days 0 and 4) and a triple-drug maintenance therapy with a calcineurin inhibitor (tacrolimus: started at 0.15 mg/kg per day or cyclosporine: started at 6 mg/kg per day), mycophenolate mofetil (2 g/day), and steroids (500 mg at day 0, 250 mg at day 1, then 20 mg/day). Acute rejection episodes were treated with steroid pulses (1g/day for 3 days).

### Detection of HLA-antibodies

In 2005, anti-HLA antibody screening and identification were performed by CDC method comprising a panel of 50 total lymphocytes from blood donors. Since 2006, in parallel with CDC, sera have also been tested with a microbead array technique, Lab Screen Mixed kit (One Lambda, Inc., Canoga Park, CA, USA). Results above a cut-off value of 4.0 (ratio value) were considered positive. Lab Screen PRA class I and/or Lab Screen PRA class II were used to determine HLA-specificities of positive sera. A specificity was retained when the normalized mean fluorescence intensity was ≥ 1000. When identification of the HLA-specificities was not possible, the Single Antigen class I or II kit was done.

### Statistical analysis

Categorical variables were expressed as number and percentage (%) and were compared by the $X^2$ test or Fisher exact test. Continuous variables were expressed as mean ± SD. Their distributions were assessed by the Shapiro–Wilk test and they were compared by the Student's *t* test or the Mann-Whitney *U* test when appropriate. A *P* value of <0.05 was considered as significant. All of the statistical tests were performed with the software package SPSS, version 13.0 (SPSS, Chicago, IL, USA).

## Results

### Baseline characteristics

The baseline characteristics and immunosuppression of the 20 ITG and 44 control recipients are summarized in Tables 1 and 2, respectively. There were no significant differences between the two groups.

### Three month clinical and histological data

At the time of the 3 month protocol biopsy, the maintenance immunosuppressive regimen and the renal function were equivalent in both groups, as shown in Table 3.

Histological data of the 3 month biopsies in the ITG and control groups are shown in Table 4. All of the biopsy specimens contained at least seven glomeruli and one artery. The mean number of glomeruli per biopsy

**Table 1.** Pretransplantation demographic characteristics of transplant recipients.

| | ITG group (*n* = 20) | Control group (*n* = 44) | *P* value |
|---|---|---|---|
| Age (year) | 47.2 ± 17.4 | 48.3 ± 11.4 | 0.789 |
| Sex (male %) | 65.0 | 61.4 | 0.967 |
| GN as cause of ESRD (%) | 20.0 | 31.8 | 0.426 |
| HCV (%) | 0 | 0 | – |
| Deceased donor (%) | 100.0 | 95.4 | 0.482* |
| Regraft (%) | 20.0 | 15.9 | 0.752 |
| DGF (%) | 9.5 | 11.4 | 0.823 |
| HLA matching (*n*) | 3.8 ± 1.1 | 3.4 ± 1.3 | 0.279† |
| HLA antibodies (%) | 40.0 | 27.3 | 0.377 |
| Class I | 10.0 | 9.1 | 0.482 |
| Class II | 5.0 | 2.3 | 0.561 |
| Class I + II | 25.0 | 15.9 | 0.387 |
| DSA (%) | 0 | 0 | – |
| CIT (hour) | 21.2 ± 9.5 | 20.2 ± 7.5 | 0.666 |
| Donor age (year) | 45.9 ± 18.4 | 46.7 ± 13.2 | 0.866 |

GN: glomerulonephritis, ESRD: end-stage renal disease, HCV: hepatitis C virus infection, DGF: delayed graft function, DSA: donor-specific antibodies, CIT: cold ischemia time.
*Fisher exact test.
†Mann-Whitney *U* test.

**Table 2.** Baseline immunosuppression.

| | ITG group % (*n*) | Control group % (*n*) | *P* value |
|---|---|---|---|
| Induction therapy | | | |
| ATG | 47 (9) | 50 (22) | 0.847 |
| Anti-IL-2R | 53 (10) | 50 (22) | |
| CNI | | | |
| Cyclosporine | 0 | 2 (1) | 0.938* |
| Tacrolimus | 100 | 98 (43) | |
| Tacrolimus trough levels (μg/l) | | | |
| Day 15 | 10.2 ± 3.3 | 11.2 ± 4.3 | 0.382 |
| Day 30 | 9.6 ± 2.9 | 9.8 ± 3.6 | 0.823 |
| Day 60 | 10.6 ± 3.4 | 10.1 ± 3.2 | 0.631 |
| Day 90 | 9.6 ± 2.9 | 9.7 ± 3.5 | 0.877 |
| MMF | 100 | 100 | – |
| 3 month steroids withdrawal | 45 (9) | 57 (25) | 0.223 |

ATG: antithymoglobulin, CNI: calcineurin inhibitors, MMF: mycophenolic acid.
*Fisher exact test.

**Table 3.** Three month clinical data.

| | ITG group | Control group | *P* value |
|---|---|---|---|
| CNI | | | |
| Cyclosporine % (*n*) | 5 (1) | 2 (1) | 0.561 |
| Tacrolimus % (*n*) | 95 (19) | 98 (43) | |
| MMF % (*n*) | 100 (20) | 89 (39) | 0.777 |
| Steroids free % (*n*) | 47 (9) | 57 (25) | 0.223 |
| Serum creatinine mg/dl | 1.6 ± 0.5 | 1.5 ± 0.4 | 0.497 |
| eGFR | 52.7 ± 20.3 | 52.2 ± 15.7 | 0.937 |
| Proteinuria/creatininuria | 0.26 ± 0.23 | 0.19 ± 0.19 | 0.137* |

CNI: calcineurin inhibitors, MMF: mycophenolic acid, eGFR: estimated glomerular filtration rate using the MDRD equation.
*Mann-Whitney *U* test.

was 20.9 ± 8.5 in the ITG-group and 21.4 ± 10.2 in the control group. In agreement with the selection criteria, PTC deposits of C4d (after both frozen and paraffin immunohistochemical preparations), significant tubulointerstitial inflammation, and duplications of glomerular basement membranes were absent. ITG was graded as g1 (involvement <25% of glomeruli) in 16 cases and as g2 (involvement of 25–75% of glomeruli) in 4 cases. In all of the ITG cases (20/20), TG was segmental, with inflammatory cells occluding less than 50% of the capillary lumens in the glomerular tuft. In 15/20 cases, immunohistochemistry with anti-CD68 and anti-CD3 antibody was performed and indicated that glomerular inflammatory cells were mainly macrophages (Fig. 2). In order to exclude recurrence of glomerulonephritis, immunofluorescence with antibodies directed against IgA, IgG, IgM, C1q, and C3 was performed in 10 of the ITG-patients and did not reveal any immune deposits.

**Table 4.** Banff 07 scores for the ITG group and the control group.

| | ITG group (*n* = 20) | Control group (*n* = 44) | *P* value |
|---|---|---|---|
| g | 1.2 ± 0.4 | 0 | <0.001 |
| i | 0 | 0 | 1.000 |
| t | 0.5 ± 0.5 | 0.2 ± 0.4 | 0.112 |
| v | 0 | 0 | 1.000 |
| ptc | 0.1 ± 0.4 | 0.1 ± 0.2 | 0.152 |
| mm | 0.7 ± 1.0 | 0.3 ± 0.7 | 0.115 |
| cg | 0 | 0 | 1.000 |
| ci | 0.8 ± 0.7 | 0.6 ± 0.7 | 0.666 |
| ct | 0.9 ± 0.6 | 0.7 ± 0.6 | 0.773 |
| cv | 0.9 ± 0.9 | 1.1 ± 0.8 | 0.110 |
| ah | 0.9 ± 1.2 | 0.5 ± 0.7 | 0.361 |
| C4d | 0 | 0 | 1.000 |

Mann-Whitney *U* test for all comparisons.
g: glomerulitis, i: interstitial inflammation, t: tubulitis, v: intimal arteritis, ptc: peritubular capillaritis, mm: mesangial matrix increase, cg: allograft glomerulopathy, ci: interstitial fibrosis, ct: tubular atrophy, cv: fibrous intimal thickening, ah: arteriolar hyaline thickening, C4d: peritubular C4d deposits.

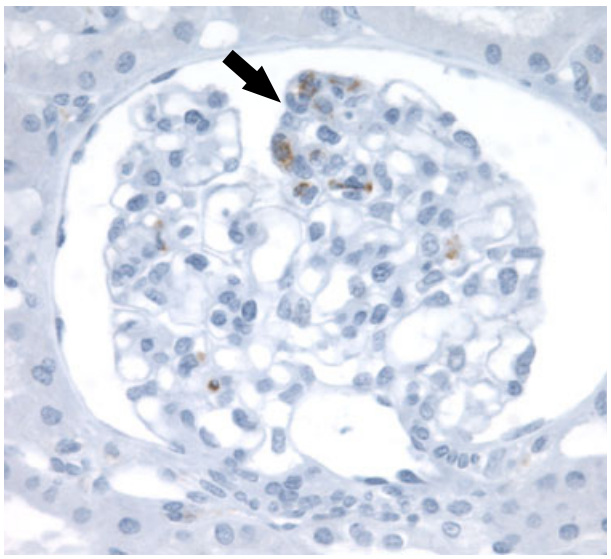

**Figure 2** Segmental macrophage infiltration (arrow). Immunostaining with anti-CD68 antibody, magnification ×40.

Peritubular capillaritis (ptc1) was present in 3/20 patients; the glomerulitis score was g2 in two of these patients.

### Three-year follow-up

The presence of ITG did not lead to any alteration of immunosuppressive treatment. From 1 to 3 year post

transplantation MDRD significantly decreased in both groups. However, serum creatinine and MDRD remained similar in ITG and control groups (Table 5). The incidence and grade of biopsy-proven acute rejection between months 3 and 36 were similar in both groups (Table 5). Two recipients developed *de novo* HLA antibodies in the control group versus none in the ITG group (NS). No patient died or lost their graft during the study period.

### Histological outcome

Among the 20 patients with ITG, 11 had follow-up biopsies at 12.4 ± 6.8 (range 5.5–25.4) months post-transplant (Table 6). Biopsies were performed for cause (renal failure defined as a 20% increase of serum creatinine from baseline or proteinuria above 0.5 g/day) in five cases and for ITG monitoring in six cases. C4d staining remained negative in all of these 11 biopsies. However, although glomerular lesions returned to normal in six patients, persistence of ITG was noted in three cases and duplication of glomerular basement membranes, consistent with chronic transplant glomerulopathy, appeared in two patients. The 3 month g score was numerically higher in these patients (g2 in three patients, and g1 in two patients) than in recipients in whom biopsies had returned normal (g1 in six patients). Notably, four of the five patients who displayed lesion progression had a second biopsy for renal failure (mean serum creatinine at the time of biopsy was 2.2 ± 1.2 mg/dl) or proteinuria. Moreover, only one of the patients in whom glomerular lesions returned to normal had preformed HLA antibodies. In contrast, preformed non-donor specific HLA antibodies were detected in 4/5 patients (Class I: 1, Class II: 0, Class I+II: 3) who had persistent ITG and/or chronic transplant glomerulopathy.

In the follow-up biopsies, the mean score of interstitial fibrosis and tubular atrophy was 1.2 ± 0.4 for the five patients with persistent glomerular lesions and 1.3 ± 0.5 for the patients without TG nor chronic glomerulopathy.

**Table 5.** Three-year follow-up data.

| | ITG group | Control group | *P* value |
|---|---|---|---|
| 12 months | | | |
| Serum creatinine (mg/dl) | 1.4 ± 0.4 | 1.4 ± 0.4 | 0.867 |
| eGFR | 60.8 ± 19.5 | 57.6 ± 18.8 | 0.768 |
| Proteinuria/creatininuria | 0.3 ± 0.6 | 0.2 ± 0.5 | 0.264* |
| 24 months | | | |
| Serum creatinine (mg/dl) | 1.6 ± 0.3 | 1.4 ± 0.4 | 0.175 |
| eGFR | 48.4 ± 13.1 | 54.2 ± 15.1 | 0.190 |
| Proteinuria/creatininuria | 0.2 ± 0.2 | 0.3 ± 0.4 | 0.376* |
| 36 months | | | |
| Serum creatinine (mg/dl) | 1.6 ± 0.4 | 1.5 ± 0.4 | 0.442 |
| eGFR | 46.6 ± 12.1[a] | 50.7 ± 14.2[a] | 0.386 |
| Proteinuria/creatininuria | 0.4 ± 0.3 | 0.3 ± 1.0 | 0.119* |
| Acute rejection | | | |
| All | 1 | 3 | 0.747 |
| T-cell mediated | 1 | 2 | – |
| Antibody-mediated | 0 | 1 | – |
| Graft failure rate (%) | 0 | 0 | – |
| *De novo* HLA antibodies | 0 | 2 | 0.938† |
| CMV disease | 3 | 6 | 0.999 |

[a]$P < 0.01$ versus 12 month eGFR.
*Mann-Whitney *U* test.
†Fisher exact test.

**Table 6.** Histological outcome of ITG groups.

| | Protocol biopsy (*n* = 6) | For cause biopsy (*n* = 5) |
|---|---|---|
| Time (mo posttransplant) | 12.5 ± 7.0 | 12.2 ± 7.3 |
| g score at 3 month | 1.2 ± 0.4 | 1.4 ± 0.5 |
| Histological outcome | | |
| g0cg0 | 5/6 | 1/5 |
| g > 0 and/or cg>0 | 1/6 | 4/5 |
| Preformed HLA antibodies | 2/6 | 3/5 |
| 1 year HLA antibodies | 2/6 | 2/5 |

g: glomerulitis, cg: allograft glomerulopathy.

## Discussion

Transplant glomerulitis is a well-known feature of AMR. In the context of protocol biopsies of DSA-positive recipients, TG may occur with or without C4d deposition, usually in association with peritubular capillaritis [8,10–13]. This finding is associated with detrimental effect on renal function and is thus considered as subclinical humoral rejection [11,13]. Conversely, the incidence and significance of TG without C4d or any other histological rejection-related changes — defined as isolated TG — has not been studied in a transplant population of non-DSA recipients using protocol biopsies. To address this question, we retrospectively evaluated clinical and immunological features and outcomes of renal transplant recipients with ITG on the 3 month protocol biopsy. Neither the immunological status (number of HLA mismatches and pretransplant non-DSA anti-HLA antibodies) nor the episodes of acute rejection were predictive of ITG occurrence. Graft outcome was not influenced by the presence of an ITG on the 3 month biopsy. Indeed, renal function, defined by serum creatinine level, estimated-GFR, and proteinuria at 3 years was not significantly different between ITG-patients and the control group (although estimated-GFR significantly decreased in both groups from 1 to 3 year post transplantation). However, the ITG-group was heterogeneous and some patients

subsequently experienced renal failure or proteinuria indicating a second graft biopsy for cause, which displayed histological progression of the lesions. The majority (4/5) of these patients were previously sensitized.

Transplant glomerulitis is defined in the Banff 97 classification as 'mononuclear cell infiltrate and endothelial cell enlargement in glomerular capillaries' [3]. It should be stressed that this definition is rather unspecified, as it does not include a minimal quantitative threshold, in contrast with other lesions, such as tubulitis and interstitial inflammation. The question of the specificity of diagnostic criteria for TG has been raised during the Tenth Banff Conference on Allograft Pathology [16]. The conference also highlighted the need for a multicenter trial to re-examine the scoring of glomerular lesions of renal allografts. The lack of accuracy for this item probably accounts for low reproducibility among pathologists in the diagnosis of TG, particularly for the g1 grade [17–19]. To avoid discrepancies in reproducibility, some authors have proposed that only g2 and g3 glomerulitis should be considered with exclusion of the g1 grade [19,20]. In our series, we accepted a diagnosis of TG if mononuclear inflammatory cells and endothelial cells occluded the glomerular capillary lumen. This criterion, previously used by others [20–22], has recently been shown to correlate with both allograft dysfunction and development of chronic transplant glomerulopathy in a 'for cause biopsy'-based study comparing different diagnostic criteria of TG [23]. In our study, two pathologists interpreted 30% of the biopsy specimens with a perfect interobserver agreement for the diagnosis of glomerulitis.

Glomerular involvement characterized by segmental hypercellularity and obliteration of capillary lumens — thus morphologically indistinguishable from rejection-related TG — has also been described in association with CMV infection [24–26]. To assess the influence of CMV in the pathogenesis of glomerular inflammation, we aimed to analyze the role of CMV infection in the study population. The incidence of CMV infection was similar in the two groups and our results do not therefore indicate a detrimental role for CMV in mediating glomerular inflammation in the ITG-group.

In renal allograft biopsies for causes, TG generally occurs in the context of AMR [6,27–29]. In early protocol biopsies, the incidence and the significance of ITG as currently defined (i.e. TG without C4d deposition or any other morphologic evidence of rejection), has not been determined in non-DSA recipients. To properly determine that TG was not associated with C4d deposition, we increased the detection sensitivity of C4d by using immunofluorescence and immunoperoxydase methods. In our transplant center, among 476 renal transplant recipients, the incidence of ITG was 4.2% on the 3 month protocol allograft biopsy. This incidence is lower than that for DSA recipients in previous reports where glomerulitis occurred in 13 to 49% of early protocol biopsies of these patients [10–12]. In these reports, TG in DSA recipients was usually associated with peritubular capillaritis [10–12].

In the present study, the absence of immunological risk factors (pretransplant anti-HLA antibodies and acute rejection episodes) suggests that ITG is not always related to a rejection process. This hypothesis is consistent with transcriptome-based analyses of renal transplant biopsy specimens that showed that ischemia or infection may mediate endothelial molecular alterations occurring in the absence of alloantibodies [30]. Therefore, ITG could reflect, at least in some cases, a microcirculatory injury (e.g. ischemia-reperfusion or microthrombosis) due to a non-specific inflammatory reaction.

Transplant glomerulitis is considered to precede the development of chronic transplant glomerulopathy, which is typically associated with decreased allograft function and proteinuria [7,20,22,31–33]. However, the favorable 3 year outcome of the ITG-group may argue against a progression to chronic rejection in these patients. Our study is nevertheless limited by the small number of patients, the short-term follow-up, and the absence of a systematic control biopsy. Indeed, only 11 of the 20 ITG-patients experienced allograft biopsies during the follow-up period, either for cause or for glomerulitis monitoring. Glomerular lesions disappeared in six patients, five of which had no pretransplant HLA antibodies. In the other five patients, isolated TG persisted or progressed to features consistent with chronic transplant glomerulopathy; it must be emphasized that 4/5 patients had preformed non-DSA. Furthermore, the patients who displayed detrimental histological evolution seemed to have more severe lesions on the 3 month biopsy, i.e. higher grades of glomerulitis. Altogether, these data could suggest a deleterious role for non-DSA. Indeed, links between non-DSA and lower graft survival, proteinuria [34], and chronic transplant glomerulopathy [35], related to unclear pathophysiological mechanism [36], have been previously reported.

In conclusion, this series is the first study to specifically focus on the significance of ITG in protocol biopsies of non DSA-recipients in the C4d era. Our results suggest that ITG on 3 month systematic transplant biopsies does not seem to influence the 3 year graft outcome in non-sensitized patients. However, this retrospective study is based on a limited number of patients and larger trials are required to determine if modifications of the immunosuppressive regimen are needed in these patients.

## Authorship

DB: designed study, performed study, collected data, analyzed data, wrote the paper. PG and FG: designed study,

wrote the paper. ML: collected data, wrote the paper. FD: collected data. DN: contributed important reagents. MCC: collected data. EB and CN: designed study. MH: designed study, collected data, analyzed data, wrote the paper.

## Funding

The authors of this manuscript have no conflict of interest to disclose.

## Acknowledgements

Part of this work has been presented at the 14th European Society of Organ Transplantation Congress, Paris, 2009.

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