Identification of clinical and serological factors during induction treatment of lupus nephritis that are associated with renal outcome

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ABSTRACT

Objective: To identify factors associated with clinical outcome in patients with lupus nephritis.

Methods: Data from the Aspreva Lupus Management Study (ALMS) were analysed. Using multivariate analysis, we assessed the prognostic value of demographic, clinical, laboratory and histopathological features on the frequency of either complete remission (CR) or treatment failure (TF) during the maintenance phase.

Results: Among the 370 subjects who entered the trial (complete population), non-Hispanic ethnicity was associated with a higher likelihood of CR (OR=2.0). Several factors were independently associated with a greater likelihood of TF, including: (1) anti-double-stranded DNA (anti-dsDNA) at trial entry (OR=12.7), (2) failure to reduce anti-dsDNA within 8 weeks (OR=2.9), and (3) failure to reduce urine protein:creatinine ratio (UP/C) by ≥25% within 8 weeks (OR=2.6). Among the 227 subjects who entered the maintenance phase (maintenance population), baseline estimated glomerular filtration rate (eGFR) ≥90 mL/min/1.73 m² was associated with a greater likelihood of CR (OR=2.0), and UP/C ≤1 at the end of induction was associated with a lower likelihood of CR (OR=0.3). Induction treatment with intravenous cyclophosphamide (IVC) was associated with a lower likelihood of CR (OR=0.5), while lack of treatment with antimalarials (OR=2.4), failure to reduce anti-dsDNA during the first 8 weeks of induction (OR=3.5), failure to reduce UP/C during the first 8 weeks of induction (OR=2.1) and anti-dsDNA positivity at the end of induction (OR=8.3) were independently associated with a greater likelihood of TF.

Conclusions: This analysis demonstrates that levels of anti-dsDNA and UP/C during induction treatment are independently associated with renal outcome over the ensuing 3 years in both the complete and maintenance populations. Ethnicity is associated with renal outcome in just the complete population, and eGFR, induction treatment and treatment with antimalarials are associated with renal outcome in just the maintenance population.

KEY MESSAGES

▸ The complexity and morbidity associated with lupus nephritis underscore the importance of determining factors that predict response to therapy.

▸ Among patients who continued into the maintenance phase of ALMS, baseline eGFR >90 mL/min/1.73 m² and UP/C <1 at the end of induction were independently associated with complete remission during the maintenance phase. Induction treatment with intravenous cyclophosphamide was associated with a lower likelihood of treatment failure.

▸ Among patients who continued into the maintenance phase of ALMS, lack of treatment with antimalarials, failure to reduce anti-dsDNA or UP/C within 8 weeks of induction, and positive anti-dsDNA at the end of induction were independently associated with treatment failure.

INTRODUCTION

Lupus nephritis continues to be a major source of morbidity and mortality in patients with systemic lupus erythematosus, with 10%–15% of patients ultimately progressing to end-stage renal disease.1 The variability of treatment regimens and time to treatment response along with the heterogeneous and unpredictable course of lupus nephritis underscore the importance of identifying factors that are associated with response to therapy. We previously identified several clinical and serological characteristics that were associated with response to treatment during the 6-month induction phase of the Aspreva Lupus Management Study (ALMS), a randomised controlled trial of 370 patients with class III–V lupus nephritis.2 Although renal response at 6 months is an important endpoint, there is a greater clinical need to determine factors that are associated with long-term renal outcomes. ALMS provides an excellent opportunity to begin to address this issue because the study included a 36-month maintenance phase that followed completion of induction therapy. The analyses presented here examine factors that are associated with clinical outcome for all 370

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subjects who entered ALMS (complete population) as well as for the 227 subjects who continued into the maintenance phase (maintenance population). Analyses were performed separately within these two populations because the patients comprising the two populations were very distinct. Only those patients who achieved a renal response at the end of the induction phase were eligible to enter into the maintenance phase of the trial.

PATIENTS AND METHODS

Study design

ALMS was a randomised, open-label, multinational, multicentre trial of mycophenolate mofetil (MMF) versus pulse intravenous cyclophosphamide (IVC) for induction therapy of lupus nephritis followed by a randomised, double-blind comparison of MMF versus azathioprine (AZA) for maintenance therapy in those patients who responded to induction. Importantly, subjects who did not meet criteria for renal response at week 24 were withdrawn from the trial. The institutional review boards at each participating centre approved the study. All of the study subjects gave written informed consent prior to randomisation into the study.

Details of the study design have been previously published. In brief, eligible patients had biopsy-proven class III–V lupus nephritis within 6 months of study entry and laboratory tests consistent with active nephritis, including evidence of an active urine sediment, proteinuria ≥1 g/day or elevated serum creatinine (>1.3 mg/dL). Patients with class III or V lupus nephritis were required to have higher levels of proteinuria (≥2 g/day) or serum creatinine >1.3 mg/dL. Only patients who met prespecified criteria for a complete or partial renal response after 24 weeks of induction therapy and were adjudicated as responders by the clinical endpoints committee (CEC) were advanced into the maintenance phase of the trial.

ALMS outcome measures

Renal response at 6 months of the induction phase was defined as a decrease in the urine protein:creatinine ratio (UP/C) on a 24 h collection to <3 in patients with baseline nephrotic-range proteinuria (UP/C >3) or by ≥50% in patients with subnephrotic-range proteinuria and stabilisation or improvement in serum creatinine levels. Complete renal remission in ALMS was defined as UP/C ≤500 mg on a 24 h collection, an inactive urine sediment and serum creatinine level within 25% of baseline. Complete renal remission was assessed at any time during the maintenance phase. Patients might have achieved complete remission at the end induction and remained in complete remission or, alternatively, achieved a partial remission at the end of induction and later developed a complete remission. Renal response (complete or partial) was required for entry into the maintenance phase of the trial. The primary outcome measure in the maintenance phase was the time to treatment failure (TF), defined by any of the following criteria as adjudicated by a CEC: (1) death, (2) end-stage renal disease, (3) sustained doubling of serum creatinine, (4) renal flare, (5) requirement for rescue therapy. The definitions of renal flare were:

- **Proteinuric renal flare**: doubling of the UP/C and proteinuria (≥1 g of protein per 24 h) in patients with urinary protein clearance of ≤0.5 g per 24 h at the end of induction and ≥2 g per 24 h in patients with urinary protein clearance of >0.5 g per 24 h at the end of induction).
- **Nephritic renal flare**: increase of 25% or more in the lowest serum creatinine level during the period from screening to the end of induction plus one or more of the following findings: simultaneous doubling of urinary protein clearance, reaching a minimum of 2 g per 24 h (or the UP/C equivalent); new or increased haematuria (≥5 red cells per high-power field or ≥2+ on a dipstick test for blood); or the appearance of cellular casts.

Baseline factors associated with renal outcome

We analysed several baseline (start of induction) demographic, clinical, serological and histopathological factors to determine whether they were associated with TF or complete renal remission during the maintenance phase. These characteristics included race, ethnicity, age, biopsy class, duration of lupus nephritis, estimated glomerular filtration rate (eGFR), anti-double-stranded DNA (anti-dsDNA) antibody level, C3 and C4 complement levels, 24 h UP/C, induction treatment (IVC or MMF) and use of antimalarials or statins. We examined these factors separately within the complete population and within the maintenance population. The characteristics of the complete and maintenance populations are described in table 1.

Changes in factors during the induction phase

In addition to the baseline variables described above, we sought to determine if changes in biological factors during the induction phase were associated with TF or complete renal remission during the maintenance phase. We examined reduction in proteinuria, reduction in anti-dsDNA levels and improvement in C3 and C4 from baseline to week 8 in both the complete and maintenance populations. We chose the time point of week 8 for two reasons: (1) we wanted to understand whether changes in biological factors very early in the induction period were associated with renal response during the maintenance phase, and (2) we wanted to be consistent with the methodology of our original manuscript in which we demonstrated that normalisation of complement by week 8 and/or reduction in proteinuria by week 8 were associated with renal response at the end of induction at week 24. In the analysis of the maintenance population, we also assessed anti-dsDNA positivity, eGFR, proteinuria and complement levels at the end of induction. Normalisation of complement was defined as a C3...
level of $<90$ mg/dL or a C4 level of $<16$ mg/dL at baseline with the corresponding week 8 value being $\geq 90$ mg/dL (C3) or $\geq 16$ mg/dL (C4). Reduction in proteinuria was defined as a decrease of $\geq 25\%$. Reduction in anti-dsDNA was defined as a decline to $\leq 60$ IU/mL for subjects with baseline anti-dsDNA of $>200$ IU/mL or to $\leq 30$ IU/mL for subjects with baseline anti-dsDNA of $>30$ IU/mL and $\leq 200$ IU/mL.

### Statistical methods

The number and percentage of successes and failures were calculated for each level of each covariate for both treatment outcome variables along with univariate ORs, 95\% CIs and p values. These univariate analyses were performed on all the patients randomised into the ALMS (complete population, n=370) and for all the patients who finished the induction phase and entered the

| Table 1 Demographic and disease characteristics (populations: complete ITT population and maintenance population) |
|-------------------------------------------------------------|
| **Complete population (N=370) n (%)** | **Maintenance population (N=227) n (%)** |
| Race | | |
| Caucasian | 370 (100.0) | 227 (100.0) |
| Black | 147 (39.7) | 99 (43.6) |
| Asian | 123 (33.2) | 76 (33.5) |
| Other | 54 (14.6) | 29 (12.8) |
| Ethnicity | | |
| Hispanic | 370 (100.0) | 227 (100.0) |
| Non-Hispanic | 131 (35.4) | 77 (33.9) |
| Age at randomisation (years) | | |
| $\leq 20$ | 50 (13.5) | 35 (15.4) |
| $>20$ and $\leq 30$ | 124 (33.5) | 73 (32.2) |
| $>30$ and $\leq 40$ | 120 (32.4) | 77 (33.9) |
| $>40$ | 76 (20.5) | 42 (18.5) |
| Biopsy class | | |
| III/IV | 370 (100.0) | 227 (100.0) |
| III/V or IV/V | 260 (70.3) | 169 (74.4) |
| V | 50 (13.5) | 23 (10.1) |
| Lupus nephritis duration (years) | | |
| $\leq 1$ | 236 (63.8) | 155 (68.3) |
| $>1$ and $<5$ | 69 (18.7) | 31 (13.7) |
| $\geq 5$ | 65 (17.6) | 41 (18.1) |
| eGFR baseline (mL/min/1.73 m$^2$) | | |
| $<90$ | 188 (56.6) | 131 (57.7) |
| $\geq 90$ | 144 (43.4) | 96 (42.3) |
| Anti-dsDNA baseline (IU/mL) | | |
| Negative ($<30$) | 325 (100.0) | 222 (100.0) |
| Positive ($\geq 30$) | 14 (4.3) | 14 (6.3) |
| C3 baseline (mg/dL) | | |
| $\geq 90$ | 328 (100.0) | 224 (100.0) |
| $<90$ | 80 (24.4) | 50 (22.3) |
| C4 baseline (mg/dL) | | |
| $\geq 90$ | 248 (75.6) | 174 (77.7) |
| $<90$ | 111 (33.9) | 70 (31.4) |
| UP/C baseline | | |
| $\leq 1$ | 216 (66.1) | 153 (68.6) |
| $>1$ and $\leq 3$ | 53 (16.3) | 33 (14.8) |
| $>3$ | 123 (37.8) | 88 (39.5) |
| Antimalarial treatment | | |
| Concomitant treatment with anti-malarials | 370 (100.0) | 227 (100.0) |
| No antimalarial treatment | 127 (34.3) | 84 (37.0) |
| Lipid-modifying agent treatment | | |
| Statin | 370 (100.0) | 227 (100.0) |
| No statin | 85 (23.0) | 52 (22.9) |
| Induction treatment | | |
| MMF | 370 (100.0) | 227 (100.0) |
| IVC | 185 (50.0) | 120 (52.9) |

Anti-dsDNA, anti-double-stranded DNA; eGFR, estimated glomerular filtration rate; ITT, intention to treat; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; UP/C, urine protein:creatinine ratio.
Association of baseline factors with complete renal remission or TF

Several baseline factors at the start of induction were independently associated with complete renal remission or TF during the maintenance phase (Table 5). In the complete population of 370 patients, non-Hispanic ethnicity was the only baseline characteristic associated with complete renal remission. A total of 40% of non-Hispanic patients achieved renal remission compared with 32% of Hispanic patients (OR 2.0, 95% CI 1.2 to 3.3, p=0.0061). Also among the complete population, the presence of anti-dsDNA was the only baseline factor that was independently associated with TF. Only 7% (1/14) of patients who were negative for anti-dsDNA at the start of induction became TFs during maintenance therapy, compared with 50% of patients who were anti-dsDNA positive (OR 12.7, 95% CI 1.6 to 101.9, p=0.0167). Within the maintenance population of 227 patients, baseline eGFR ≥90 mL/min/1.73 m² was independently associated with complete renal remission (65% vs 58%, OR 2.0, 95% CI 1.0 to 3.8, p=0.0407). Also within the maintenance population, induction treatment with IVC and treatment with antimalarials were independently associated with reduced risk of TF. A total of 19.6% of patients induced with IVC were TFs during the maintenance phase, compared with 28.3% of patients induced with MMF (OR 0.5, 95% CI 0.2 to 1.0, p=0.05). Patients who were not treated with antimalarial agents during induction were more likely to be TFs during the maintenance period (26% vs 21%, OR 2.4, 95% CI 1.1 to 5.1, p=0.0209).

None of the other baseline characteristics examined was independently associated with either complete renal remission or TF during the maintenance phase. These characteristics included race, age, duration of lupus nephritis, biopsy class, C3 concentration, C4 concentration, UP/C and statin use. Although not achieving statistical significance in the multivariate model, biopsy class was associated with complete remission and TF in the univariate analysis. Among the complete population of 370 patients (Table 2), mixed proliferative and membranous biopsy were negatively associated with complete renal remission in that only 22% of patients with mixed class biopsy achieved complete renal remission versus 40% of the pure class III or IV patients (OR 0.4, 95% CI 0.2 to 0.8, p=0.0161). A total of 70% of patients with biopsies containing mixed proliferative and membranous features (III/V or IV/V) became TFs, compared with 50% of patients with pure class III or IV (OR 2.4, 95% CI 1.2 to 4.5, p=0.0095). Lastly, in the univariate analysis of the maintenance population, patients with baseline age ≥40 years were less likely to be TFs during the maintenance period. A total of 14% of patients >40 years versus 40% of patients ≤20 years were TFs during maintenance (OR 0.3, 95% CI 0.1 to 0.7, p=0.0133). These comparisons did not achieve statistical significance in the multivariate analysis.

**RESULTS**

The ALMS demonstrated that there was no difference in efficacy between MMF and IVC for the induction treatment of lupus nephritis at 24 weeks.3 However, during the subsequent 36-month maintenance phase, MMF was shown to be superior to AZA in preventing TF in patients who initially responded to induction therapy.5

Tables 2 and 3 show the results of the univariate analyses for the baseline characteristics examined. Table 4 shows the results of the univariate analyses of clinical changes that occurred during induction therapy. Table 5 shows the results of the multivariate analysis.
Changes in biological parameters during the induction phase and association with renal outcome in the maintenance phase

Changes in anti-dsDNA

Failure to reduce anti-dsDNA by week 8 of induction therapy was associated with TF during the maintenance phase in the multivariate analysis of both the complete and maintenance populations (Table 5). Among patients in the complete population who did not have an early reduction in anti-dsDNA by week 8, 50% developed TF compared with 27% who did have an early reduction in anti-dsDNA.

Table 2 Univariate baseline parameters associated with renal outcome (population: complete population (n=370))

| Treatment failure | Complete renal remission |
|-------------------|--------------------------|
| n (%)             | OR (95% CI)              | p Value | n (%)             | OR (95% CI)              | p Value |
| Race              |                          |         |                   |                          |         |
| Caucasian (ref)   | 75 (51.0)                |         | 59 (40.1)         |                          |         |
| Black             | 31 (67.4)                | 2.0 (1.0 to 4.0) | 0.0538 | 13 (28.3)         | 0.6 (0.3 to 1.2) | 0.1487 |
| Asian             | 62 (50.4)                | 1.0 (0.6 to 1.6) | 0.9200 | 49 (39.8)         | 1.0 (0.6 to 1.6) | 0.9602 |
| Other             | 30 (55.6)                | 1.2 (0.6 to 2.2) | 0.5685 | 17 (31.5)         | 0.7 (0.4 to 1.3) | 0.2634 |
| Ethnicity         |                          |         |                   |                          |         |
| Hispanic (ref)    | 78 (59.5)                |         | 42 (32.1)         |                          |         |
| Non-Hispanic      | 120 (50.2)               | 0.7 (0.4 to 1.1) | 0.0858 | 96 (40.2)         | 1.4 (0.9 to 2.2) | 0.1238 |
| Age at randomisation (years) | | | | | |
| ≤20 (ref)         | 29 (58.0)                |         | 20 (40.0)         |                          |         |
| >20 and ≤30       | 69 (55.6)                | 0.9 (0.5 to 1.8) | 0.7769 | 38 (30.6)         | 0.7 (0.3 to 1.3) | 0.2376 |
| >30 and ≤40       | 60 (50.0)                | 0.7 (0.4 to 1.4) | 0.3421 | 51 (42.5)         | 1.1 (0.6 to 2.2) | 0.7633 |
| >40               | 40 (52.6)                | 0.8 (0.4 to 1.7) | 0.5539 | 29 (38.2)         | 0.9 (0.4 to 1.9) | 0.8356 |
| Biopsy class      |                          |         |                   |                          |         |
| III/V (ref)       | 129 (49.6)               |         | 105 (40.4)        |                          |         |
| III/V or IV/V     | 35 (70.0)                | 2.4 (1.2 to 4.5) | 0.0095 | 11 (22.0)         | 0.4 (0.2 to 0.8) | 0.0161 |
| V                 | 34 (56.7)                | 1.3 (0.8 to 2.3) | 0.3256 | 22 (36.7)         | 0.9 (0.5 to 1.5) | 0.5959 |
| Lupus nephritis duration (years) | | | | | |
| ≤1 (ref)          | 119 (50.4)               |         | 95 (40.3)         |                          |         |
| >1 and ≤5         | 44 (63.8)                | 1.7 (1.0 to 3.0) | 0.0521 | 19 (27.5)         | 0.6 (0.3 to 1.0) | 0.0567 |
| ≥5                | 35 (53.8)                | 1.1 (0.7 to 2.0) | 0.6251 | 24 (36.9)         | 0.9 (0.5 to 1.5) | 0.6268 |
| eGFR baseline (mL/min/1.73 m²) | | | | | |
| <90 (ref)         | 88 (46.8)                |         | 76 (40.4)         |                          |         |
| ≥90               | 72 (50.0)                | 1.1 (0.7 to 1.8) | 0.5642 | 62 (43.1)         | 1.1 (0.7 to 1.7) | 0.6299 |
| Anti-dsDNA baseline (IU/mL) | | | | | |
| Negative (<30) (ref) | 1 (7.1)              |         | 8 (57.1)          |                          |         |
| Positive (≥30)    | 157 (50.5)               | 13.3 (1.7 to 103) | 0.0133 | 128 (41.2)        | 0.5 (0.2 to 1.5) | 0.2427 |
| C3 baseline (mg/dL) |                          |         |                   |                          |         |
| ≥90 (ref)         | 43 (53.8)                |         | 32 (40.0)         |                          |         |
| <90               | 115 (46.4)               | 0.7 (0.4 to 1.2) | 0.2517 | 106 (42.7)        | 1.1 (0.7 to 1.9) | 0.6658 |
| C4 baseline (mg/dL) |                          |         |                   |                          |         |
| ≥16 (ref)         | 53 (47.7)                |         | 45 (40.5)         |                          |         |
| <16               | 104 (48.1)               | 1.0 (0.6 to 1.6) | 0.9453 | 92 (42.6)         | 1.1 (0.7 to 1.7) | 0.7224 |
| UP/C baseline     |                          |         |                   |                          |         |
| ≤1 (ref)          | 27 (50.9)                |         | 23 (43.4)         |                          |         |
| >1 and ≤3         | 59 (48.0)                | 0.9 (0.5 to 1.7) | 0.7168 | 59 (48.0)         | 1.2 (0.6 to 2.3) | 0.5772 |
| >3                | 70 (47.0)                | 0.9 (0.5 to 1.6) | 0.6198 | 54 (36.2)         | 0.7 (0.4 to 1.4) | 0.3578 |
| Antimalarial treatment concomitantly during induction | | | | | |
| Antimalarial (ref) | 61 (48.0)              |         | 56 (44.1)         |                          |         |
| No antimalarial   | 137 (56.4)               | 1.4 (0.9 to 2.2) | 0.1270 | 82 (33.7)         | 0.6 (0.4 to 1.0) | 0.0513 |
| Lipid-modifying agent treatment concomitantly during induction | | | | | |
| Statin (ref)      | 47 (55.3)                |         | 33 (38.8)         |                          |         |
| No statin         | 151 (53.0)               | 0.9 (0.6 to 1.5) | 0.7083 | 105 (36.8)        | 0.9 (0.6 to 1.5) | 0.7403 |
| Induction treatment |                          |         |                   |                          |         |
| MMF (ref)         | 99 (53.5)                | 1.0 (0.7 to 1.5) | >0.9999 | 72 (38.9)         |                  |         |
| IVC               | 99 (53.5)                |         | 66 (35.7)         | 0.9 (0.6 to 1.3) | 0.5190 |

Percentages show the proportion of TFs or complete remitters within the applicable covariate category. Ref: reference category for ORs. ORs >1 imply more TF or complete remission in comparison category than in reference category. Anti-dsDNA, anti-double-stranded DNA; eGFR, estimated glomerular filtration rate; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; TF, treatment failure; UP/C, urine protein:creatinine ratio.
anti-dsDNA (OR 2.9, 95% CI 1.5 to 5.4, p=0.0012). Among those in the maintenance population who did not have an early reduction in anti-dsDNA by week 8, 30% developed TF during the maintenance phase compared with 10% of patients who did have an early reduction in anti-dsDNA (OR 3.5, 95% CI 1.4 to 9.2, p=0.0097). Lastly, in the maintenance population, positive anti-dsDNA at the end of induction was associated with TF. Among patients in the maintenance population who were anti-dsDNA positive at the end of induction, 26.9% developed TF during the maintenance phase compared with 4.8% of patients who were anti-dsDNA

| Table 3 Univariate baseline parameters associated with renal outcome (population: maintenance population (n=227)) |
|---------------------------------------------------------------|
| **Treatment failure**                                      | **Complete renal remission**                      |
| | n (%) | OR (95% CI) | p Value | n (%) | OR (95% CI) | p Value |
| Race |
| Caucasian (ref) | 27 (27.3) | 1.4 (0.5 to 3.7) | 0.4745 | 59 (59.6) | 1.5 (0.4 to 2.2) | 0.7872 |
| Black | 8 (34.8) | 0.7 (0.3 to 1.3) | 0.2490 | 13 (56.5) | 0.9 (0.4 to 2.2) | 0.5108 |
| Asian | 15 (19.7) | 0.6 (0.2 to 1.6) | 0.2772 | 49 (64.5) | 1.2 (0.7 to 2.3) | 0.9251 |
| Other | 5 (17.2) | 0.6 (0.3 to 1.1) | 0.1282 | 17 (58.6) | 1.0 (0.4 to 2.2) | 0.1681 |
| Ethnicity |
| Hispanic (ref) | 24 (31.2) | 1.8 (0.7 to 4.7) | 0.1998 | 42 (54.5) | 1.5 (0.8 to 2.6) | 0.7441 |
| Non-Hispanic | 31 (20.7) | 1.2 (0.5 to 2.8) | 0.6799 | 96 (64.0) | 1.0 (0.5 to 2.2) | 0.9356 |
| Age at randomisation (years) |
| ≤20 (ref) | 14 (40.0) | 0.7 (0.3 to 1.3) | 0.7611 | 20 (57.1) | 0.9 (0.4 to 1.8) | 0.5383 |
| >20 and ≤30 | 18 (24.7) | 1.1 (0.5 to 2.5) | 0.7812 | 38 (52.1) | 0.8 (0.4 to 1.8) | 0.5108 |
| >30 and ≤40 | 17 (22.1) | 0.4 (0.2 to 1.0) | 0.0524 | 51 (66.2) | 1.5 (0.6 to 3.3) | 0.3557 |
| Biopsy class |
| III/V (ref) | 38 (22.5) | 1.8 (0.7 to 4.7) | 0.1998 | 105 (62.1) | 1.0 (0.5 to 2.2) | 0.7441 |
| III/V or IV/V | 8 (34.8) | 1.2 (0.5 to 2.8) | 0.6799 | 11 (47.8) | 0.6 (0.2 to 1.3) | 0.3557 |
| V | 9 (25.7) | 0.3 (0.1 to 0.7) | 0.0133 | 22 (62.9) | 1.7 (0.7 to 4.3) | 0.2812 |
| Lupus nephritis duration (years) |
| ≤1 (ref) | 38 (24.5) | 4.6 (0.6 to 35.7) | 0.1484 | 128 (61.5) | 1.2 (0.4 to 3.6) | 0.7441 |
| >1 and ≤5 | 6 (19.4) | 1.1 (0.5 to 2.5) | 0.7611 | 19 (61.3) | 1.0 (0.5 to 2.2) | 0.9356 |
| ≥5 | 11 (26.8) | 0.9 (0.4 to 1.8) | 0.7227 | 24 (58.5) | 0.9 (0.4 to 1.8) | 0.9356 |
| eGFR baseline (mL/min/1.73 m²) |
| <90 (ref) | 31 (23.7) | 1.1 (0.6 to 2.0) | 0.8165 | 76 (58.0) | 1.3 (0.8 to 2.3) | 0.3171 |
| ≥90 | 24 (25.0) | 1.1 (0.6 to 2.0) | 0.8165 | 62 (64.0) | 1.3 (0.8 to 2.3) | 0.3171 |
| Anti-dsDNA baseline (IU/mL) |
| Negative (<30) (ref) | 1 (7.1) | 1.2 (0.5 to 2.8) | 0.6799 | 22 (62.9) | 1.0 (0.5 to 2.2) | 0.9356 |
| Positive (≥30) | 54 (26.0) | 4.6 (0.6 to 35.7) | 0.1484 | 128 (61.5) | 1.2 (0.4 to 3.6) | 0.7441 |
| C3 baseline (mg/dL) |
| ≥90 (ref) | 13 (26.0) | 0.9 (0.4 to 1.8) | 0.7227 | 45 (64.3) | 0.8 (0.5 to 1.5) | 0.5543 |
| <90 | 41 (23.6) | 1.1 (0.5 to 2.5) | 0.7611 | 92 (60.1) | 0.8 (0.5 to 1.5) | 0.5543 |
| C4 baseline (mg/dL) |
| ≥16 (ref) | 12 (17.1) | 0.9 (0.3 to 3.6) | 0.1189 | 56 (66.7) | 0.9 (0.5 to 1.7) | 0.6931 |
| <16 | 41 (26.8) | 1.8 (0.9 to 3.6) | 0.1189 | 45 (64.3) | 0.9 (0.5 to 1.7) | 0.6931 |
| UP/C baseline |
| ≤1 (ref) | 7 (21.2) | 1.3 (0.7 to 2.4) | 0.4510 | 23 (69.7) | 0.7 (0.4 to 1.2) | 0.1657 |
| >1 and ≤3 | 24 (27.3) | 1.4 (0.5 to 3.6) | 0.4976 | 59 (67.0) | 0.9 (0.4 to 2.1) | 0.7812 |
| >3 | 23 (22.5) | 1.1 (0.4 to 2.8) | 0.8725 | 54 (52.9) | 0.5 (0.2 to 1.1) | 0.0945 |
| Antimalarial treatment concomitantly during induction |
| Antimalarial (ref) | 18 (21.4) | 1.3 (0.7 to 2.4) | 0.4510 | 56 (66.7) | 0.7 (0.4 to 1.2) | 0.1657 |
| No antimalarial | 37 (25.9) | 1.3 (0.7 to 2.4) | 0.4510 | 82 (57.3) | 0.7 (0.4 to 1.2) | 0.1657 |
| Lipid-modifying agent treatment concomitantly during induction |
| Statin (ref) | 14 (26.9) | 0.8 (0.4 to 1.7) | 0.6059 | 50 (60.0) | 0.9 (0.5 to 1.6) | 0.6536 |
| No statin | 41 (23.4) | 0.8 (0.4 to 1.7) | 0.6059 | 105 (60.0) | 0.9 (0.5 to 1.6) | 0.6536 |
| Induction treatment |
| MMF (ref) | 34 (28.3) | 0.6 (0.3 to 1.1) | 0.1282 | 66 (61.7) | 1.1 (0.6 to 1.8) | 0.7956 |
| IVC | 21 (19.6) | 0.6 (0.3 to 1.1) | 0.1282 | 66 (61.7) | 1.1 (0.6 to 1.8) | 0.7956 |

Percentages show the proportion of TFs or complete remitters within the applicable covariate category. Ref: reference category for ORs. ORs >1 imply more TF or complete remission in comparison category than in reference category. Anti-dsDNA, anti-double-stranded DNA; eGFR, estimated glomerular filtration rate; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; TF, treatment failure; UP/C, urine protein:creatinine ratio.
negative at the end of induction (OR 8.3, 95% CI 1.0 to 66.0, p=0.0464).

Changes in proteinuria

Early changes in UP/C within 8 weeks and UP/C at the end of induction were independently associated with renal outcome (table 5). Among the complete population and the maintenance population, lack of reduction of UP/C by ≥25% within 8 weeks of induction was associated with TF. A total of 59% of patients in the complete population without UP/C reduction versus 35% with UP/C reduction were TFs (OR 2.7, 95% CI 1.5 to 4.4, p=0.0001). A total of 33% of patients in the maintenance population without UP/C reduction versus 21% with UP/C reduction were TFs (OR 2.1, 95% CI 1.0 to 4.2, p=0.0471). Lastly, among patients in the maintenance population who entered the maintenance phase with UP/C of >1, 43% achieved complete renal remission. In contrast, 68% of patients with UP/C ≤1 achieved complete renal remission (OR 0.3, 95% CI 0.2 to 0.6, p=0.0008).

Changes in complement levels

While changes in complement levels during induction were not independently associated with clinical outcomes during maintenance (p>0.05), a number of these measures did demonstrate association in the univariate analysis (table 4). C3 and C4 normalisation by week 8 (both individually and combined as a single measure of normalisation) showed association with TF in the complete population. Depending on the complement measure, patients who did not normalise complement had between 1.7 and 2.7 times the odds of TF compared with patients who did normalise complement. A similar magnitude of effect was seen in the analysis of the maintenance population for C4 level at the end of induction.
A total of 33% of patients with C4 <16 mg/dL versus 20% of patients with C4 ≥16 mg/dL at the end of induction were TFs during maintenance (OR 2.0, 95% CI 1.0 to 3.8, p=0.0388) (table 4).

**DISCUSSION**

In this study, we analysed data from the ALMS of IVC versus MMF for induction treatment followed by MMF versus AZA for maintenance treatment of lupus nephritis. Our objectives were twofold: (1) to determine if baseline factors present at the beginning of induction were associated with renal outcome and (2) to determine if changes in biological factors in the early weeks of induction or by the end of induction were associated with renal outcome during a 36-month maintenance period. We studied the complete intent-to-treat population (complete population) as well as the population of patients who continued into the maintenance phase after having achieved a complete or partial renal response after induction treatment (maintenance population). It is important to note that patients who did not respond to the induction treatment regimen were not randomised into the maintenance phase. Thus, the patients in the maintenance phase were inherently different from those who started the trial in the induction phase. In order to mitigate this potential source of bias, we chose to analyse the complete population and the maintenance population separately and report the results for both populations. For purposes of the analysis, we made the conservative decision that those patients who were not randomised into the maintenance phase were assumed to have a treatment outcome of either TF or non-complete remission, as appropriate.

In the multivariate analysis of the complete population, only non-Hispanic ethnicity was independently associated with complete remission during the maintenance phase. Three factors were independently associated with TF: (1) anti-dsDNA positivity at baseline, (2) failure to reduce anti-dsDNA within 8 weeks of induction and (3) failure to reduce UP/C by at least 25% within 8 weeks of induction.

In the multivariate analysis of those patients who continued into the maintenance phase of the trial (the maintenance population), baseline eGFR ≥90 mL/min/1.73 m² and UP/C ≤1 at the end of induction were independently associated with complete remission during the maintenance phase. Induction treatment with IVC was associated with a lower likelihood of TF, while lack of treatment with antimalarials, failure to reduce anti-dsDNA or UP/C within 8 weeks of induction, and positive anti-dsDNA at the end of induction were independently associated with TF.

### Table 5

| Analysis covariate                  | Comparison                                      | OR (95% CI)        | p Value |
|-------------------------------------|------------------------------------------------|--------------------|---------|
| **Population: complete population (n=370)** | **TF**                                        |                    |         |
| Anti-dsDNA at baseline              | Positive (≥30) vs negative (<30)               | 12.7 (1.6 to 101)  | 0.0167  |
| UP/C reduction by week 8            | No 25% reduction vs 25% reduction              | 2.6 (1.5 to 4.4)   | 0.0006  |
| Anti-dsDNA reduction by week 8      | No reduction vs reduction                      | 2.9 (1.5 to 5.4)   | 0.0012  |
| C3 normalisation by week 8          | No normalisation vs normalisation              | 1.7 (0.9 to 3.1)   | 0.0766  |
| **Complete renal remission**        |                                              |                    |         |
| Ethnicity                           | Non-Hispanic vs Hispanic                      | 2.0 (1.2 to 3.3)   | 0.0061  |
| C4 normalisation by week 8          | No normalisation vs normalisation              | 0.6 (0.4 to 1.1)   | 0.0794  |
| **Population: maintenance population (n=227)** | **TF**                                        |                    |         |
| Induction treatment                 | IVC vs MMF                                     | 0.5 (0.2 to 1.0)   | 0.0500  |
| Antimalarial treatment              | No treatment vs treatment                      | 2.4 (1.1 to 5.1)   | 0.0209  |
| UP/C reduction by week 8            | No 25% reduction vs 25% reduction              | 2.1 (1.0 to 4.2)   | 0.0471  |
| Anti-dsDNA reduction by week 8      | No reduction vs reduction                      | 3.5 (1.4 to 9.2)   | 0.0097  |
| Anti-dsDNA at end of induction      | Positive (≥30) vs negative (<30)               | 8.3 (1.0 to 66.0)  | 0.0464  |
| **Complete renal remission**        |                                              |                    |         |
| Age group*                          | >20 and ≤30 vs ≤20                             | 0.7 (0.3 to 1.8)   | 0.4801  |
|                                     | >30 and ≤40 vs ≤20                             | 1.8 (0.7 to 4.5)   | 0.2303  |
|                                     | >40 vs ≤20                                     | 2.7 (0.9 to 8.3)   | 0.0804  |
| eGFR at baseline                    | ≥90 vs <90                                     | 2.0 (1.0 to 3.8)   | 0.0407  |
| UP/C at end induction               | >1 vs ≤1                                       | 0.3 (0.2 to 0.6)   | 0.0008  |

TF: ORs >1 imply more failure in first factor level of comparison.

Complete renal remission: ORs >1 imply more remission in first factor level of comparison.

C3 (mg/dL) normalisation: change from <90 at baseline to ≥90 at week 8.

C4 (mg/dL) normalisation: change from <16 at baseline to ≥16 at week 8.

Anti-dsDNA (IU/mL) reduction: >200 at baseline to ≤30 at week 8.

Anti-dsDNA reduction by week 8: No reduction vs reduction.

Anti-dsDNA at baseline: Positive (>30) vs negative (<30).

UP/C at end induction: No reduction vs reduction.

UP/C reduction by week 8: No 25% reduction vs 25% reduction.

TF: ORs >1 imply more failure in first factor level of comparison.

*Overall p value for age: p=0.0279.

Anti-dsDNA, anti-double-stranded DNA; eGFR, estimated glomerular filtration rate; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; TF, treatment failure; UP/C, urine protein:creatinine ratio.
Our current findings build upon those from our previous study in which we found that early reduction in proteinuria and early normalisation of complement were independently associated with renal response at 6 months. Consistent with our previous findings, we have now shown that early improvement in proteinuria is independently associated with renal outcome during the 36-month maintenance phase following completion of induction therapy. Differing from our previous study, we found that early changes in complement levels were not independently associated with long-term renal outcome. Although we previously found that early reduction in anti-dsDNA was not predictive of response at 6 months, our current study demonstrates that changes in anti-dsDNA during induction are predictive of response during the longer maintenance phase. Given our extended period of observation out to 36 months after the completion of induction, it is not surprising that some of our results differ from our previous short-term study. It is well described and currently accepted that response to treatment of lupus nephritis continues to occur well beyond induction therapy. Thus, it is often difficult to draw a clear distinction between when induction treatment ends and maintenance treatment begins. For example, one study suggested that the median time to renal response with intravenous Cytoxan was 10 months. A study of oral Cytoxan for induction treatment of lupus nephritis followed by MMF or AZA as maintenance therapy demonstrated that improvement in proteinuria and renal function continues well into the maintenance phase. These observations highlight both the importance of long-term follow-up when assessing renal response to treatment in lupus nephritis trials and the somewhat arbitrary nature of our current definitions of disease response.

Our findings demonstrating a higher likelihood of renal remission in patients of non-Hispanic ethnicity in the complete population expand upon previous studies that have shown a greater incidence and worse prognosis in patients of Hispanic ethnicity with lupus nephritis. Our data showing a reduced likelihood of TF in patients in the maintenance population treated with antimalarials are consistent with the results of a study of patients with MMF-treated membranous lupus nephritis in which patients who received concomitant treatment with hydroxychloroquine were more likely to achieve renal remission by 12 months as compared with patients who did not receive hydroxychloroquine. These results are consistent with a broader accumulating literature demonstrating the many benefits of treatment with antimalarials, including the reduction of flares and prevention of renal damage. Although ALMS showed similar efficacy of IVC and MMF, inducing a renal response at 6 months, our current study suggests that patients treated with IVC during induction are less likely to develop TF during maintenance. A similar observation was made in a subgroup analysis of the ALMS maintenance phase, in which there was a trend of reduced incidence of TF during maintenance in those patients who received IVC as the induction agent. This trend of improved efficacy with IVC was present in patients regardless of whether they received MMF or AZA during maintenance. In our present analysis, the magnitude of the difference in response between the IVC and MMF groups is not profound enough to currently dictate individual patient treatment decisions. It is also important to recognise that various factors play a role in the choice of an induction agent for a particular patient, including the documented and perceived risks of a potential medication. However, these observations emphasise the importance of long-term renal outcome data in assessing the efficacy of induction regimens for the treatment of lupus nephritis.

Several other studies have examined predictors of renal response to treatment. Consistent with our findings, an early response to treatment as defined by proteinuria has been shown to be associated with improved long-term patient and renal survival. An analysis of 90 patients from the Euro-Lupus Nephritis Trial of low-dose IVC versus high-dose IVC for the induction treatment of lupus nephritis demonstrated that a decrease in serum creatinine and proteinuria of <1 g at 6 months predicted a favourable renal outcome at 10 years. Another study of 85 patients showed that change in proteinuria at 1 year was predictive of long-term renal survival and overall mortality.

A strength of our study is that we used data from ALMS, a randomised controlled trial that collected data throughout a 36-month maintenance phase following a 6-month induction phase. ALMS is one of the largest controlled trials for the treatment of lupus nephritis. By including data from the 36-month maintenance phase, we expanded upon our previous manuscript using only the 6-month induction phase data. Similar to our previous manuscript, a limitation of our current study is the fact that it is a post hoc analysis.

In conclusion, using long-term data from the ALMS, we identified several factors during the induction phase that are associated with renal outcome during the maintenance phase. Because of the differing patient composition of the complete and maintenance populations, we analysed the two populations separately. The maintenance population was composed of only those patients who achieved a renal response at the end of the induction phase of the trial and continued into the maintenance phase of the trial. The data from both populations are presented separately in this manuscript.

Although our findings contribute to the understanding of predictors of renal outcome in lupus nephritis, we believe that the associations described in this study are not strong enough to directly impact therapeutic decision making in individual patients in the clinic. Better biomarkers are needed to achieve this important goal. Lastly, in future controlled trials of lupus nephritis, studying the factors identified in our present analysis in a prespecified fashion might serve to further elucidate their association with renal response to treatment.
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