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Review

Treatment for refractory lupus nephritis: Rituximab vs triple target therapy

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1. Introduction

The clinical picture of systemic lupus erythematosus (SLE) is characterized by a wide variety of clinical manifestations with variable severity, among these lupus nephritis (LN) is a severe manifestation occurring in more than half of patients (range 27–74%) [1–6].

The treatment of LN has greatly changed over the decades, with the reduction of the steroid dose, the optimization of cyclophosphamide (CYC) protocols and the introduction of new drugs such as mycophenolate mofetil (MMF) and, more recently, tacrolimus (TAC). Recently published European League Against Rheumatism (EULAR) recommendations suggest that MMF and low dose CYC are the treatments of choice for the induction of remission and should be followed by a maintenance therapy with MMF, azathioprine (AZA) or calcineurin inhibitors (CNI) [7].

Thanks to the improvements in diagnosis, monitoring and management, the prognosis of LN has greatly improved over the past decades; however, recently, Tektonidou et al. [5] have shown that the occurrence of end stage renal disease (ESRD) has plateaued around 10% and had an apparent increase in 2000s. Moroni et al. [6] have reported an improved outcome of LN; however, a complete renal remission was achieved in only 58.5% of patients with LN diagnosed in the period 2002–2016, while 32.1% of patients appeared in partial remission and 9.4% had a poor outcome (either chronic kidney disease, end stage renal disease or death).

LN is associated with an increased morbidity and mortality. It has been shown, in fact, that LN is associated with a reduced probability of reaching lupus low disease activity (LLDAS), increased damage accrual, increased infection risk and has an impact on patients' survival [3,8,9]. In the Euro- Lupus cohort, 10-years survival of patients with LN was 88% vs 94% in patients without [1]. More recently, Mok et al. [4] have shown that life expectancy is reduced of 15 years in patients with LN.

Last but not least, LN has an impact on healthcare costs; in fact, the mean cumulative cost per patient with renal damage is estimated around $ 99.544 vs $20,337 in patients with no renal damage [10].

These data highlight the fact that LN is still an unmet need in the management of SLE and suggest the need of alternative therapeutic approaches able to better control disease activity, prevent the occurrence of flares and reduce damage accrual.

2. Rituximab in systemic lupus erythematosus and in lupus nephritis

Rituximab is a high-affinity chimeric monoclonal IgG1 antibody directed against the CD20 antigen, an integral membrane protein which is expressed on B cells from the pre-B stage to the mature B stage but not on hematopoietic stem cells and normal plasma cells. It exerts its lithic effect through three pathways: by binding C1q and activating complement-dependent cytotoxicity, by binding to Fc receptor and mediating cell killing through antibody-dependent cellular toxicity, and finally by stimulating the apoptotic pathway. It has shown to effectively and selectively deplete B cells in peripheral blood and lymph nodes [11]. Although initially approved by the US Food and Drug Administration (FDA) for its use in non-Hodgkin lymphoma [12], it did not take long to make the leap to other disciplines, and as such, by the beginning of the new millennium rituximab proved its value in the field of autoimmune diseases, first in rheumatoid arthritis [13] followed closely by SLE [14].

Since the first report published in 2002 regarding its use in life-threatening autoimmune haemolytic anaemia in a patient with SLE [14], a compelling body of evidence has accumulated, relying on large number of case reports and several open-label studies, even in spite of the discouraging results of two randomized clinical trials. Important learnings have derived from all these studies with rituximab, particularly regarding pharmacokinetics and pharmacodynamics, but also about its safety profile and the impact over the immune system.

It is worth noting that, supported by findings in animal models, rituximab has manifested a capability to deplete the B cells also in the tissue in humans, and interestingly, although not fully corroborated, there seems to be a close correlation between the findings in tissue and

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peripheral blood compartment [15]. The timing and the mode the repopulation occur are also important, to the point that shorter periods and repopulation with higher proportion of memory cells, lower transitional and lower naïve cells correlated with poor clinical response. Also, higher basal levels of CD19+ cells have been associated with poor clinical responses [16].

The high number of publications and the accumulation of evidence led to the development of two large phase III randomized, double-blind, placebo-controlled, multicentre studies designed to evaluate the efficacy and safety of rituximab in patients with moderate to severe SLE: the EXPLORER [17] in SLE without renal involvement and the LUNAR [18] in LN. Surprisingly, both failed to meet their primary endpoints of significantly reducing the disease activity when compared to placebo. This generated a broad debate, since clinicians felt a gap between the clinical trials and the real world experience, and the learnings derived from these failures made a clear impact in the design and outcome of future studies in SLE. The main deficiencies implied can be summarized in three points: Firstly, errors when selecting the target population, regarding both ethnic factors and severity of the disease; secondly, an excessive background immunosuppressive treatment, including very high doses of corticosteroids; and thirdly, the election of unrealistic endpoints that would set the bar too high for rituximab to achieve meaningful differences in such complicated context. However, in spite of these discouraging results, rituximab has been, and is being employed in SLE, particularly in complicated cases, virtually with involvement of any possible organ.

Regarding the management of LN, the results of various prospective clinical trials conducted at the US National Institutes of Health (NIH) were published in 1986, and they had such a profound impact worldwide that meant a change of paradigm in the treatment of LN and in the natural course of the disease [19], revealing a dramatical decrease in mortality with the use of certain immunosuppressive agents, especially intravenous CYC together with corticosteroids.

Later on, several other trials, such as the Euro-Lupus Nephritis Trial [20,21], the ALMS trial [22,23] and the MAINTAIN Nephritis Trial [24,25] ended up configuring the hallmark of the treatment of LN as it is known today: intravenous CYC at low doses or MMF, both in combination with corticosteroids, can be used as induction treatment, whereas MMF or AZA can be used as a maintenance treatment [7,26–28].

It is important to note that complete renal response can take up to 2 years to establish and only a small to moderate percentage of patients achieve it in the short term. Different studies show that the majority of patients that will respond will do it within a year, with only 50% reaching that goal, and another 5–25% of patients remitting at 24 months [26]. The identification of predictors of favourable long-term renal outcomes is of the utmost importance, such as the achievement of a rapid drop in the proteinuria to levels below 1 g/24 h at 6 months [21] or below 0.7 g/24 h [29] or 0.8 g/24 h [30] at one year, in order to help the clinician to identify those patients who are unlikely to respond. However, the sooner we identify the non-responders, the lesser the potential damage accrual, and indeed, in those patients whose nephritis fail to improve or worsens in 4–6 months, it is advised to switch therapy from CYC to MMF or vice-versa [27,28].

3. Rituximab in refractory lupus nephritis

In spite of the best efforts, there is a subset of patients who do not achieve complete renal response and are classified as refractory patients. The definition of refractoriness is in itself a matter of debate nowadays, given the wide variety of terms employed, and the main SLE clinical guidelines do not universally address this problem, leaving the decision on when to consider as such to the discretion of the clinician. Independently of the disparity of criteria, it could be understood, as a concept, as the inability to achieve remission of the inflammatory process that is generating damage in the kidney despite the treatment employed. In any case, once a patient has failed previously to MMF and intravenous CYC, there is no strategy supported by strong evidence, although the EULAR and the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) [7,27] and the American College of Rheumatology (ACR) [28] guidelines agree in pointing to rituximab as the next option of treatment, whereas Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [26] also contemplate, in addition to rituximab, other options with lower quality of evidence, such as immunoglobulins or CNI.

The weight of evidence regarding the treatment of refractory LN comes from open-label, non-randomized, non-controlled, prospective and retrospective studies, and likely, and unfortunately, there will not be evidence of higher quality, since the drug companies are no longer interested in conducting another randomized controlled trial due to economic reasons. Up to the present, nearly 1110 patients with SLE have been reported to have been treated with rituximab [31], with > 300 patients among them with LN [31,32]. Despite having been used in all histopathological classes, the experience for class I or II is anecdotical, and in a considerable percentage of cases (nearly 30%) this was not reported. All the cases were considered refractory, and although, as previously stated, the definitions of refractoriness varied among studies, in general all of them showed unresponsiveness to 1–3 immunosuppressive drugs in addition to corticosteroids. There was some variability in the rituximab regimen employed, but in general were the lymphoma-based protocol (375 mg/m2 weekly for four weeks) or the rheumatoid arthritis-based protocol (1000 mg on day 1 and 15), with slight modifications in some cases. Other background treatment was administered in conjunction with rituximab in approximately half of the cases, being the most frequent corticosteroids, followed by CYC in almost one third of the cases, and MMF in one fourth of the cases. Also, heterogeneity was noted regarding the method of evaluating the response to rituximab, since in some trials it was used the SLAM activity index, while in others the BILAG or the SLEDAI index. According to a well conducted meta-analysis [32], the mean follow-up was 60 weeks (12–120 weeks), and rituximab achieved a striking overall response rate of 74% (40% of complete response and 34% partial response) in such refractory cases. The authors also demonstrated a better response rate (complete response or any response) in class III, and less frequent in class IV and V. Another meta-analysis [31] estimated a rate of 51% and 27% of complete and partial response, respectively. Taken together, all this evidence strongly supports the use of rituximab in patients that are insufficiently controlled with the standard of care.

Given the key role that B cells play in the pathogenesis of the disease, and the remarkable results that rituximab has shown in such complicated scenarios, the future is still looking at the depletion of B cells as a target. The RItuximab for lupus Nephritis with remission as a Goal, the RING study (NCT01673295), is a randomized international clinical trial promoted by independent investigators that is currently enrolling patients with the aim to test whether rituximab is effective to achieve complete renal response in LN patients with persistent proteinuria (≥1 g/d) despite at least 6 months of standard of care. Obinutuzumab is a fully humanized monoclonal antibody directed against CD20 that has demonstrated superiority over rituximab in lymphoma animal models, while producing similar depletion of normal B cells in peripheral blood, but deeper depletion in spleen and lymph nodes. A phase II trial with 127 patients with LN is currently ongoing (NCT02550652). CFZ533 (Iscalimab) is a fully humanized monoclonal antibody directed against CD40, a glycoprotein constitutively expressed on B cells and antigen presenting cells such as monocytes, macrophages, and dendritic cells (DCs). It has a notable capability to deplete the B cells, especially in tissue, and has already produced promising results in other autoimmune diseases. Currently, a phase II trial in non-renal SLE is already ongoing.

4. Calcineurin inhibitors in lupus nephritis

CNI plus corticosteroids have emerged as an effective induction treatment in LN according to several studies [33,35]. Although TAC and cyclosporine (CsA) share similar immunosuppressive mechanisms of action, TAC has shown to be a more potent and safer agent [36]. The inhibition of calcineurin is achieved through intracellular competitive binding of TAC to FKBP12 (cyclophilin in the case of CsA) with consequent suppression of interleukin-2 (IL-2), which in turn regulates T-cell activation [37,38]. Moreover, CNI have shown non-immunologic actions in several proteinuric kidney diseases. These effects include: a) protection of podocytes from apoptotic stimuli (by inhibition of genetic activation of the calcineurin effector NFAT [nuclear factor of activated T cells]); b) stabilizing the actin cytoskeleton of podocytes (by preventing synaptopodin degradation); and c) downregulation of angiopoietin-like-4 expression (a potential mediator of proteinuria in some types of glomerulopathy) [39,40]. These antiproteinuric effects are relevant since proteinuria at 1 year has been described as the best predictor of long-term renal outcome in LN [29,30,41]. On the other hand, achieving more complete remissions has an important implication since patients who achieve partial remission have a worse renal survival at 10 years compared to patients who reach complete remission [42].

Although numerous studies have demonstrated that CNI have a good safety profile, several caveats should be considered. Its intrinsic hemodynamic effect (decreased glomerular filtration rate by intrarenal vasoconstriction) could lead in some cases to acute kidney injury or even chronic irreversible nephrotoxicity [43]. Another consideration is the difficulty of dose adjustment since blood concentration should be through-level monitoring. Hypertension and hyperglycemia are some other feared complications [44]. Finally, secondary hemolytic-uremic syndrome because of direct endothelial damage is a potential adverse event [45].

The combination of T-cell activation blockers, such as CNI, with drugs such as corticosteroids and MMF, which induce their immunosuppressive and anti-inflammatory effects through other pathways, could theoretically be more effective than the current standard of care protocols for induction therapy in LN and for refractory cases. This regimen, termed as multi-target or triple target therapy (TTT), has been evaluated through several prospective randomized trials that have shown promising results.

5. Clinical trials of corticosteroids plus calcineurin inhibitors and triple target therapy in lupus nephritis

5.1. Corticosteroids plus CNI

In a multicenter randomized clinical trial in Chinese patients, prednisone (PDN) plus TAC ($n = 42$) was compared to PDN plus intravenous CYC ($n = 39$) as induction therapy for active LN classes III, IV, or V. After 6 months, complete remission and response (partial or complete remission) were higher in the PDN + TAC group (52% vs 38% and 90% vs 82%, respectively), although differences did not reach statistical significance. Noteworthy, proteinuria was significantly lower after the first month of treatment in the PDN + TAC group [34]. Similar results have been published by other group, although in a non-randomized prospective cohort study [46]. In a randomized Chinese trial (three-arm trial) of 60 patients with similar baseline characteristics as the above-mentioned studies, response rates and complete remission rates were similar between induction therapy with TAC/PDN, CYC/PDN or MMF/PDN, ($p > .05$) [33]. In another open randomized controlled study for induction therapy, PDN + TAC was not inferior compared to PDN + MMF [35]. Even though individual studies have not showed firm statistically significant differences, a recent meta-analysis suggest that complete remission and response are increased in patients treated for induction therapy with TAC as compared to those treated with CYC, with no difference observed between MMF vs TAC [47].

5.2. TTT

In prospective trials performed in China the combination of corticosteroids (intravenous methylprednisolone pulse therapy for 3 days followed by oral PDN 0.6 to 0.8 mg/kg per day for 4 wk. and then tapered until a maintenance dosage of 10 mg/d) with TAC (initial dosage 4 mg/d, then titrated to maintain a blood concentration within 5 to 7 ng/ml) and MMF (1.0 g/d) was compared to standard PDN + CYC regimen. In a first small trial, higher rates of complete remission with TTT at both 6 and 9 months (50% and 65%, respectively) were found in comparison with PDN + CYC (5 and 15%, respectively) [48]. These results were confirmed in a second larger trial that showed complete remission at 24 weeks in 46% of patients treated with TTT versus 25% in patients receiving PDN + CYC [49].

TTT has been also evaluated as maintenance treatment in LN. In an extension trial, patients who achieved remission with TTT continued with the same regimen as maintenance treatment while the CYC-based group received AZA. At 18 months, both groups had similar renal function and relapse rates (TTT 5.4%, AZA 7.6%) [50].

Voclosporin, a new CNI with a better metabolic profile that lead to less adverse effects and no need for therapeutic drug monitoring [51], has been evaluated for patients with LN in the AURA-LV trial [52]. This multicenter and international trial compared placebo, low-dose and high-dose voclosporin in addition to MMF and PDN, in 265 patients with LN. Patients of all races were included, most of them with normal renal function and high proteinuria. At 24 weeks, the rate of complete remission was significantly higher in the low-dose group compared to placebo (32% vs 19%, p 0.04). Also, at 48 weeks both low-dose and high-dose voclosporin were superior than placebo (49%,39% and 23%, respectively). The rate of serious adverse events including death were significantly higher in the treatment arm, however, as most of the deaths occurred in only two centers, these results will need further confirmation in future trials. A phase 3 randomized, placebo-controlled trial (Aurinia Renal Response in Active Lupus with Voclosporin, NCT03021499) is currently ongoing.

6. Calcineurin inhibitors and triple target therapy in refractory lupus nephritis

The EULAR/ERA-EDTA guidelines considers refractory disease as the failure to improve within 3–4 months or the inability to achieve partial remission after 6–12 months or complete remission after 2 years of treatment [27]. Some studies have assessed the efficacy of monotherapy with CNI for refractory LN. In an observational study including 26 patients previously treated with CYC with persistent proteinuria > 1.5 g/day, rates of partial and complete remission at 6 months with CNI were 50% and 38.5%, respectively [53]. Another small study with 9 patients found complete remission in 78% of the cases and a significant decline in proteinuria (urine protein to creatinine ratio from 2.19 to 0.44) after CNI initiation [54].

The efficacy of TTT in refractory LN has been reported in several studies. In a study with 70 patients treated with MMF, TAC was added to 17 cases with refractory or relapsing disease. At 2 years, 35% of these patients achieved a completed remission and 35% a partial remission [55]. The efficacy of this combination was also tested in an observational study that included 29 patients, with a response rate of 55.5% at 12 months [56]. Gordon et al. [57] also described 8 cases that achieved remission (5 complete and 3 partial remission) after the addition of TAC to MMF. In a small case series, response was achieved in 4 of 7 patients, but in contrast to other studies, adverse effects limited the use of triple target therapies [58]. Another study with CsA showed a complete remission in 4 patients, partial remission in 1 patient, and only 1 case did not respond [59].

7. Profile of patients with refractory lupus nephritis susceptible to present a better response to calcineurin inhibitors or triple target therapy

As commented above, CNI possess a very potent antiproteinuric effect, partially independent of their immunosuppressive properties. Most patients included in the prospective studies that demonstrated the superiority of TTP over standard of care treatments presented severe proteinuria at baseline and normal renal function [48,49,52]. These clinical data are important, considering that kidney function impairment is a common side effect of CNI and that CNI treatment in patients with acute kidney injury or unstable kidney function is often difficult. For all these reasons, those patients with stable and normal or only mildly impaired kidney function who present persistently high amounts of proteinuria after several months of standard of care treatment are probably the ideal cases for TTT.

8. Combination of calcineurin inhibitors and rituximab

The combination of rituximab and CNI could also be of interest in refractory LN, as it has proven to be effective in other glomerulopathies such as membranous nephropathy (MN) [60,61]. It has been shown that rituximab is excreted in the urine in patients with nephrotic syndrome, resulting in significantly lower plasma levels [62–64]. Previous treatment with CNI could reduce proteinuria and consequently enhance the effect of rituximab. However, this therapeutic approach has not been evaluated in LN.

9. Concluding remarks

In view of all the above, rituximab has ceased to be a promising treatment to become a solid option in patients with severe SLE, not restricted to nephritis, refractory to various previous treatments.

On the other hand, induction treatment with TTT has shown to be more effective than standard of care treatments to achieve complete and partial remission in LN [48,49,52]. However, it is unknown if a more sequential approach, adding CNI only to those patients who maintain unacceptable proteinuria while on PDN plus MMF treatment, could offer similar results. Observational studies [55–59] have reported good results with this gradual approach, but prospective trials comparing both TTT schedules are needed.

Finally, further studies are needed to fully assess which refractory LN patients will benefit more from the addition of rituximab or of CNI in the form of TTT.

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