Safety and efficacy of Methotrexate Monotherapy Vs Methotrexate plus Leflunomide Combination Therapy in Early Active Rheumatoid Arthritis Patients

Mariane S. Boshra[1], Amira S. Ahmed[1], Heba F. Salem[2], Mohamed N. Abd El Hamid[3]

[1] Clinical Pharmacy Department, Faculty of Pharmacy, Benisuef University, Beni-Suef, Egypt

[2] Pharmaceutics and IndustrialDepartment, Faculty of Pharmacy, Benisuef University, Beni-Suef, Egypt

[3] Internal Medicine and Immunology Department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt

Abstract

MTX has long been considered as the anchor drug in RA treatment, however given, its high failure rate and the multifactorial nature of the disease, there has been a great interest in developing more powerful and tolerable MTX combination therapies. Leflunomide has long been appreciated to be a suitable alternative therapy used on its own or with MTX due to its equivalent efficacy and structural similarity. The aim of the study was to compare the efficacy and safety of a combination therapy of MTX plus LEF to MTX alone when used with early active rheumatoid arthritis patients. Fifty female patients with early active RA were randomly allotted in two groups each of 25. Patients were randomized to receive MTX or MTX+LEF. Analysis of DAS28 score, complete blood picture, and liver enzymes at the start and post 24 weeks of treatment with study regimens was performed, compared and evaluated. MTX+LEF combination therapy was more significantly beneficial in decreasing DAS28 score compared to MTX alone. The combination therapy showed less effect on blood components, but was more hepatotoxic compared to MTX monotherapy. The combination therapy is a more attractive option to consider especially in early active RA patients than MTX monotherapy. It is more clinically beneficial in improving disease outcomes, well tolerated, with less hematological toxicity and only showed mild hepatotoxicity. Taken into consideration proper monitoring of liver toxicity, this combination is safe and more effectively intervene early in the disease process thus preventing future possible complications and aiming better remission.

Keywords: Rheumatoid, methotrexate, leflunomide, liver enzymes, DAS_{28}

(Rec.Date: Jul 19, 2015 Accept Date: Sep 18, 2015)

Corresponding Author*:* Amira S. Ahmed, Department of Clinical Pharmacy, Faculty of Pharmacy, University of Beni Suef, Beni Suef, Egypt
E-mail: amirashaban19@yahoo.com **Phone:** 00201279330813. **Fax:** 0020822317953

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease that causes substantial morbidity and mortality throughout the world. It is a highly progressive disease that may rapidly complicate to permanent disability within only three years of diagnosis if untreated [1]. To date, no cure for RA has been found, and treatment is only targeted to decreasing pain, limiting joint damage, and functional decline [2]. According to the most recent European League Against Rheumatism (EULAR) guidelines updated 2013, the proper management of RA depends primarily on the use of disease-modifying anti-rheumatic drugs (DMARDs) singly or combined as early as possible in the disease process for achieving remission, and stepping up treatment as necessarily needed to reach this target [3].

Methotrexate (MTX) has long been considered as the anchor drug in all rheumatoid arthritis treatment strategies, due to itsefficacy, low toxicity and better tolerability [4]. MTX is still regarded as the clinician first favorable therapeutic approach to newly diagnosed RA patients prior to stepping up treatment to a combination therapy. However, several clinical studies have shownits high failure rate as a mono therapy in some patients even with maximal tolerated doses [5]. Thus, many MTX combination regimens have been studied and even found more appealing and promising in treating RA patients [6]. Leflunomide combined with methotrexate has been appreciated to be used as an alternative therapy to RA after failure of the initial monotherapy [7,8]. However, the data regarding the efficacy and safety profile of this combination compared to MTX monotherapy is still limited, conflicting and non-conclusive and warrants further investigation.

In this study we compared the efficacy and tolerability of a combination therapy of MTX plus LEF, with MTX as monotherapyin early, active rheumatoid arthritis patients. We investigated the effect of either treatment on rheumatoid arthritis disease activity scores, blood pictures and liver enzymes.

Material and Methods

Fifty female patients were randomly allotted in two groups of 25 patients in each. Patients were collected from Beni-Suef University teaching hospital outpatient clinic throughout one year study period. All subjects recruited for this study aged between (18-45) years old and

were diagnosed with early (<2 years' duration) active (DAS28>4.4) rheumatoid arthritisaccording to American College of Rheumatology (ACR) criteria [3]. Participation was voluntary, and written informed consent was obtained.

The study was approved by the Ethics Committee of Beni-Suef University teaching hospital. Patients were excluded from the study if they had any coexistent anaemia, any other immune systemdis orders, hypertension, diabetes, heart disease, osteoporosis, pre-existing liver disease, hematologic system disease, pregnant, lactating, or had drug allergies.

Study design

On admission, the selected patients were subjected to a complete and detailed medical history, physical and clinical examination. All their baseline demographic and clinical data were recorded. Subjects were randomized to receive either MTX monotherapy or MTX + LEF combination therapy. All patients were scheduled for a weekly follow-up visit, for confirmation of using the assigned medication doses of a 20 mg daily tablet of Leflunomide (Arthfree®, Eva Pharma), and MTX 1.5cm(15mg/week) in the form of Methotrexate injection 50 mg vial manufactured by ACDIMA. Previous use of other DMARDs was permitted only if they were discontinued at least 28 days before trial enrolment.

The primary outcome assessed in this study was the DAS28 score in 28 joints at baseline and at 24 weeks post each treatment regimen. The DAS28 is a composite index of the number of swollen and tender joints, the erythrocyte sedimentation rate, and a visual-analogue scale of patient-reported disease activity. DAS28 was calculated using the following formula: DAS28=0.56 × sqrt (tender 28) + 0.28 × sqrt (swollen 28) + 0.70 × ln (ESR) + 0.014 × VAS [9]. A decrease in the DAS28 of 1.2 or more from baseline was considered to be a clinically meaningful improvement [10]. In rheumatology practice, physicians assess disease activity levels using a DAS28 cutoff value of >4.4 to define active disease, and assess treatment response according to changes in disease activity [3].

The hematological adverse events of each treatment were evaluated at baseline and at 24 weeks post treatment. Hematological parameters assessed included; Hemoglobin (Hgb), RBCs count, hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBCs, neutrophils, basophils,

eosinophils, lymphocytes, monocytes, aspartate aminotransferases (AST), and alanine aminotransferases (ALT).

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS version20; SPSS, Inc, Chicago). The comparisons between the study groups were performed with the use of Student's t-test for continuous measures and the chi-square test (or Fisher's exact test) for categorical measures.

Results

A total of 50 female patients diagnosed with active rheumatoid arthritis with disease duration of at least 3 months but not more than 2 years were recruited. Patients were randomised into two groups, each of 25 that received either MTX or MTX+ LEF combination therapywith mean age of 42.5 ± 9.8 and 45.3 ± 11.2 years old, respectively. The patients' demographic details were illustrated in Table 1. The two recruited groups at baseline showed no significant difference in age, sex, disease duration and baseline DAS.

Data of complete blood picture, liver enzymes and DAS28at baseline and post 24 weeks of each treatment regimen were illustrated in Table 2.

When comparing the baseline data of two treatment groups regarding haematological parameters, AST, ALT, and DAS28, the results were non-significant.

As shown in Table 2 and Figure1, both treatment regimens significantly decreased DAS28 compared to baseline data ($p<0.001$), however, MTX+LEF combination therapy was found to more significantly ($p<0.01$) decrease DAS28 than MTX alone.

In addition, as illustrated in Table 2 and Figure 2, results of hematological analysis showed that, MTX treatment for six months significantly decreased ($p<0.001$) Hgb, RBCs, Hct, MCH, MCHC, WBCs and neutrophils count and significantly increased ($p<0.001$) monocytes, eosinophils, basophils and lymphocytes.

However, it was observed that less blood components were affected by MTX+ LEF combination therapy, where a significant decrease was only noted with Hgb ($p< 0.001$), RBCs

(p< 0.001), and MCHC (p< 0.05); Whereas only monocytes were significantly increased (p< 0.01).

The increase in liver enzymes from its initial basal values post each treatment regimen was calculated by subtracting the initial values from post treatment values and presented in table 3. The difference was statistically analysed.

As shown in Figure 3 and Table 3, both treatment significantly increased AST and ALT (p<0.001). However, MTX + LEF therapy was observed to be more hepatotoxic and caused a more significant increase in both AST and ALT levels at p<0.05 and at p<0.01, respectively.

Table 1. Baseline demographic characteristics of the study group

Parameter	MTX study group	MTX+ LEF study	p<0.05
No. of patients	25	25	NA
Sex	Females		NA
Age	42.5 ± 9.8	45.3 ± 11.2	NS
Disease duration	1.5 ± 1.2	1.9 ± 0.8	NS
Baseline DAS score	4.9 ± 0.4	5.0 ± 0.4	NS

DAS= Disease activity score, NS= non-significant, NA= not applicable

Table 2. The mean ±SD hematological analysis, liver enzymes and DAS_{28} before and post24 weeks of MTX and MTX + LEF therapy in RA patients

Parameter	MTX		MTX + LEF	
	Mean ± SD Baseline parameters	**Mean ± SD parameters post 24 weeks treatment**	**Mean ± SD Baseline parameters**	**Mean ± SD parameters post 24 weeks treatment**
Hgb (gm/dl)	11.3± 0.7	10.5± 0.5	11.3 ± 0.6	10.4 ± 0.5
RBCs count ($x10^6$/µl)	4.2 ± 0.5	2.0 ± 1.0	4.2 ± 0.4	3.8 ± 0.4
Hct (%)	35.1 ± 3.8	29 ± 2.6	33.9 ± 7.1	32.6 ± 3.1
MCV (fl)	84.1 ± 8.1	98.2 ±12.5	84.1 ±8.2	81.4 ± 10.1
MCH (pg)	28.2 ± 3.1	26.5 ± 8.5	28.2 ± 3.1	27.3 ± 4.1
MCHC (gm/dl)	33.1 ± 2.1	25 ± 1.8	32.7 ± 2.8	31.3 ± 3.8
WBCs ($x10^3$/µl)	6.5 ± 2.5	3.5 ± 2.3	6.7 ± 2.33	6.5 ± 1.7
Neutrophils (%)	59.4 ± 10.9	37.1 ± 4.3	58.5 ± 9.2	57.6 ± 5.5
Lymphocytes (%)	33.0 ± 10.2	55.0 ± 9.5	34.8 ± 9.9	34.2 ± 5.5
Monocytes %)	5.6 ± 2.5	7.5 ± 3.2	5.1 ± 2.4	6.2 ± 2.1
Eosinophils (%)	1.9 ± 1.1	3.9 ± 0.5	1.6 ± 0.8	1.6 ± 0.8
Basophils (%)	0.2 ± 0.5	1.0 ± 0.5	0.4 ± 0.5	0.5 ± 0.5
DAS_{28}	4.9 ± 0.4	4.2 ± 0.4	5.0 ± 0.4	3.8 ± 0.5
AST (IU/L)	24.8 ± 4.7	31.4 ± 4.1	23.5 ± 3.7	31.8 ± 3.7
ALT (IU/L)	22.2 ± 7.2	34.4 ± 7.6	21.1 ± 6.1	37.0 ± 7.7

Hgb= haemoglobin, g/dl= gram/decilitre, Hct= hematocrit, MCV= mean corpuscular volume, fl=femtolitre, MCH= mean corpuscular haemoglobin, Pg= picogram, MCHC= mean corpuscular hemoglobin concentration, DAS= Disease activity score, AST= aspartate aminotransferase, ALT= alanine aminotransferase

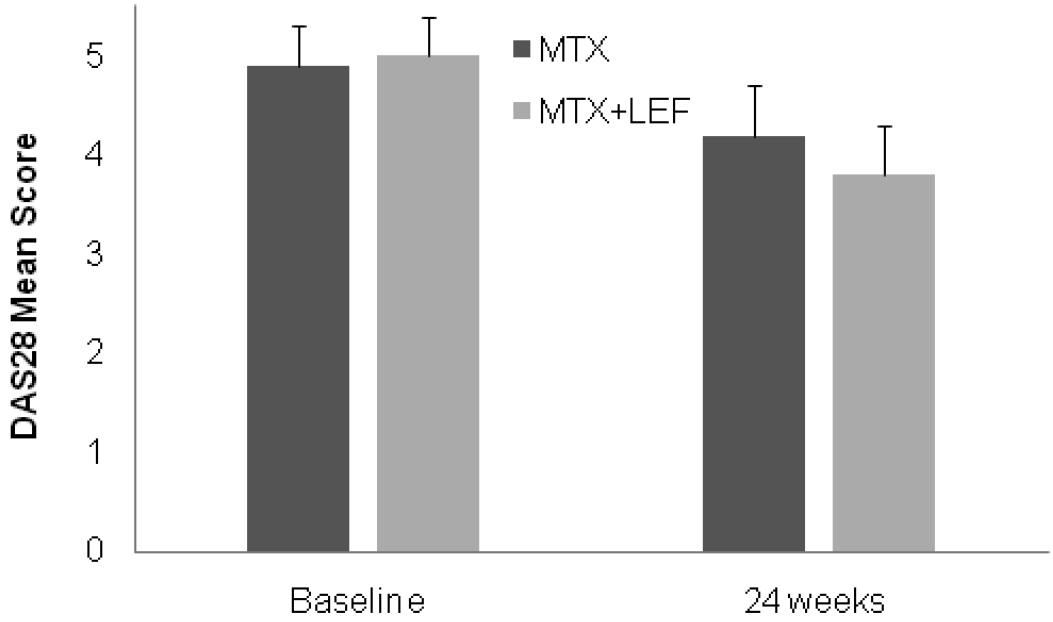

Figure 1.

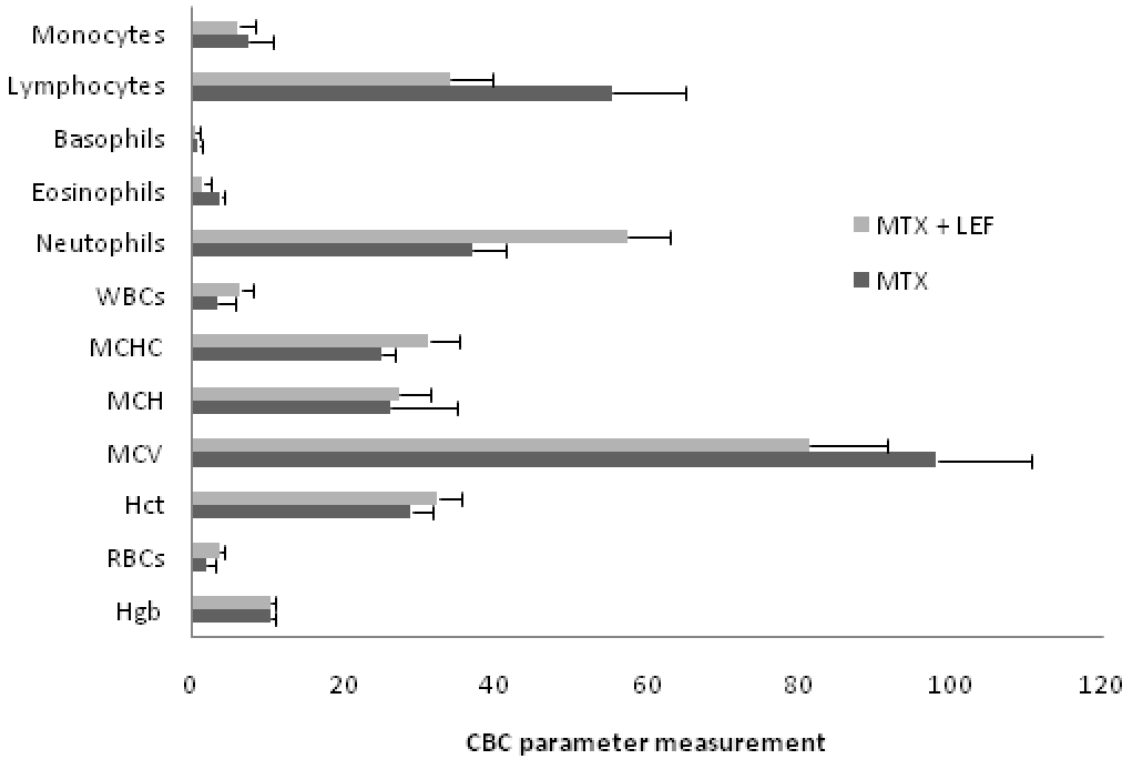

Figure 2.

Table 3. The mean ±SD increase in AST and ALT levels post six months treatment of MTX monotherapy and MTX+LEF combination therapy

Mean±SD (IU/L)	**MTX**	**MTX +LEF**
AST	6.7± 2.1	8.4 ±3.4
ALT	12.2± 3.7	15.8± 5.2

AST= aspartate aminotransferase, ALT= alanine aminotransferase

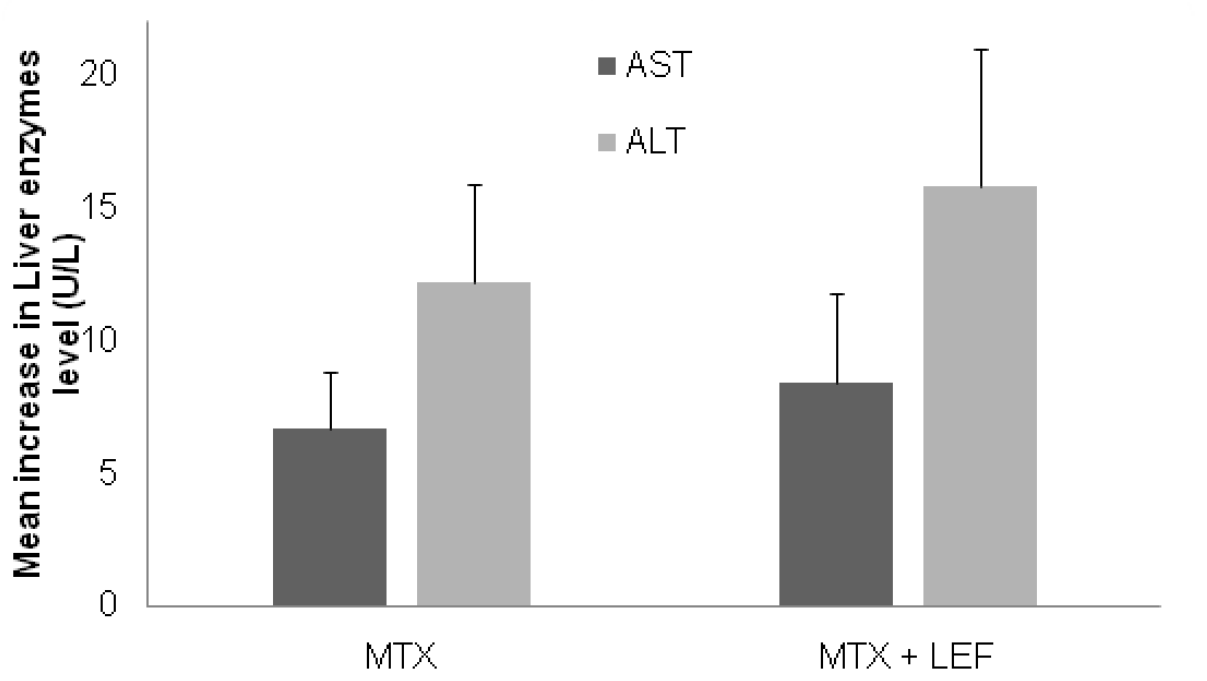

Figure 3.

Discussion

Despite the advent of the newer revolutionary management of RA by biologic therapies, several drug formularies in different countries still recommend MTX either singly or combined with other disease-modifying anti-rheumatic drugs (DMARDs)as first line therapy prior to stepping up to the more expensive biologic drugs [11,12]. However, prioritizing first line treatment with DMARDs is not only based on cost issues, but also combinations such as MTX +LEF have been considered safer regarding long term use, when compared to biologics, though there are no strict data to support this assumption [13].

Despite the central role that (DMARDs) continue to play in RA treatment, questions regarding the relative benefit and toxicity of using mono MTX versus MTX+LEF combination therapy still need to be addressed. According to a recent review by Singer et al

(2011), studies examining the previously demonstrated added benefit of combination MTX+LEF over either alone are still few. In addition, the safety profile of this combination has been re-examined in several studies, but showed conflicting results and remains a highly debatable issue [8].

Our study showed a significant decrease in mean DAS_{28}score with the combined therapy over that of MTX monotherapy. The mean±SD level of DAS_{28} obtained with LEF+MTX combination therapy were 3.8±0.5 vs 4.2 ± 0.4 obtained when using MTX alone post 24 weeks treatment period. Actually, the DAS_{28} score obtained with the combination therapy is closer to the remission value of DAS <3.2 which is the commonly used value to determine remission in several randomized clinical trials [14].

This confirms the substantial clinical benefit obtained when adding Leflunomide to MTX therapy and agrees with previous findings that similarly showed an added therapeutic benefit of concomitant use of LEF + MTX over either as monotherapy [15]. Similarly, another Cochrane analysis reported the superiority of MTX+LEF combination therapy over MTX mono therapy in improving DAS_{28} score and significantly reducing pain over 24 weeks [16].

Another randomized study by Kremer et al, have shown that the combination of MTX + LFN is superior to treatment with MTX alone where withdrawals due to adverse events were more frequent in the MTX+LEF group, while withdrawals owing to loss of efficacy were higher in the MTX group. Also in the very same study the MTX+ LEF group showed higher levels of aminotransferases, mild leucopenia and neutropenia [17].

Studies could not conclusively agree on safety of LEF alone or in combination with MTX. Although many randomized controlled trials to this point proved that LEF safety and efficacy profiles were comparable to MTX [18-22], other studies showed serious concerns regarding LEF hepatotoxicity that still needs clarification [22,23]. Several previous findings have suggested a greater toxicity (up to 5 fold increased incidence of LFT abnormalities) with LEF + MTX combination over MTX monotherapy [24] or increased risk of fibrosis when using this combination [25]. In addition another study showed a more effective therapy of MTX + LEF but with high incidence of hepatotoxicity than MTX alone [26].

Our findings indicated that MTX+LEF combination therapy may show more possible hepatotoxicity as evidenced by the more significant elevation in ALT ($p<0.01$) and AST ($p<0.05$) liver enzymes compared to the MTX monotherapy. MTX is a well-known hepatotoxic drug and LEF has also been associated with risk of liver disorders [27], therefore liver dysfunction is more likely to develop when LEF and MTX are used in combination. Noteworthy that our results with the LEF + MTX combination therapy only showed mild elevation in liver enzymes and none of our study patients withdrew from the study.

Although many studies agreed on the more hepatotoxicity of the MTX+LEF combination which is somewhat expected , it should be stressed that many of these studies that detected such elevated transaminase levels found them to be reversible with dose reduction or discontinuation of medication [13].

In fact, the European Agency for the Evaluation of Medicinal Products reported that the major hepatic adverse effects with LEF were only associated with other patient risk factors such as concomitant use of hepatotoxic drug, history of alcohol abuse, liver function disturbance, acute heart failure, severe pulmonary disease or pancreatic carcinoma [28].

Regarding the hematological parameters, MTX monotherapy showed more hematological toxicity represented by leucopenia (decreased WBC count $<3.5/mm^3$), anemia (hematocrit <30%), neutropenia (<54%), and mild macrocytosis (high MCV).

Despite that MTX mechanism of action in RA pathogenesis is yet unclear, it is assumed that its action as an anti-folate have been widely recognized as risk factor for its potential toxicity. Moreover, high MCV value (as observed in this study) has been recognized as a possible predictor of potential hematological toxicity and should be closely monitored and regulated [29,30].

Theoretically, it seems reasonable that folate supplementation with MTX should reduce such toxicological effects, and clinically, several studies have shown it to reduce the incidence of liver function abnormalities and hematological toxicity during MTX treatment. Folate supplementation does not affect MTX efficacy, inferring that MTX efficacy and toxicity are mediated by different metabolic pathways [29].

Contradicting, several previous studies results were reassuring about the safety of MTX+LEF combination such as the SMILE study [26] that reported no significant difference in LFTs abnormalities and incidence of neutropenia between either treatments alone or in combination. Other studies have shown that using MTX+LEF combination for a period of 25 months may cause rare LFT abnormalities that are reversible within 3 months of cessation of therapy [13]. Another study similarly showed patients using MTX + LEF therapy for a period of 8 months did not show any LFT abnormalities, hematologic disorders, or gastrointestinal complaints [8]. Similarly other studies also showed comparable safety profile of MTX+LEF versus MTX alone [10].

The contradiction in literature regarding each treatment regimen safety and efficacy is evident and the absolute preference for each treatment regimen over the other is still debatable.

The results of our study clearly suggested that MTX + LEF combination provides a potential clinical benefit with acceptable tolerability compared to MTX monotherapy. Nevertheless, the risk of liver toxicity from the combination should be strictly monitored throughout the whole treatment period and avoiding using it for patients with liver disease or with additional risk for hepatotoxicity.

Indeed, MTX + LEF is a more attractive option compared to MTX monotherapy especially in managing early RA patients provided that liver toxicity is effectively implemented throughout the treatment period.

For decades, it has been known that early therapeutic intervention in RA reduces long-term disability and joint damage. Since the ultimate goal of RA treatment is remission or approaching remission, thus, it seems quite reasonable to suggest that early intervention with the more effective combination treatment (MTX+LEF) in early active RA patients is the best strategy to yield less damage on the long run. Several clinical studies have emphasized the superiority of using DMARDs combination compared to a monotherapy in decreasing radiographic progression and improving remission without increasing toxicity especially in early RA patients. Noteworthy that according to literature, there is not a single early RA trial where combination treatment is not superior to monotherapy and not even more hazardous at the first 6 months of treatment [14].

Another privilege for the combination therapy is that while treatment delay is detrimental for the monotherapy in yielding better treatment outcomes, this was not the case with combination therapy where both early and delayed treatment similarly improved long term outcomes and delayed disease progression [14]. An advantage that must be considered to decrease potential damage in patients with treatment delays or in those whom early therapy with monotherapy was not feasible [32].

Furthermore, this combination therapy represents an economic alternative to the most costly biologic regimens. A previous study reported about five times more increase in medical annual costs for a RA patient treated with anti-TNF-αbiological agents than when the same patient treated with MTX + LEF.

However our study may be limited by including only female patients, as according to literature males are more responsive to treatment than females [31], however, yet, there is no conclusive evidence that sex is associated with a differential response to a definite therapy. In addition the small sample size and the short follow up time may be insufficient to identify all possible complications associated with LEF + MTX combination.

Conclusion

In summary, our results showed that MTX + LEF combination therapy is an attractive option to consider especially in early active RA patients more than the monotherapy. This is not only because it's more substantial clinical benefit in improving disease outcomes but also to aim remission as soon as possible where rapid effective treatment intervention becomes a priority to prevent future possible disease complications.

With respect to safety, the combination of MTX + LFN was reasonably well tolerated where it caused less hematological toxicity than MTX monotherapy. Despite that MTX+ LEF combination therapy showed more hepatotoxicity than MTX monotherapy, however, both agents should be preferably avoided in patients with underlying liver disease or those on other hepatotoxic medication. Close monitoring for liver enzymes is advised for both drugs especially when they are combined. Noteworthy that despite these limitations, so far, there is no evidence that leflunomide treatment is associated with the development of clinically significant liver disease.

Author Disclosure Statement

No Conflict of Interest and no competing financial interests exist.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23):2205-19.
2. Ding CZ, Yao Y, Feng XB, Fang Y, Zhao C, Wang Y. Clinical analysis of chinese patients with rheumatoid arthritis treated with leflunomide and methotrexate combined with different dosages of glucocorticoid. Curr Ther Res Clin Exp. 2012;73(4-5):123-33.
3. Smolen JS, Landewé R, Breedveld FC, Buch M, Burmester G, Dougados M, Emery P, Gaujoux-Viala C, Gossec L, Nam J, Ramiro S, Winthrop K, de Wit M, Aletaha D, Betteridge N, Bijlsma JW, Boers M, Buttgereit F, Combe B, Cutolo M, Damjanov N, Hazes JM, Kouloumas M, Kvien TK, Mariette X, Pavelka K, van Riel PL, Rubbert-Roth A, Scholte-Voshaar M, Scott DL, Sokka-Isler T, Wong JB, van der Heijde D. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis. 2014;73(3):492-509.
4. Van Vollenhoven RF. Treatment of rheumatoid arthritis: state of the art 2009. Nat Rev Rheumatol. 2009;5(10):531-41.
5. Emery P, Breedveld FC, Hall S, Durez P, Chang DJ, Robertson D, Singh A, Pedersen RD, Koenig AS, Freundlich B. Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): a randomised, double-blind, parallel treatment trial. Lancet. 2008;372(9636):375-82.
6. Dale J, Alcorn N, Capell H, Madhok R. Combination therapy for rheumatoid arthritis: methotrexate and sulfasalazine together or with other DMARDs. Nat Clin Pract Rheumatol. 2007;3(8):450-8; quiz, following 478.
7. Li EK, Tam LS, Tomlinson B. Leflunomide in the treatment of rheumatoid arthritis. Clin Ther. 2004;26(4):447-59.
8. Singer O, Gibofsky A. Methotrexate versus leflunomide in rheumatoid arthritis: what is new in 2011? Curr Opin Rheumatol. 2011;23(3):288-92.
9. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995;38(1):44-8.

10. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum. 1996;39(1):34-40.
11. Caporali R, Conti F, Alivernini S, Atzeni F, Seriolo B, Cutolo M, Valesini G, Ferraccioli G, Sarzi-Puttini P, Salvarani C, Guiducci S, Zampogna G, Gremese E, Pipitone N, Scrivo R, Bugatti S, Montecucco C, Matucci-Cerinic M; Italian Society for Rheumatology. Recommendations for the use of biologic therapy in rheumatoid arthritis: update from the Italian Society for Rheumatology I. Efficacy. Clin Exp Rheumatol. 2011;29(3 Suppl 66):S7-14.
12. Favalli EG, Biggioggero M, Meroni PL. Methotrexate for the treatment of rheumatoid arthritis in the biologic era: still an "anchor" drug? Autoimmun Rev. 2014;13(11):1102-8.
13. Londono J, Santos AM, Santos PI, Cubidez MF, Guzman C, Valle-Oñate R. Therapeutic efficacy and safety of methotrexate + leflunomide in Colombian patients with active rheumatoid arthritis refractory to conventional treatment. Rev Bras Reumatol. 2012;52(6):837-45.
14. Haraoui B, Pope J. Treatment of early rheumatoid arthritis: concepts in management. Semin Arthritis Rheum. 2011;40(5):371-88.
15. Kremer JM, Genovese MC, Cannon GW, Caldwell JR, Cush JJ, Furst DE, Luggen ME, Keystone E, Weisman MH, Bensen WM, Kaine JL, Ruderman EM, Coleman P, Curtis DL, Kopp EJ, Kantor SM, Waltuck J, Lindsley HB, Markenson JA, Strand V, Crawford B, Fernando I, Simpson K, Bathon JM. Concomitant leflunomide therapy in patients with active rheumatoid arthritis despite stable doses of methotrexate. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2002;137(9):726-33.
16. Katchamart W, Trudeau J, Phumethum V, Bombardier C. Methotrexate monotherapy versus methotrexate combination therapy with non-biologic disease modifying antirheumatic drugs for rheumatoid arthritis (Review). Cochrane Database Syst Rev. 2010;(4):CD008495.
17. Kremer J, Genovese M, Cannon GW, Caldwell J, Cush J, Furst DE, Luggen M, Keystone E, Bathon J, Kavanaugh A, Ruderman E, Coleman P, Curtis D, Kopp E, Kantor S, Weisman M, Waltuck J, Lindsley HB, Markenson J, Crawford B, Fernando I, Simpson K, Strand V. Combination leflunomide and methotrexate (MTX) therapy for patients with active rheumatoid arthritis failing MTX monotherapy: open-label extension of a randomized, double-blind, placebo controlled trial. J Rheumatol. 2004;31(8):1521-31.
18. Osiri M, Shea B, Robinson V, Suarez-Almazor M, Strand V, Tugwell P, Wells G. Leflunomide for treating rheumatoid arthritis. Cochrane Database Syst Rev. 2003;(1):CD002047.

19. Mladenovic V, Domljan Z, Rozman B, Jajic I, Mihajlovic D, Dordevic J, Popovic M, Dimitrijevic M, Zivkovic M, Campion G Safety and effectiveness of leflunomide in the treatment of patients with active rheumatoid arthritis. Results of a randomized, placebo-controlled, phase II study. Arthritis Rheum. 1995;38(11):1595-603.
20. Smolen JS, Kalden JR, Scott DL, Rozman B, Kvien TK, Larsen A, Loew-Friedrich I, Oed C, Rosenburg R. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. European Leflunomide Study Group. Lancet. 1999;353(9149):259-66.
21. Strand V, Cohen S, Schiff M, Weaver A, Fleischmann R, Cannon G, Fox R, Moreland L, Olsen N, Furst D, Caldwell J, Kaine J, Sharp J, Hurley F, Loew-Friedrich I. Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group. Arch Intern Med. 1999;159(21):2542-50.
22. Martin K, Bentaberry F, Dumoulin C, Dehais J, Haramburu F, Bégaud B, Schaeverbeke T. Effectiveness and safety profile of leflunomide in rheumatoid arthritis: actual practice compared with clinical trials. Clin Exp Rheumatol. 2005;23(1):80-4.
23. Siva C, Eisen SA, Shepherd R, Cunningham F, Fang MA, Finch W, Salisbury D, Singh JA, Stern R, Zarabadi SA. Leflunomide use during the first 33 months after food and drug administration approval: experience with a national cohort of 3,325 patients. Arthritis Rheum. 2003;49(6):745-51.
24. Curtis JR, Beukelman T, Onofrei A, Cassell S, Greenberg JD, Kavanaugh A, Reed G, Strand V, Kremer JM. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. Ann Rheum Dis. 2010;69(1):43-7.
25. Lee SW, Park HJ, Kim BK, Han KH, Lee SK, Kim SU, Park YB. Leflunomide increases the risk of silent liver fibrosis in patients with rheumatoid arthritis receiving methotrexate. Arthritis Res Ther. 2012;14(5):R232.
26. Bird P, Griffiths H, Tymms K, Nicholls D, Roberts L, Arnold M, Burnet S, de Jager J, Scott J, Zochling J, Littlejohn G. The SMILE study -- safety of methotrexate in combination with leflunomide in rheumatoid arthritis. J Rheumatol. 2013;40(3):228-35.
27. Gupta R, Gupta SK. Severe Hepatotoxicity in a Rheumatoid Arthritis Patient Switched From Leflunomide to Methotrexate. MedGenMed. 2005;7(3):9.
28. The European Agency for the Evaluation of Medicinal Products. EMEA issues public statement on leflunomide. Reactions Weekly. 2001;844:2. Updated 2014.
29. Whittle SL, Hughes RA. Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review. Rheumatology (Oxford). 2004;43(3):267-71.
30. Weinblatt ME, Fraser P. Elevated mean corpuscular volume as a predictor of hematologic toxicity due to methotrexate therapy. Arthritis Rheum. 1989;32(12):1592-6.

31. öttönen T, Hannonen P, Korpela M, Nissilä M, Kautiainen H, Ilonen J, Laasonen L, Kaipiainen-Seppänen O, Franzen P, Helve T, Koski J, Gripenberg-Gahmberg M, Myllykangas-Luosujärvi R, Leirisalo-Repo M; FIN-RACo Trial Group. FINnish Rheumatoid Arthritis Combination therapy. Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. Arthritis Rheum. 2002;46(4):894-8.
32. National Institute for Clinical Excellence. Guidance on the use of etanercept and infliximab for the treatment of rheumatoid arthritis: technology appraisal guidance, no 36. London: National Institute for Clinical Excellence. 2002. http://www.spitjudms.ro/_files/protocoale_terapeutice/reumatologie/ghid%20de%20tratament%20in%20par.pdf access date 03.07.2015