Long-term efficacy of azathioprine treatment for proliferative lupus nephritis

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Abstract

Background. Combination therapy with cytotoxic drugs and corticosteroids reduces the risk for renal failure in patients with proliferative lupus nephritis (PLN), but uncertainty remains about the best mode of immunosuppression and its long-term effects. We report long-term results of combined azathioprine–prednisolone treatment for PLN, which has been the therapy of choice for the treatment of PLN at our centre for 15 yr.

Patients and methods. A retrospective cohort study was carried out of 26 lupus patients, seen between 1978 and 1993, with histological and/or clinical evidence of PLN. Therapy consisted of prednisolone 1 mg/kg daily, tapered after 4 weeks to the lowest possible maintenance dose combined with azathioprine up to 2.5 mg/kg. Median duration of azathioprine treatment was 53 months. Standard statistical lifetable analyses were performed.

Results. Median follow-up on 1 January 1998 was 119 months. Patient survival estimates after 5, 10 and 15 yr of follow-up were 96, 91 and 82%, respectively. Four patients (15%) developed end-stage renal failure and three received renal transplants after a mean period of 27 months on haemodialysis. Renal survival estimates after 5, 10 and 15 yr of follow-up were 92, 87 and 87%, respectively. No malignancies were seen during the study period.

Conclusion. Azathioprine treatment for 4 yr was well tolerated in this cohort of Caucasian patients with PLN and was associated with outcomes similar to those reported for pulse cyclophosphamide therapy.

Key words: Proliferative lupus nephritis, Azathioprine, Long-term survival.

Therapy for proliferative lupus nephritis (PLN) is still controversial, despite nearly 50 yr of clinical experience and research. Whereas treatment with low-dose corticosteroids was soon found to be ineffective, high-dose corticosteroids (introduced only after an accidental observation on overdosing) slowed the progression of renal lesions and increased the 5-yr survival rate to 31% [1, 2]. The subsequent addition of immunosuppressive agents to corticosteroid therapy has led to further improvements in prognosis, although PLN still carries a 10–25% risk of end-stage renal failure (ESRF) [3, 4]. As a result of several reports from the National Institutes of Health (NIH) group, intermittent intravenous (‘pulse’) administration of cyclophosphamide has become the standard of therapy in PLN [5–7], although it is not equally successful in all hands in reducing the risk of ESRF [8–10]. Several studies found azathioprine to be an effective adjuvant to corticosteroids in the treatment of PLN [4, 11–15]. In fact, no clear difference in efficacy between azathioprine and i.v. cyclophosphamide therapy has been demonstrated in clinical trials, and a recent meta-analysis concluded that ‘no superiority of any specific immunosuppressant is established’ [16]. With equal efficacy of the various agents, toxicity becomes an important consideration, and i.v. cyclophosphamide carries a significantly higher risk for serious morbidity than azathioprine [4, 17–19]. Concerns about the efficacy:toxicity ratio combined with practical problems in administering i.v. cyclophosphamide to patients living several hundred kilometres away from this hospital are the reason that azathioprine has been the standard treatment for PLN patients in this hospital for 15 yr, and this report describes the results of that policy.

Patients and methods

Patients

This department of rheumatology became fully operational in 1978 and serves a population of 225 000 in the two most northern counties of Norway. All patients seen since then who fulfilled at least four American College of Rheumatology ACR criteria for the classification of SLE [20] and had clinical evidence of lupus nephritis (LN) in the form of persistent, otherwise unexplained proteinuria (>0.5 g per day), haematuria and/or the presence of cellular urinary casts were included. Patients seen after 1993, when departmental changes led to the acceptance of i.v. cyclophosphamide as the standard therapy, were not included (n = 8).
Of 35 patients with a clinical diagnosis of PLN between 1978 and 1993, five had mesangial LN (WHO class IIa/b) and two membranous PLN (WHO class V, \( n = 3 \)) upon renal biopsy and were excluded from further analysis. PLN was treated with prednisolone 1 mg/kg daily, tapered after 4 weeks to the lowest possible maintenance dose, and azathioprine at a starting dose of 100 mg daily, followed by dose adjustments up to a dose of 2.5 mg/kg. Laboratory monitoring was carried out weekly in the first 2 months and subsequently at gradually increasing intervals. In the event of gastrointestinal or haematological side-effects, dose reductions were made to 50 mg daily followed by 25 mg dose increases at longer intervals. The median duration of azathioprine treatment was 53 months (range 2–184). In 20 (77%), the first occurrence of PLN was documented by renal biopsy (WHO class III, \( n = 5, \) WHO class IV, \( n = 15 \)). Six patients did not have histological confirmation of PLN. Three had rapidly progressive glomerulonephritis (and received methylprednisolone 1000 mg i.v. on 3 consecutive days as initial treatment), one patient refused biopsy, one had thrombocytopenia \(<50,000\) (with WHO class III lesions on renal biopsy during a later renal relapse) and one had a severe psychosis, precluding biopsy. All six patients had a nephritic–nephrotic presentation with positive tests for anti-double-stranded DNA (anti-dsDNA) antibodies and/or hypocomplementaemia, indicative of PLN [4]. Two patients with biopsy-proven PLN were treated otherwise: one received oral cyclophosphamide initially and was switched to azathioprine first after 6 months, and one patient received prednisolone monotherapy as she refused any cytotoxic medication. Both patients were excluded from this analysis, which thus included 26 patients. Disease activity was measured by SLEDAI (systemic lupus erythematosus disease activity index) [21], and damage development was assessed by the Systemic Lupus International Collaborating Clinics (SLICC)–ACR damage index [22]. Hypertension was considered present if blood pressure exceeded 150/90 in two separate measurements and was considered sustained if present for \( >6 \) months. Hypertension was managed in a step-up fashion using vasodilators, diuretics and beta-blocking agents. Renal flares were defined as a \( >50\% \) increase in proteinuria with an active urinary sediment and were managed initially by increasing the prednisolone dose to 0.5–1 mg/kg. Anti-dsDNA testing was performed by \textit{C. luciliae} immunofluorescence (titre >1:8 were considered positive), while serum complement factors were measured by nephelometry (normal values: C3 >0.82 g/l and/or C4 >0.08 g/l).

**Statistical analysis**

Data were analysed by nonparametric tests: the Kruskal–Wallis test for continuous variables and the \( \chi^2 \) test for grouped variables. Data analysis was done on an intention-to-treat basis, and all patients starting azathioprine were included. Life-table analyses were performed by the use of Kaplan–Meier estimates and differences in survival curves analysed by log-rank testing. Censoring took place at the last known follow-up, the start of dialysis or death in the life-table analysis. Proportional hazards analysis was performed with the Cox regression technique. A 5% cut-off alpha level was used as the criterion of significance.

**Results**

**Patient characteristics**

Patient characteristics at the time of LN diagnosis are summarized in Table 1. The female: male ratio was 12:1, and male patients tended to be older (mean age 51.3 yr vs. 26.5 for females, \( P = 0.09 \)). Fourteen patients (54\%) presented with LN at systemic lupus erythematosus (SLE) diagnosis, and 12 developed LN after a median disease duration of 84 months (range 11–276). Nineteen per cent of patients had renal insufficiency with serum creatinine levels \( >150 \mu\text{mol/l} \) at diagnosis; nephrotic range proteinuria (\( >3.5 \) g per day) was present in 10 patients (39\%), and high disease activity (SLEDAI score \( >10 \)) in 18 patients (62\%). The median follow-up time to the last visit or death was 119 months (range 5–277). Four patients discontinued azathioprine because of persistent clinical (\( n = 2 \)) or laboratory-detected side-effects (\( n = 2 \)) despite dose reductions. The median dose of azathioprine was 125 mg (range 75–200) and the median prednisolone dose 7.5 mg (range 0–40) 12 months after PLN diagnosis. Nineteen renal flares occurred in 12 patients, with a mean interval of 414 days between LN diagnosis and first flare; 16 flares (85\%) were accompanied by positive anti-dsDNA and/or low serum complement factors. Figure 1 shows the cumulative frequencies of various disease manifestations in this cohort.

**Survival analysis**

Seven patients (27\%) died, after a mean disease duration of 180 months. Patient survival rate at 5, 10 and 15 yr was 96, 91 and 82\%, respectively (Fig. 2A). In a univariate survival analysis we found that age \( <30 \) yr (\( P < 0.04 \)), male sex (\( P < 0.001 \)), persistence of proteinuria for \( >6 \) months (\( P = 0.02 \)) and the presence at any time of discoid lesions (\( P < 0.03 \)) were associated with poorer survival, but only the last two variables remained independent predictors in a multivariate analysis.

<table>
<thead>
<tr>
<th>No. males/females (%)</th>
<th>2/24 (8/92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) (range)</td>
<td>30.4 (15–68)</td>
</tr>
<tr>
<td>Duration of SLE (months) (range)</td>
<td>0 (0–276)</td>
</tr>
<tr>
<td>Serum creatinine (( \mu\text{mol/l} )) (range)</td>
<td>91 (58–514)</td>
</tr>
<tr>
<td>Proteinuria (g/24 h) (range)</td>
<td>2.7 (0.5–8.5)</td>
</tr>
<tr>
<td>SLEDAI score (range)</td>
<td>11.5 (5–29)</td>
</tr>
<tr>
<td>ESR (mm/h) (range)</td>
<td>52 (13–144)</td>
</tr>
<tr>
<td>No. with CRP &gt; 10 mg/l (%)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg) (range)</td>
<td>135 (110–180)</td>
</tr>
<tr>
<td>No. with systolic blood pressure ( &gt;160 ) mmHg (%)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) (range)</td>
<td>85 (60–120)</td>
</tr>
<tr>
<td>No. with diastolic blood pressure ( &gt;100 ) mmHg (%)</td>
<td>3 (11)</td>
</tr>
</tbody>
</table>
analysis (\(P = 0.04\) and 0.02, respectively). Four patients (15%) developed end-stage renal failure and required chronic haemodialysis. One patient died after 6 months on dialysis, and three received successful renal transplants after a mean time of 27 months on dialysis. Renal survival rates at 5, 10 and 15 yr (Fig. 2B) were 92, 87 and 87%, respectively. Poor renal survival was independently associated with the presence of sustained arterial hypertension (\(P = 0.005\)), but not with renal flares (\(P = 0.09\)) or any of the lupus manifestations included in Fig. 1 (all \(P\) values >0.1). We performed a separate life-table analysis to determine the risk of any bad outcome (end-points of death or ESRF), which showed an 11.5% risk for either death or ESRF 2 yr after LN diagnosis, that increased to nearly 30% after 10 yr and to 47% after 15 yr (Fig. 2C). Patient and renal survival estimates for the six patients without histological evidence of PLN did not differ from survival estimates in the 20 patients with biopsy-proven PLN (\(P > 0.2\) for both curves, data not shown).

**Latest recorded findings**

Table 2 summarizes the clinical findings in the surviving patients at the last follow-up. In addition to the four patients progressing to ESRF, two patients developed renal insufficiency, their latest recorded serum creatinine levels being 278 and 165 \(\mu\)mol. The number of patients with diastolic hypertension had increased to 10 (38%), and serological evidence of disease activity (presence of anti-dsDNA and/or low C3/C4 levels) was still detected in almost 20% of patients. Sixteen patients were still using azathioprine and all patients were still using prednisolone (median dose 7.5 mg, range 5–40). No malignancies were diagnosed during the time of observation.

**Discussion**

When the mercaptopurine derivative azathioprine was found to reduce antibody production, prolong graft survival in renal transplant patients and reduce the severity of experimental lupus nephritis, it soon found its place in the treatment of patients with SLE [14, 19, 23–25]. Although its role in has been overshadowed by the NIH reports on the renal efficacy of i.v. cyclophosphamide [26], our findings support the notion that azathioprine has a place in the treatment of PLN [15, 16, 27]. The 10-yr patient survival rate of 91% in our cohort not only represents a large improvement over rates reported 40 yr ago using high-dose steroid monotherapy [2] but is among the highest reported in studies on various treatment regimens in recent decades [23, 28]. In this respect it is important to remember that long-term studies reporting patient survival with i.v. cyclophosphamide treatment are scarce at best. We chose not to censor patients reaching ESRF from this survival analysis as patient survival no longer requires renal survival if access to dialysis is available. ESRF, although still unwelcome, is now a treatable condition and lupus patients do at least as well as other patient groups in renal replacement programmes [29, 30].

![Fig. 1.](image) Cumulative frequency of non-renal SLE manifestations in patients with PLN (\(n = 26\)).
prolonged proteinuria and hypertension with poorer prognosis indicates a substantial role for such supportive therapies in improving the prognosis [31, 32].

Four of our patients developed ESRF (15%), which is well within the range of rates reported in the literature, although it is nearly double the 8% rate reported in the latest NIH study [7, 8, 15]. In that study, however, follow-up was relatively short; renal failure in PLN usually results from a slow decline in renal function, which may be independent of the initial insult, while renal lupus flares may accelerate this decline [33, 34]. In contrast to crude ESRF rates, survival analysis allows evaluation of the time-dependence of this complication of PLN, and a minimum observation time of 100 months for therapeautic studies on PLN has been proposed [35]. This study’s single-centre design allowed consistent long-term follow-up. The renal survival at 5 yr was 92% in this cohort, which compares favourably with the 75–88% reported in a recent review [25]; the 10- and 15-yr renal survival rates of 87% are similar to that reported for i.v. cyclophosphamide in the longer-term NIH study [35]. Angiotensin converting enzyme-inhibiting agents are now known to have renoprotective effects in various forms of glomerulonephritis [33], but as only three (hypertensive) patients were treated with ACE inhibitors in this study no conclusion can be drawn.

Our study patients were not remarkable in terms of age, gender, renal function, other lupus manifestations or autoimmune serology, but the regional aspect of this study is nonetheless of importance. All our patients were Caucasians of Nordic descent, whereas most US-based studies include a considerable number of patients of African heritage, who tend to have a worse disease outcome because of both intrinsic medical and socioeconomic factors [28]. This considerably limits one’s ability to extrapolate the NIH data on the benefit of cyclophosphamide therapy for PLN [36] and calls for homogeneous study populations. More than half of our patients were treated at the very beginning of their disease, which may be an additional reason for the good results of azathioprine therapy in our cohort. The timing of immunosuppression has been considered an important prognostic factor in LN, and providing early immunosuppression may be even more important than the type of immunosuppression given [37, 38].

Our results indicate that 15 yr after diagnosis, and despite the relative efficacy of azathioprine, patients had a risk of nearly 50% of an adverse outcome in the form of death or the need for renal replacement. This illustrates that PLN remains a serious, although less acute, threat, and patients are in need of better treatment regimens or drugs that reduce this long-term threat [39, 40]. The limitations of this study are obvious: the lack of a control group, its retrospective nature and the relatively small number of cases. This notwithstanding, we feel that the homogeneity of our cohort, the consistency in patient management and the long-term follow-up make these findings worthwhile.

In conclusion, treatment of PLN with prednisolone and azathioprine was well tolerated and associated with
5- and 10-yr survival rates similar to those for cyclophosphamide-based therapies. Nonetheless, it still carried a 50% risk of a poor outcome after 15 yr in this group of Nordic patients.

References
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