**Analysis of HLA-G expression in renal tissue in Lupus nephritis: a pilot study**

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**ABSTRACT**

**Background.** To investigate whether HLA-G antigen is expressed in the kidneys of patients affected by lupus nephritis (LN) and whether its detection in renal biopsies could be adopted as a marker of treatment response and prognosis.

**Methods.** Thirty renal biopsies from patients with LN were selected and analyzed through immunohistochemistry. Laboratory and clinical data were retrospectively collected at baseline, 6, 12 months and at the latest clinical appointment. 63.3% of patients were treated with Rituximab (RTX) +/- methylprednisolone in the induction phase. The expression of HLA-G in glomeruli, tubules and infiltrating cells was examined and compared between lupus patients who achieved either complete or partial renal response and those who did not respond to treatment.

**Results.** HLA-G staining was observed in the glomeruli of twenty of thirty samples of LN. The expression of the antigen was detected in podocytes, along glomerular capillary walls, on parietal glomerular epithelial cells and within the juxtaglomerular apparatus. 70% of patients whose glomeruli expressed HLA-G achieved partial or complete response at 6 months and 75% at the latest available follow up compared with 30% and 40%, respectively, of those who did not show any expression. The pattern of staining in tubules and infiltrating cells was highly variable precluding any clinical correlation.

**Conclusion.** This study demonstrates that HLA-G is expressed in renal tissue in LN. Our retrospective data suggest that its expression could correlate with response to treatment.

**Key words:** Systemic Lupus Erythematosus, HLA-G, Lupus nephritis, Rituximab, immunohistochemistry

## INTRODUCTION

Lupus nephritis (LN) is an organ-threatening complication of Systemic Lupus Erythematosus (SLE) which may develop in 30-60% of patients. The identification of novel molecules involved in the immunopathogenesis of SLE might help to both predict the treatment response and select an adequate treatment. Several studies have looked for a possible role for human leukocyte antigen-G (HLA-G) in SLE [1-10].

HLA-G is a major histocompatibility complex class I antigen encoded on chromosome 6p21.3. It belongs to the non-classical HLA-class I (or class Ib) genes that are characterized by a restricted tissue distribution and limited polymorphism in the coding region. In physiological conditions HLA-G is expressed in cytotrophoblast, cornea, thymus, erythroid and endothelial precursors, T-cells, antigen-presenting cells (APCs) and mesenchymal stem cells (MSCs) [11-15]. In some pathological contexts (e.g. viral infections, cancers, transplantation, inflammatory and autoimmune diseases) the production of the antigen is induced by stimuli such as interferon (IFN)-α, -β, and –γ, interleukin (IL)-10 and other soluble factors [16].

By alternative splicing of primary transcript seven isoforms of HLA-G are produced: four membrane-bound (HLA-G1, -G2, -G3, -G4) and three soluble (HLA-G5, -G6, -G7) forms [17]. It has been suggested that HLA-G gene could be implicated in SLE within the chromosome 6-associated region [7]. The different polymorphisms that influence HLA-G expression have been variously associated with the development of SLE, but the mechanism of these associations remains unclear [7]. A recent study using mouse transplantation models showed that administration of microbeads coated with HLA-G5-β2m attenuated LN through the inhibition of dendritic cell maturation and B1 cell depletion [18]. HLA-G expression has also been demonstrated in kidneys in a significant proportion of renal cancers [19], where it is involved in the inhibition of the immune response against cancer cells. It is also detected in renal transplants [20], where high levels of the antigen

are a marker of allograft acceptance. HLA-G also acts as an immunomodulatory molecule at the maternal-fetal interface, preserving tolerance and suppressing inflammation [21].

In this study we investigated the expression of HLA-G in LN and its relationship to treatment response.

## PATIENTS AND METHODS

*Preliminary analysis:* In order to evaluate whether the expression of HLA-G could be detected in renal tissue from biopsies of patients with LN, we conducted a preliminary analysis at the Rheumatology Unit of S. Anna Hospital in Ferrara (Italy), in collaboration with the Department of Medical Sciences, Section of Microbiology and Medical Genetics, and Section of Pathology, University of Ferrara, Italy. We analysed nineteen samples from patients with LN (mainly class IV and V) and fourteen samples of unaffected renal tissue obtained from nephrectomies of patients with renal cancer or with traumatic injury.

The specimens were stained manually using an anti-HLA-G primary monoclonal antibody (4H84 Exbio), diluted 1:50, or the isotype-matched IgG1 antibody (Santa Cruz), with the ABC Detection kit (Leica Biosystems, Newcastle, UK). Cytotrophoblast from first trimester of human placenta was used as a positive control.

To obtain larger sample size analysis, we subsequently utilised the Bond automated staining system in collaboration with Imperial Lupus Centre, Imperial College Healthcare NHS Trust (ICHNT).

*Patients:* Thirty kidney biopsies were selected from 30 LN patients enrolled at the Imperial Lupus Centre, Imperial College Healthcare NHS Trust (ICHNT). Informed consent was obtained according to the Declaration of Helsinki. Ethical approval was obtained from the ICHNT Tissue Bank (NRES approval 12/WA/0196, R14042). All patients met the following

inclusion criteria: age >18 years; diagnosis of SLE according to the revised American College of Rheumatology criteria [22] and/or the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [23]; and a minimum follow up period of 12 months. The renal samples were obtained from the first biopsy or from biopsies performed at the time of a renal flare (mean SELENA-SLEDAI score 8.3 ± 3.4). LN was classified using the International Society of Nephrology/Renal Pathology Society criteria [24]. Patients whose biopsies showed high degree of sclerosis and tubular atrophy were excluded from the study. Control renal tissue included sections from non-SLE renal diseases (thin basement membrane nephropathy, TBM). Clinical data was collected at baseline, +6 and +12 months after the renal biopsy. We also included data from the most recent clinical assessment. The demographic features of study population are reported in Table 1**.** The treatment response was defined as follows: Complete renal response (CR): urine protein:creatinine ratio (uPCR) ≤50 mg/mmol in a spot urine and eGFR ≥60 ml/min; or if ≤60 ml/min at baseline, not fallen by >20% compared to baseline. Partial renal response (PR): eGFR no more than a 20% decrease from the baseline value; and if not nephrotic at baseline (uPCR <300 mg/mmol) 50% improvement in spot uPCR; or if nephrotic at baseline (uPCR >300 mg/mmol), 50% improvement in spot uPCR and uPCR <300 mg/mmol. Non-response (NR) was used if neither CR nor PR was achieved.

*Immunohistochemistry:* Sections (4 micron) were cut from paraffin-embedded renal biopsy blocks. The samples were stained with a Bond automated system which utilizes controlled polymerization technology and Leica-BondTM Polymer Refine Detection system. Heat-induced retrieval system and the same primary monoclonal antibody used for the manual staining (anti-HLA-G, 4H84 Exbio) diluted 1:50, were adopted. Sections of placental tissue were stained as positive controls and an isotype-matched IgG1 (Santa Cruz) antibody was used under similar conditions to control for non-specific staining. 4H84 moAb is able to

recognize all the b-2-microglobulin associated HLA-G isoforms, including the G1 and G5 isoform, known to exert the major biological functions. The slides were observed under an Olympus light microscope. In light microscopy, positive staining appears brown. The intensity of staining was graded from "-" to "+++". A score of "+++" represented intensity comparable to the staining of the first-trimester cytotrophoblast sections. A score of "-" was comparable to the staining of the corresponding negative control using isotype-matched IgG1. The scores "+" and "++" were increasing gradations between "-" and "+++" scores. The degree of interstitial inflammation was scored using a similar semi-quantitative scale. The renal pathologist who interpreted HLA-G staining results was blinded to the diagnostic categories. The IHC protocol performed in this study used the 4H84 antibody, that is able to recognize all the b-2-microglobulin associated HLA-G isoforms, both soluble and membrane bounded. Furthermore, there is a permeabilization step in the protocol that allows to stain not only the membrane-bound HLA-G, but also the soluble isoform that is stored in the cytoplasm endosomes.

*Statistical analysis:* Results were expressed as mean with standard deviation (SD) or percentages. Categorical variables were compared using Fisher's exact test while comparisons between the median values of two defined patient groups were performed using Mann-Whitney U test. $p<0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA).

## RESULTS

**Preliminary analysis using manual staining on renal biopsy tissue from the Italian cohort.** Positive staining was observed in tubules and interstitium, but not in glomeruli of

fifteen LN biopsies, whilst four samples were completely negative. The higher positivity for HLA-G expression was correlated to the presence on inflammation (Figure 1). We found only one specimen that was HLA-G positive without inflammation. Renal tissue from nephrectomies were all negative, despite a variable degree of inflammation in the tubulointerstitial compartment (Figure 1).

**Baseline clinical characteristics and HLA-G expression in glomeruli using automated staining on renal biopsy tissue from the UK cohort.** Thirty LN samples were stained and glomerular HLA-G was detected in twenty out of thirty (Table 2 and Figure 2). We observed different staining patterns after immunohistochemistry assay: positive staining in the glomerular capillary walls (n=6); in podocytes (n=12); and in both the capillary walls and podocytes (n=2). Positive HLA-G staining was also observed in parietal glomerular epithelial cells (PECs) of Bowman's capsule in 10 samples. Since PECs can be involved in crescent formation, it was notable that eight of the ten samples were proliferative lesions (class III n=2, class IV n=2, class IV+V n=4), whilst only two were class V LN. Four out of ten patients had low eGFR <59 ml/min and in three serum creatinine was >110 μmol/l.

In five of the thirty samples HLA-G expression was present within the juxtaglomerular apparatus (JGA) (Figure 2D). These five lesions comprised class V (n=2), class III+V (n=2) and one class III. Two samples did not show any staining in other parts of the glomeruli. Two samples had a positive staining in podocytes and one in PECs, but none in the capillary walls. In four patients the renal function was normal, while one patient presented with acute kidney injury.

**HLA-G expression in tubules and infiltrating cells.** HLA-G staining was detected in tubules (Figure 3C-D) in all specimens of LN and in some infiltrating cells in the

tubulointerstitial space (Figure 3A-B). In these sites different cells can express HLA-G. These include distal [20] and proximal tubule epithelial cells [25], infiltrating cells such as CD4+ and CD8+ T cells [26], monocytes, DC-10 cells. Furthermore, other cells could acquire HLA-G expression through trogocytosis [4]. Different patterns of tubular staining were observed (Figure 3C-D). As shown in figure 2C-D, in some samples the staining of tubules resulted focal and in others diffuse. Moreover, some samples had a uniform staining while in others it was heterogeneous. Again, the staining in the tubular epithelial cells had different aspects and distribution. This included granular or uniformly diffuse patterns, perinuclear or cytoplasmic staining (Figure 3C) and staining within the brush border of the proximal tubules. 75% of samples of class V had diffuse staining in tubules (Figure 3D), with weak and moderate intensity. In contrast, class III, IV, III+V and IV+V had a more frequent irregular tubular staining. Due to the extreme variability of the patterns of HLA-G expression in tubules, potential correlations with clinical data and outcome were not investigated. Several studies have demonstrated a correlation between the severity of tubulointerstitial inflammation, kidney damage and risk of renal failure [27]. Since HLA-G can be expressed on inflammatory cells, biopsies with tubulointerstitial cell aggregates were assessed. Although a higher intensity of staining in infiltrating cells was frequently observed in samples with a concurrent diffuse staining in glomeruli and tubules, there were also some sections with rich interstitial inflammatory infiltrates which did not display any expression of HLA-G (Figure 3B).

**HLA-G expression in other renal biopsies.** Nine biopsies from patients with TBM were analysed. One sample did not contain glomeruli. Three TBM samples showed weak staining in podocytes and three showed moderate staining in the glomerular capillary walls while two did not show any glomerular staining. The expression of the antigen in tubules was variable (data not shown). We found no correlation with the inflammation status.

**Correlation of clinical characteristics and glomerular HLA-G staining.** We next looked for associations between clinical parameters and glomerular HLA-G staining (Table 2**)**. All the patients in the HLA-G positive group were female, while there were two males in the negative group. Due to the small number of subjects belonging to ethnical groups other than Caucasians, the influence of ethnicity on HLA-G production was not investigated. No significant differences were seen between the two groups in terms of renal function at the time of the renal biopsy. Although proteinuria was greater in the HLA-G positive group, this did not reach statistical significance. HLA-G expression was detected in the glomeruli of 87.5% (7/8) of patients with pure class V and in 50% of patients with pure proliferative GN (class III n=2/4; class IV n=3/6).

Treatments between the two groups were similar (Table 2). In the HLA-G positive group twelve patients were treated with Rituximab (RTX) +/- methylprednisolone (MP) followed by mycophenolate mofetil (MMF); two patients were treated with RTX + MP followed, respectively, by methotrexate (MTX) and by hydroxychloroquine (HCQ), two with MMF + steroids, two with RTX + MP alone, two with cyclophosphamide (CYC) according to the Eurolupus protocol (one after an infusion reaction to RTX). In the HLA-G negative group three patients were treated with RTX +/- MP followed by MMF; one patient with RTX + MP followed by Azathioprine (AZA); two with Tacrolimus + MTX or MMF; two with MMF + steroids and two with CYC followed by MMF (one after 1 cycle of RTX). The rituximab regimen was RTX (1 g) and MP (500 mg) on days 1 and 15 [28]; MP was omitted in those cases where concomitant oral prednisolone was given.

At 6 months post-renal biopsy (T6) the percentage of patients classified as non-responders was lower in the HLA-G positive group (30%, n=6/20) compared with the

negative group (70%, n=7/10, p=0.05). This difference persisted when data were analysed at the last available follow up (25% vs 60%, data not shown). 70% of patients in the HLA-G positive group achieved a partial or complete response at 6 months compared with 30% in the negative group (p=0.05).

## DISCUSSION

Previous studies have shown a potential role of HLA-G in pathogenesis and susceptibility to SLE with contrasting results [7]. This might reflect the different genetic background of the study populations and/or to the lack of a standardised method to measure the soluble levels of the antigen. The role of cell membrane-bound HLA-G in LN is unclear. One mouse study suggested a role of the antigen in suppressing the inflammatory process in kidney, thereby improving renal function [18]. We have demonstrated that HLA-G is expressed in a significant proportion of kidney biopsies from LN patients in glomeruli (66,7%, n. 20/30). Moreover, the antigen can be produced by infiltrating cells and in some specialized structures, such as the JGA. HLA-G staining was detected in endothelial cells of the capillaries, podocytes and PECs of the Bowman's capsule. These cells probably do not express the antigen in physiological conditions, but it is well known HLA-G expression can be induced by different stimuli. These include hypoxia, indolamine 2,3-dioxygenase (IDO) and some inflammatory mediators that play a central role in LN (IL-10, IFNs) [29]. In particular, it has been shown that IFN-α and IFN-γ are synthesized by both infiltrating and resident renal cells, including glomerular endothelial cells [30].

Despite its protective effect against rejection and cardiac allograft vasculopathy after heart transplantation, it has been demonstrated that HLA-G is not expressed at baseline in vascular endothelial and smooth muscle cells, but it can be induced by exposure to

progesterone [31]. Similarly, we hypothesized that in LN the inflammatory IFN-rich milieu could induce HLA-G production in the glomerular capillary walls. In our study we detected the expression of HLA-G by immunohistochemistry in endothelial cells of six out of thirty LN samples: among these samples, only 1 was a pure class V, while the others were class III (1), IV (1), III+V (2) and IV+V (1). This is consistent with the fact that endothelial cell activation and damage is commonly found in severe proliferative LN where complement activation is localized in the subendothelial space [32]. The HLA-G expression is not modified in control patients, suggesting a different impact of the microenvironment .

Another interesting finding was the detection of HLA-G in the afferent and efferent arterioles in the JGA. This is a specialized structure that plays a central role in the regulation of renal functions and blood pressure through the control of renin release and glomerular hemodynamics. It is possible that this reflects an association between stimuli that regulate the glomerular blood flow and HLA-G expression.

Regarding the epithelial staining, we hypothesized that, similar to endothelial cells, the high level of IFNs and other inflammatory cytokines that characterized the renal environment in LN induced HLA-G expression in podocytes. In a study based on a murine model of necrotizing crescentic glomerulonephritis (NCGN) the authors reported that an *in vitro* IFN-γ treatment stimulated podocyte expression of MHC classes I and II and ICAM-1. This suggested that when podocytes are activated, they participate to the inflammatory process through antigen presentation and expression of adhesion molecules that can promote infiltration of inflammatory cells [33]. In our cohort we have demonstrated the expression of HLA-G in podocytes in 12/30 samples of LN. Six of these were pure class V membranous LN that is mainly characterized by the presence of subepithelial immune deposits and podocyte injury [34].

In ten of thirty samples the production of HLA-G was also detected in PECs. Emerging data suggest that this cell population is directly involved in the pathogenesis of certain glomerular diseases, such as diabetic nephropathy, crescentic glomerulonephritis and focal segmental glomerulosclerosis where increased cellular activity of PECs has been observed [35]. Different signalling pathways are involved in PEC activation. Activation results in increased proliferation, migration and extracellular matrix production [36]. The role of PECs during glomerular inflammation is still complex. They can proliferate obstructing the urine flow resulting in an impairment in the glomerular function. They can also have a reparative and regenerative role because they are able to migrate from Bowman's capsule to the capillary tuft and replace lost podocytes [37,38]. In NCGN PECs are involved in cellular crescent formation. Crescents are a typical feature of proliferative GN and are due to the accumulation of PECs, podocytes and infiltrating macrophages within Bowman's space. PECs that form cellular crescents may undergo epithelial-to-mesenchymal transition [39]. Since HLA-G can be expressed in both epithelial and MSCs [15], it would be interesting to understand if it has a role in these processes.

The expression of HLA-G has also been identified in aggregates of infiltrating cells in the periglomerular and peritubular interstitial space. We observed patchy and weak staining in some samples, diffuse and strong in others. This could be due to different cell populations (CD4+ and CD8+ T cells, monocytes, DCs) that form the aggregates and/or to different cytokine patterns produced locally. The precise mechanisms regulating the expression of the antigen in the renal tissue in LN remain to be elucidated. Both genetic (polymorphisms in the promoter and in the 3' untranslated region) and non-genetic factors, such as medications (steroids, methotrexate, cyclosporine) may be involved [40-42]. One limitation of this research was the inability to genotype the samples for HLA-G polymorphisms

implicated in HLA-G expression. Further studies are necessary to elucidate the clear mechanisms at the basis of the differential HLA-G expression in LN patients.

Notable, high levels of sHLA-G or of its transcripts in renal tissue are considered a good prognostic factor of renal allograft acceptance [20] and they seem to have a protective role against the development of LN. In fact, in a recent study investigating the upregulation of T regulatory cells in SLE by MSCs, lower levels of sHLA-G were found in lupus patients with nephritis compared to those without renal involvement [43]. Because of the variability in tubular staining, we focused on correlations between glomerular expression and treatment outcome. Interestingly, 70% of patients who did not show any glomerular HLA-G expression were classified as non-responders at 6 months compared with 30% in the positive group.

The study has limitations with respect to performing a clinic-pathological analysis: small sample size, different distribution of proliferative and membranous GN between the two groups, possible influence of medications at baseline and different induction therapies. Due to the retrospective nature of the study, the potential effect of genetic polymorphisms on HLA-G expression and the levels of sHLA-G at the time of renal biopsy were not analysed. Due to the small number of subjects belonging to different ethnic groups, we did not analyse the impact of ethnicity on HLA-G expression in kidneys and renal response.
To our knowledge there are no previous studies investigating the expression of HLA-G through immunohistochemistry in renal tissue of human LN.

In conclusion, we have demonstrated the diversity and extent of HLA-G renal staining in a group of LN patients. Our retrospective data suggest that glomerular HLA-G staining in LN may associate with treatment outcome. The limited number of patients unable a significant

analysis on the impact of the therapy on HLA-G expression. This explain our decision to point our attention on therapy outcome in 6 months treatment, independently from the medical regimens that has been followed. Prospective studies with uniform treatment protocols should be performed to determine if glomerular HLA-G staining has prognostic utility in LN. A confirm of these prelinimary data might suggest the HLA-G staining during the routine automated IHC protocols that are commonly used for LN diagnosis, to identify a priori the treatment outcome. This could facilitate a more personalized and efficient therapeutical approach.

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The data are available upon request.

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## CONFLICT OF INTEREST STATEMENT

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## TABLES

### Table 1. Cohort characteristics at the time of the renal biopsy (n=30)

| **Characteristics** (T0) | **Value** |
|---|---|
| Age (years) | 39 (±14.1) |
| Caucasian (%) | 30% (9/30) |
| African (%) | 13.33% (4/30) |
| Afro-Caribbean (%) | 16.67% (5/30) |
| Asian – Indian subcontinent (%) | 16.67% (5/30) |
| Oriental (%) | 6.67% (2/30) |
| Mixed (%) | 16.67% (5/30) |
| Gender (F/M) | 28/2 |
| SLE duration (months) | 116.9 (±115.9) |
| LN duration (months) | 78.3 (±90) |
| Lupus nephritis class[1]: | |
| III | 13.33% (4/30) |
| IV | 20% (6/30) |
| V | 26.67% (8/30) |
| III+V | 20% (6/30) |
| IV+V | 20% (6/30) |
| uPCR (mg/mmol) | 404.6 (±385.6) |
| eGFR (ml/min) | 72.4 (±20.6) |
| Creatinine level (µmol/l) | 86.1 (±36.7) |
| Albumin (g/l) | 26.6 (±7.4) |
| Serum C3 (g/l) | 0.8 (±0.3) |
| Serum C4 (g/l) | 0.2 (±0.1) |
| Anti-dsDNA positivity (%) | 73.33% (22/30) |
| SELENA-SLEDAI (mean±SD) | 8.3±3.4 |
| Medications (T0) | |
| CYC | 3.3% (1/30) |
| MMF | 60% (18/30) |
| Tac | 3.3% (1/30) |
| MTX | 10 % (3/30) |
| AZA | 10 % (3/30) |
| HCQ | 63.3% (19/30) |
| RTX (<6 months) | 10% (3/30) |
| Steroids | 36.7% (11/30) |

F – female; M – male; uPCR - urine protein to creatinine ratio; eGFR- estimated glomerular filtration rate; CYC - cyclophosphamide; MMF - mycophenolate mofetil; MTX - methotrexate; Tac – tacrolimus; AZA - azathioprine; HCQ - hydroxychloroquine; RTX - rituximab; LN – lupus nephritis; SELENA-SLEDAI - Estrogen in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index . [1] LN was classified using the International Society of Nephrology/Renal Pathology Society criteria.

### Table 2. Cohort characteristics (T0) and glomerular HLA-G expression

| | **HLA-G positive** 66.7% (20/30) | **HLA-G negative** 33.3% (10/30) | P value |
|---|---|---|---|
| Sex (number, M/F) | 0/20 | 2/8 | 0.10 |
| Caucasian/other ethnicities | 8/12 | 1/9 | 0.20 |
| Age (years) | 40 ±15 | 37 ±12.4 | 0.71 |
| SLE duration (months) | 109.7 ±110.7 | 131.4 ±130.7 | 0.67 |
| LN duration (months) | 85.9 ±103.2 | 63 ±57.1 | 0.99 |
| Lupus nephritis class[1]: | | | |
| III | 10% (2/20) | 20% (2/10) | |
| IV | 15% (3/20) | 30% (3/10) | |
| V | 35% (7/20) | 10% (1/10) | |
| III+V | 20% (4/20) | 20% (2/10) | |
| IV+V | 20% (4/20) | 20% (2/10) | |
| uPCR (mg/mmol) | 462 ±440.5 | 289.8 ±218 | 0.31 |
| Serum creatinine (µmol/l) | 83.75 ±36.96 | 90.7 ±37.7 | 0.66 |
| eGFR (ml/min) | 72.75 ±20.56 | 63.7 ±21.8 | 0.88 |
| Albumin (g/l) | 26.7 ±7.2 | 26.4 ±8.1 | 0.72 |
| Serum C3 (g/l) | 0.8 ±0.3 | 0.9 ±0.4 | 0.22 |
| Serum C4 (g/l) | 0.2 ±0.1 | 0.3 ± 0.2 | 0.06 |
| Corticosteroids at T0 | 30% (6/20) | 50% (5/10) | 0.42 |
| Prednisone dosage (mg/day) | 2.75 ±5.25 | 7.2 ±10.9 | 0.16 |
| RTX 6 months before | 1/20 | 2/10 | 0.25 |
| Treatment at T0: | | | |
| CYC | 0% | 10% (1/10) | |
| MMF | 60% (12/20) | 60% (6/10) | |
| AZA | 10% (2/20) | 10% (1/10) | |
| Tacrolimus | 5% (1/20) | 0% | |
| MTX | 10% (2/20) | 10% (1/10) | |
| HCQ | 55% (11/20) | 80% (8/10) | |
| Treatment at T6: | | | |
| RTX + MP + MMF | 60% (12/20) | 30% (3/10) | 0.24 |
| RTX + MP + MTX/AZA/HCQ | 10% (2/20) | 10% (1/10) | >0.99 |
| MMF + corticosteroids | 10% (2/20) | 20% (2/10) | 0.58 |
| CYC | 10% (2/20) | 20% (2/10) | 0.58 |
| MMF/MTX + Tac | 0% (0/20) | 20% (2/10) | 0.10 |
| RTX+MP | 10% (2/20) | 0% (0/10) | 0.54 |

**Table 2. Continued**

| | | | |
|---|---|---|---|
| Response to treatment at T6: | | | |
| Complete response | 25% (5/20) | 20% (2/10) | >0.99 |
| Partial response | 45% (9/20) | 10% (1/10) | 0.10 |
| Complete + partial response | 70% (14/20) | 30% (3/10) | 0.05 |
| Non-response | 30% (6/20) | 70% (7/10) | 0.05 |

Values are mean +/- standard deviation or % with numbers. T0 – time of renal biopsy, T6 – 6 months post-renal biopsy. uPCR - urine protein to creatinine ratio; eGFR- estimated glomerular filtration rate; CYC - cyclophosphamide; MMF - mycophenolate mofetil; MTX - methotrexate; Tac – tacrolimus; AZA - azathioprine; MP – methylprednisolone; HCQ - hydroxychloroquine; RTX - rituximab; LN – lupus nephritis. [1] LN was classified using the International Society of Nephrology/Renal Pathology Society criteria. P values were derived using Mann-Whitney U test and Fisher's exact test. Response to treatment was determined as described in the materials and methods section.

## LEGENDS TO FIGURES

**Figure 1. Correlation between HLA-G positivity and inflammation.** HLA-G positivity in LN and control (CTR) renal biopsies was defined as high (3), medium (2), low (1) or absent (-1) in inflamed (red spots) or not inflamed (black spots) samples.

**Figure 2. Glomerular HLA-G immunostaining in lupus nephritis.** Examples of staining of HLA-G (4H84 mAb; A-D) and isotype-matched IgG1 antibody (a-d) in different cell populations in the glomeruli. (A) HLA-G staining in capillary walls in class III LN; (B) HLA-G staining in podocytes in class V LN; (C) HLA-G staining in podocytes and PECs (arrow) in class III LN; (D) HLA-G staining in the juxtaglomerular apparatus (arrow) in class V LN. PECs – parietal epithelial cells; LN – lupus nephritis. Original magnification 200x.

**Figure 3. HLA-G staining of infiltrating cells and tubules**. The 4H84 mAb enabled the detection of variable degree and distribution of HLA-G staining in infiltrating cells (A-B) and tubules (C-D), while no staining was observed in the same structures with the isotype-matched IgG1 antibody (a-d): (A) cells of a large interstitial cell aggregate are strongly and diffusely stained in a sample of

class III+V LN; (B) isolated cells are labelled in a big follicular aggregate in class IV+V lupus LN. Different patterns of staining were detected in tubules: (C) cytoplasmic staining in class III+V LN; (D) diffuse staining in class V LN. LN – lupus nephritis. Original magnification 200x.