DMARDs–Gut Microbiota Feedback

Subjects: Biochemistry & Molecular Biology

Contributor: Iris Guzmán-Guzmán, Gloria Pérez Rubio

Evidence suggests that the increase or decrease of microorganism communities has an effect on the production of metabolites that are related with immunomodulatory functions. This review suggests that there is feedback between DMARDs and gut microbiota, based on the evidence that supports that DMARDs favor intestinal dysbiosis, as well as on the evidence that some bacterial genera participate in DMARDs-type xenobiotics' metabolism and in the production of metabolites with an immunomodulatory effect. This document sets a precedent in which DMARDs-promoted dysbiosis could cause, in time, variability of response to different therapeutic schemes.

Keywords: DMARDs ; Gut microbiota ; Response to therapy

1. Introduction

The clinical practice guides for the treatment of rheumatoid arthritis (RA) establish different therapeutic schemes to diminish the clinical activity, limit the articular radiological damage progression, and the functional incapacity. However, response to treatment is variable and this effect can be attributed to genetic factors as well as clinical or serologyc factors, and presence of comorbidities, among others[1][2].

Technological evolution has opened up the possibility of studying gut microbiota and also allows for the potential role in clinical characteristics and the response variability to RA treatment to be established. Originally, the microorganism recount in fecal culture allowed for the identification of big bacterial groups in the gut microbiota of RA patients[3][4][5][6]. The use of gas–liquid chromatography facilitated the identification of metabolites from bacteria[7][8] and real-time polymerase chain reaction (qPCR) analysis to quantitatively identify bacterial species [9][10][11][12][13]. Similarly, the analysis of bacterial 16s ribosomal ribonucleic acid sequencing (rRNA) [14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32] revolutionized the study of microbial diversity and the bacterial metagenome in RA[17][19][25][26][33][34][35][36][37][38]. In humans, the gut microbiome plays an important role in immunologic mechanisms and the inflammatory process. Changes in microbiota, influenced also by lifestyle and diet, may promote intestinal increased permeability and local inflammation, causing a spread of inflammation to the joints. Several nutrients such as polyunsaturated fatty acids, vitamin D, antioxidant, flavonoids, and probiotics present anti-inflammatory properties, featuring a protective role for RA development, while others such as red meat, high sugary drinks, and salt have a harmful effect[39]. Furthermore, the combination of probiotics and methotrexate (MTX) have been proven to contribute with the efficiency of the response to treatment[40][41] as well as in the specific variability of clinically relevant microbial species in the inflammatory process associated with RA [33].

Particularly, a great variability of bacterial species is shown in RA patients throughout the different clinical stages of the analyzed studies. However, cohort studies[6][25][33][37] allowed for an association between gut microbiota and pharmacological response variability in RA to be established. It is important then, to know the mechanisms that DMARDs put gut microbiota through and gut dysbiosis implications in the modulation of response to treatment as well as the strategies that should be followed to restore microbial symbiosis in RA.

2. Disease-Modifying Antirheumatic Drugs (DMARDs) Usage in RA

International guides of clinical practice recommend the use of DMARDs as a pharmacological treatment in RA. These could have a conventional synthetic origin (csDMARDs) such as methotrexate (MTX), sulfasalazine (SSZ), or leflunomide (LEF) and could be administered in monotherapy or a combined therapy. They could also be administered along with the gradual and temporal use of corticosteroids (Cs) [42]. Hydroxychloroquine (HCQ) and chloroquine (CLQ) are also recommended drugs[43][44]. Recently, treat to target therapy (T2T), which includes the combined use of MTX + SSZ + HCQ[45], has been suggested as the best therapy because of its efficiency at accomplishing clinical remission in RA patients through the rational use of drugs and because it is economically feasible[46]. The use of biological DMARDs

(boDMARDs)[42][47][48][49][50] or biosimilar DMARDs (bsDMARDs) is also included in treatment schemes when faced with a poor response to conventional drugs[51]. Nonetheless, the use of csDMARDs is still the principal strategy in RA treatment globally.

3. Gut Microbiota and csDMARDs' Metabolism

In vivo and in vitro studies revealed that gut microbiota has a role in the metabolism of approximately 50 drugs [52]. Zimmermann et al.[53] evaluated in an in vitro study 76 bacterial strains in gut microbiota and the metabolism of 271 drugs. They demonstrated that each bacterial strain metabolized from 11 to 95 drugs and that 176 drugs presented substantial metabolic change through the reduction of the drugs' active molecules by some bacterial strain, which would allow the suggestion that the bioavailability of DMARDs is subjected to bacterial metabolism.

MTX is the key drug in the T2T scheme. It is used orally or parenterally in RA treatment[54]. MTX's metabolism occurs through three different pathways. (1) Metabolized by gut bacteria in 2,4-diamino-N(10)-methylpteroic acid (DAMPA); a metabolite that represents less than 5% of MTX administered doses[55]. It has been proven that carboxypeptidase-G2 (CPDG2), a bacterial enzyme, induces the hydrolysis of MTX and non-toxic metabolite production such as DAMPA and glutamate[56][57][58][59]. In an in vitro study, it was proven that the species *Pseudomonas* catalyzed the synthesis of glutamate thorough CPDG2 from MTX[60], by which gut bacteria, which already modulates the drugs' active metabolite availability, can also possibly modulate its effects. (2) The second metabolic pathway occurs in the liver, where MTX biotransforms into 7-OH-MTX[61]. This metabolite is considered an inhibitor of human dihydrofolate reductase enzyme (DHFR). Gut microbiota also includes the DHFR enzyme, so it can modulate the drug's metabolism, and at the same time, the drug can modulate microbial metabolism, thus creating a strong relationship. (3) The third pathway happens through the intracellular conversion of MTX into polyglutamates. This pathway is considered the most important one given that it contains the principal mechanism of immunomodulation[62]. The principal cells and tissue where MTX is metabolized into polyglutamate derivates are: fibroblasts, myeloid precursors, keratinocytes, cortical and trabecular bone, and enterocytes[63], so homeostasis and intestinal barrier integrity are essential for the principal active form of MTX synthesis.

Gut microbiota is a key element in intestinal mucus' homeostasis and, aside from the fact that it has a direct participation in MTX's metabolism, it can indirectly regulate pharmacological metabolism through the maintenance of intestinal barrier integrity. It is known that microbial dysbiosis has an impact on mechanisms of translocation, immunomodulation, metabolism, and enzymatic degradation on a gut level and it compromises microbial diversity[64]. It was reported that administering high doses of MTX produces antibacterial activity, thus in vivo diminishing *Bacteroidetes* abundancy and the increase of *Firmicutes*[25][65]. Similarly, it was observed in murine models that treatment with MTX diminishes the abundance of *Bacteroides fragilis* [66], but not the one from the order *Lactobacillales*[67].

On the other hand, it was also proven that SSZ has effects over gut microbiota when it is administered in monotherapy or in combination with MTX[42][68]. SSZ is metabolized by gut microbiota through chemical reactions that are mediated by azoreductases, which reduce SSZ into sulfapyridine and 5-aminosalicylic acid (5-ASA/mesalazine). This last one is considered an anti-inflammatory component [52]. Most of 5-ASA is held in the colon and experiences enterohepatic recirculation and finally is excreted in the feces [69]. At the same time, 5-ASA could be inactivated by microbial arylamine of N-acetyltransferases (NATs)[70], inhibiting the anti-inflammatory effects of the drug. Sulfapyridine, on the other hand, has anti-microbial effects[71] and is also metabolized in the liver through the acetylation by the arylamine NAT-2, hydroxylation, and glucuronidation[72][73]. Due to all of the above, the abundance of bacteria that produces azoreductases—*Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Clostridium*, *Eubacterium*, and *Bacteroides* genus[74][75][76], and bacteria that produces NATs could have a relevant impact on the markers that define the response to RA treatment.

Regarding the use of LEF, it was established that its mechanism of immunomodulatory action happens through its active metabolite A 77 1726, which participates in the inhibition of pyrimidine's synthesis de novo by inhibiting dihydroorotate dehydrogenase and as a consequence, the lymphocyte proliferation[77][78]. To date, there is no direct evidence between LEF treatment and gut microbiota modulation, however, it is known that *Eggerthella lenta* uses ornithine as a substrate to generate energy, thus producing citrulline and carbamoyl-phosphate synthase. This last one is involved in the pyrimidine pathway, so its production by microbial species could be related to the active metabolism of LEF[78], and the production of citrulline along with the presence of citrullinated antigens of bacterial origin. Nonetheless, in the study of Chen et al.[19] the elevated abundance was not related to the citrulline serum levels.

CLQ and HCQ are drugs that are included in the treatment scheme for RA[43] because of their effects in the inhibition of the processing and presentation of antigens[79] and because they limit the activation and proliferation of T-lymphocytes and the synthesis of pro-inflammatory cytokines[80] such as TNF-α, IL-1β, IL-6[81], IL-17, and IL-22[82]. In a model of arthritic rats' K/BxN, it was shown that the consumption of HCQ increased the intestinal abundance of *Akkermansia* and

Parabacteroides while diminishing *Clostridium sensu stricto*-1[83]. In RA patients, Chen et. al. [19] reported that the intake of HCQ increased the bacterial diversity. Recently, it was described that the T2T scheme modulated the presence of bloodstream bacteria in RA patients by promoting the abundance of the genera *Haemophilus, Alloprevotella, Eremococcus*, and *Lachnospiraceae_UCG001*, possibly translocated from classic niches of the human microbiome[31].

The effect of boDMARDs on gut microbiota has also been shown in an arthritic rat model DBA/IJ, treated with etanercept (ETN). It was reported that there was a decrease in the relative abundance of bacterial genus *Escherichia/Shigella* and the genera *Lactobacillus*, *Clostridium XVIa,* and *Tannerella*[84]. This only strengthens the evidence of the intimate relationship between gut microbiota and the immune system, given that boDMARDs can have a direct immunomodulatory effect, local or systemic, over their cellular targets as well as the indirect way through gut microbiota modulation.

Therefore, DMARDs-promoted dysbiosis could cause, in time, variability of response to different therapeutic schemes.

References

1. Katchamart, W.; Johnson, S.; Lin, H.J.; Phumetthum, V.; Salliot, C.; Bombardier, C. Predictors for remission in rheumat oid arthritis patients: A systematic review. Arthritis Care Res. 2010, 62, 1128–1143.
2. Wijbrandts, C.A.; Tak, P.P. Prediction of Response to Targeted Treatment in Rheumatoid Arthritis. Mayo Clin. Proc. 201 7, 92, 1129–1143.
3. Shinebaum, R.; Neumann, V.C.; Cooke, E.M.; Wright, V. Comparison of faecal florae in patients with rheumatoid arthriti s and controls. Rheumatology 1987, 26, 329–333.
4. Neumann, V.C.; Shinebaum, R.; Cooke, E.M.; Wright, V. Effects of sulphasalazine on faecal flora in patients with rheum atoid arthritis: A comparison with penicillamine. Br. J. Rheumatol. 1987, 26, 334–337.
5. Dearlove, S.M.; Barr, K.; Neumann, V.; Isdale, A.; Bird, H.A.; Gooi, H.C.; Wright, V. The effect of non-steroidal anti-infla mmatory drugs on fecal flora and bacterial antibody levels in rheumatoid arthritis. Br. J. Rheumatol. 1992, 31, 443–447.
6. Bradley, S.M.; Neumann, V.C.; Barr, K.; Troughton, P.R.; Astbury, C.; Bird, H.A.; Gooi, H.C.; Wright, V. Sequential study of bacterial antibody levels and faecal flora in rheumatoid arthritis patients taking sulphasalazine. Br. J. Rheumatol. 199 3, 32, 683–688.
7. Eerola, E.; Möttönen, T.; Hannonen, P.; Luukkainen, R.; Kantola, I.; Vuori, K.; Tuominen, J.; Toivanen, P. Intestinal flora in early rheumatoid arthritis. Br. J. Rheumatol. 1994, 33, 1030–1038.
8. Peltonen, R.; Nenonen, M.; Helve, T.; Hänninen, O.; Toivanen, P.; Eerola, E. Faecal microbial flora and disease activity in rheumatoid arthritis during a vegan diet. Br. J. Rheumatol. 1997, 36, 64–68.
9. Maeda, Y.; Matsushita, M.; Katayama, M.; Yoshimura, M.; Watanabe, A.; Tanaka, E.; Tsuji, S.; Kitatobe, A.; Harada, Y.; Oshima, S.; et al. SAT0079 The analysis of fecal microbiota in rheumatoid arthritis patients compared to healthy volunt eers using bacterial RRNA-targeted reverse transcription-quantitative PCR. EULAR Abstracts. Ann. Rheum. Dis. 2012, 71, 496.
10. Liu, X.; Zou, Q.; Zeng, B.; Fang, Y.; Wei, H. Analysis of fecal lactobacillus community structure in patients with early rhe umatoid arthritis. Curr. Microbiol. 2013, 67, 170–176.
11. Maeda, Y.; Matsushita, M.; Yura, A.; Teshigawara, S.; Katayama, M.; Yoshimura, M.; Watanabe, A.; Tanaka, E.; Tsuji, S.; Kitatobe, A.; et al. OP0191 The Fecal Microbiota of Rheumatoid Arthritis Patients Differs from that of Healthy Volunt eers and is Considerably Altered by Treatment with Biologics. EULAR Abstracts. Ann. Rheum. Dis. 2013, 72, A117.
12. Maeda, Y.; Motooka, D.; Nii, T.; Matsumoto, Y.; Matsushita, M.; Saeki, Y.; Narazaki, M.; Kumanogoh, A.; Nakamura, S.; Takeda, K. AB0139 Investigation of prevotella copri from rheumatoid arthritis patients. EULAR Abstracts. Ann. Rheum. Dis. 2018, 77, 1261.
13. Rodrigues, G.S.P.; Cayres, L.C.F.; Gonçalves, F.P.; Takaoka, N.C.; Lengert, A.H.; Tansini, A.; Brisotti, J.L.; Sasdelli, C. B.G.; Oliveira, G.L.V. Detection of increased relative expression units of Bacteroides and Prevotella, and decreased Cl ostridium leptum in stool samples from Brazilian rheumatoid arthritis patients: A pilot study. Microorganisms 2019, 7, 41 3.
14. Toivanen, P.; Vartiainen, S.; Jalava, J.; Luukkainen, R.; Möttönen, T.; Eerola, E.; Mannienen, R. Intestinal anaerobic ba cteria in early rheumatoid arthritis (RA). Arthritis Res. 2002, 4, 5.
15. Vaahtovuo, J.; Munukka, E.; Korkeamäki, M.; Luukkainen, R.; Toivanen, P. Fecal microbiota in early rheumatoid arthriti s. J. Rheumatol. 2008, 35, 1500–1505.
16. Gul'neva, M.; Noskov, S.M. Colonic microbial biocenosis in rheumatoid arthritis. Klin. Med. 2011, 89, 45–48.

17. Scher, J.U.; Sczesnak, A.; Longman, R.S.; Segata, N.; Ubeda, C.; Bielski, C.; Rostron, T.; Cerundolo, V.; Pamer, E.G.; Abramson, S.B.; et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. eLife 2 013, 2, e01202.

18. Tap, J.; Abou-Ghantous, J.; Leboime, A.; Nahal, R.S.; Langella, P.; Garchon, H.J.; Chiocchia, G.; Furet, J.P.; Breban, M. Gut microbiota variations correlate with disease activity in spondylarthritis (SpA) and rheumatoid arthritis (RA). ACR Meeting Abstracts. Arthritis Rheumatol. 2014, 662. Available online: https://acrabstracts.org/abstract/gut-microbiota-vari ations-correlate-withdisease-activity-in-pondyloarthritis-spa-and-rheumatoid-arthritis-ra/ (accessed on 24 January 202 0).

19. Chen, J.; Wright, K.; Davis, J.M.; Jeraldo, P.; Marietta, E.V.; Murray, J.; Nelson, H.; Matteson, E.L.; Taneja, V. An expan sion of rare lineage intestinal microbes characterizes rheumatoid arthritis. Genome Med. 2016, 8, 43.

20. Maeda, Y.; Kurakawa, T.; Umemoto, E.; Motooka, D.; Ito, Y.; Gotoh, K.; Hirota, K.; Matsushita, M.; Furuta, Y.; Narazaki, M.; et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. Arthritis Rh eumatol. 2016, 68, 2646–2661.

21. Breban, M.; Tap, J.; Leboime, A.; Said-Nahal, R.; Langella, P.; Chiocchia, G.; Furet, J.P.; Sokol, H. Faecal microbiota st udy reveals specific dysbiosis in spondyloarthritis. Ann. Rheum. Dis. 2017, 76, 1614–1622.

22. Piccianti-Diamanti, A.; Penebianco, C.; Salemi, S.; Sorgi, M.L.; Rosa, R.D.; Tropea, A.; Sgrulleti, M.; Salerno, G.; Terrac ciano, F.; D'Amelio, R.; et al. Analysis of gut microbiota in rheumatoid arthritis patients: Disease-related dysbiosis and modifications induced by etanercept. Int. J. Mol. Sci. 2018, 19, 2938.

23. Forbes, J.D.; Chen, C.Y.; Knox, N.C.; Marrie, R.A.; El-Gabalawy, H.; Kievit, T.; Alfa, M.; Bernstein, C.N.; Domselaar, G. V. A comparative study of the gut microbiota in immune-mediated inflammatory diseases-does a common dysbiosis exi st? Microbiome 2018, 6, 221.

24. Alpizar-Rodríguez, D.; Lesker, T.R.; Gronow, A.; Gilbert, B.; Raemy, E.; Lamacchia, C.; Gabay, C.; Finckh, A.; Strowig, T. Prevotella copri in individuals at risk for rheumatoid arthritis. Ann. Rheum. Dis. 2019, 78, 590–593.

25. Nayak, R.R.; Alexander, M.; Stapleton-Grey, K.; Ubeda, C.; Scher, U.U.; Turnbaugh, P.J. Perturbation of the human gut microbiome by a non-antibiotic drug contributes to the resolution of autoimmune disease. bioRxiv 2019.

26. Jeong, Y.; Kim, J.W.; You, H.J.; Park, S.J.; Lee, J.; Ju, J.H.; Park, M.S.; Jin, H.; Cho, M.L.; Kwon, B.; et al. Gut microbio ta composition and function are altered in patients with early rheumatoid arthritis. J. Clin. Med. 2019, 8, 693.

27. Lee, J.Y.; Mannaa, M.; Kim, Y.; Kim, J.; Kim, G.T.; Seo, Y.S. Comparative analysis of fecal microbiota composition betw een rheumatoid arthritis and osteoarthritis patients. Genes 2019, 10, 748.

28. Sun, Y.; Chen, Q.; Lin, P.; Xu, R.; He, D.; Ji, W.; Bian, Y.; Shen, Y.; Li, Q.; Liu, C.; et al. Characteristics of gut microbiota in patients with rheumatoid arthrtis in Shangai, China. Front. Cell. Infect. Microbiol. 2019, 9, 369.

29. Chiang, H.I.; Li, J.R.; Liu, C.C.; Liu, P.Y.; Chen, H.H.; Chen, Y.M.; Lan, J.L.; Chen, D.Y. An association of gut microbiota with different phenotypes in chinese patients with rheumatoid arthritis. J. Clin. Med. 2019, 8, 1770.

30. Tong, Y.; Bai, Y.; Zhao, Y.; Lui, Y.; Luo, Y. Intestinal microbiota dynamics in the progression of rheumatoid arthritis. ACR Meeting Abstracts. Arthritis Rheumatol. 2019, 71. Available online: https://acrabstracts.org/abstract/intestinal-microbiota -dynamics-in-theprogression-of-rheumatoid-arthritis/ (accessed on 24 February 2020).

31. Hammad, D.B.M.; Hider, S.L.; Liyanapathirana, V.C.; Tonge, D.P. Molecular Characterization of Circulatins Microbiome sigantures in Rheumatoid Arthritis. Front. Cell. Infect. Microbiol. 2020, 9, 440.

32. Mena-Vázquez, N.; Ruiz-Limón, P.; Moreno-Indias, I.; Manrique-Arija, S.; Tinahones, F.J.; Fernández-Nebro, A. Expans ion of Rare and Harmful Lineages is Associated with Established Rheumatoid Arthritis. J. Clin. Med. 2020, 9, 1044.

33. Zhang, X.; Zhang, D.; Jia, H.; Feng, Q.; Wang, D.; Liang, D.; Wu, X.; Li, J.; Tang, L.; Li, Y.; et al. The oral and gut micro biomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat. Med. 2015, 21, 895–905.

34. Muñiz-Pedrogo, D.A.; Chen, J.; Hillmann, B.; Jeraldo, P.; Al-Ghalith, G.; Taneja, V.; Davis, J.M.; Khights, D.; Nelson, H.; Faubion, W.A.; et al. An increased abundance of Clostridiaceae characterizes arthritis in inflammatory bowel disease a nd rheumatoid arthritis: A cross-sectional study. Inflamm. Bowel Dis. 2019, 25, 902–913.

35. Isaac, S.; Artacho, A.; Nayack, R.; Flor, A.; Abramson, S.; Rosenthal, P.; Puchades, L.; Scher, J. OP0119 The pre-treat ment gut microbiome predicts early response to rheumatoid therapy. EULAR Abstracts. Ann. Rheum. Dis. 2019, 78, 13 3–134.

36. Cedola, F.; Coras, R.; Sanchez-Lopez, E.; Mateo, L.; Pedersen, A.; Brandy-García, A.; Prior-Español, Á.; Rosental, B. S.; Martínez-Morillo, M.; Guma, M. Choline metabolite is associated with inflammation in arthritis in the elderly. ACR Me eting Abstracts. Arthritis Rheumatol. 2019, 71. Available online: https://acrabstracts.org/abstract/choline-metabolite-is-a ssociated-withi-inflammation-in-arthritis-in-the-elderly/ (accessed on 13 December 2019).

37. Isaac, S.; Artacho, A.; Nayack, R.; Abramson, S.B.; Alexander, M.; Koo, I.; Rosenthal, P.; Izmirly, P.; Petterson, A.; Pineda, A.; et al. The pre-treatment gut microbiome predicts early response to metrothexte in rheumatoid arthritis. ACR Meeting Abstracts. Arthritis Rheumatol. 2019, 71. Available online: https://acrabstracts.org/abstract/the-pre-treatment-gut-microbiomepredicts-early-response-to-methotrexate-in-rheumatoid-arthritis/ (accessed on 2 March 2020).

38. Kishikawa, T.; Maeda, Y.; Nii, T.; Motooka, D.; Matsumoto, Y.; Matsushita, M.; Matsuoka, H.; Yoshimura, M.; Kawada, S.; Teshigawara, S.; et al. Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the japanese population. Ann. Rheum. Dis. 2019, 79, 103–111.

39. Gioia, C.; Lucchino, B.; Tarsitano, M.G.; Iannuccelli, C.; Di Franco, M. Dietary Habits and Nutrition in Rheumatoid Arthritis: Can Diet Influence Disease Development and Clinical Manifestations? Nutrients 2020, 12, 1456.

40. Rovenský, J.; Svík, K.; Stancíková, M.; Istok, R.; Ebringer, L.; Ferencík, M. Treatment of experimental adjuvant arthritis with the combination of methotrexate and lyophilized Enterococcus faecium enriched with organic selenium. Folia Microbiol. 2002, 47, 573–578.

41. Lowe, J.R.; Briggs, A.M.; Whittle, S.; Stephenson, M.D. A systematic review of the effects of probiotic administration in inflammatory arthritis. Complement. Ther. Clin. Pract. 2020, 40, 101207.

42. Smolen, J.S.; Landewé, R.; Bijlsma, J.; Burmester, G.; Chatzidionysiou, K.; Dougados, M.; Nam, J.; Ramiro, S.; Voshaar, M.; van Vollenhoven, R.; et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann. Rheum. Dis. 2017, 76, 960–977.

43. Aviña-Zubieta, J.A.; Galindo-Rodríguez, G.; Newman, S.; Suarez-Almazor, M.E.; Russell, A.S. Long term effectiveness of antimalarial drugs in rheumatics diseases. Ann. Rheum. Dis. 1998, 57, 582–587.

44. Cardiel, M.H.; Pons-Estel, B.A.; Sacnun, M.P.; Wojdyla, D.; Saurit, V.; Marcos, J.C.; Pinto, M.R.C.; Cordeiro de Aceveda, A.B.; da Silveria, I.G.; Radominski, S.C.; et al. Treatment of early rheumatoid arthritis in a multinational inception cohort of Latin American Patients: The GLADAR experience. J. Clin. Rheumatol. 2012, 18, 327–335.

45. Smolen, J. Treat to target in rheumatology: A historical account on occasion of the 10th aniversary. Rheum. Dis. Clin. N. Am. 2019, 45, 477–485.

46. Drosos, A.A.; Pelechas, E.; Voulgari, P.V. Treatment strategies are more important than drugs in the management of rheumatoid arthritis. Clin. Rheumatol. 2020, 39, 1363–1368.

47. Emery, P.; Keystone, E.; Tony, H.P.; Cantagrel, A.; van Vollenhoven, R.; Sanchez, A.; Alecock, E.; Lee, J.; Kremer, J. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumor necrosis factor biological: Results from a 24-week multicenter randomized placebo-controlled trial. Ann. Rheum. Dis. 2008, 67, 516–523.

48. Smolen, J.S.; Beaulieu, A.; Rubbert-Roth, A.; Ramos-Remus, C.; Rovensky, J.; Alecock, E.; Woodworth, T.; Alten, R.; OPTION Investigators. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): A double-blind, placebo-controlled, randomised trial. Lancet 2008, 371, 987–997.

49. Smolen, J.S.; Kay, J.; Doyle, M.K.; Landawé, R.; Matteson, E.L.; Wollenhaupt, J.; Gaylis, N.; Murphy, F.T.; Neal, J.S.; Zhou, Y.; et al. Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor alpha inhibitors (GO-AFTER study): A multicentre, randomised, double-blind, placebo-controlled, phase III trial. Lancet 2009, 374, 210–221.

50. Smolen, J.S.; Landewé, R.; Bijlsma, J.W.J.; Burmester, G.R.; Dougados, M.; Kerschbaumer, A.; McIness, I.B.; Sepriano, A.; van Vollenhoven, R.F.; de Wit, M.; et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann. Rheum. Dis. 2020, 79, 685–699.

51. Kay, J.; Schoels, M.M.; Dörner, T.; Emery, P.; Kvien, T.K.; Smolen, J.S.; Breedveld, F.C. Task Force on the Use of Biosimilars to Treat Rheumatological Diseases. Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. Ann. Rheum. Dis. 2018, 77, 165–174.

52. Haiser, H.J.; Turnbaugh, P.J. Developing a metagenomic view of xenobiotic metabolism. Pharmacol. Res. 2013, 69, 21–31.

53. Zimmermann, M.; Zimmermann-Kogadeeva, M.; Wegmann, R.; Goodman, A.L. Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature 2019, 570, 462–467.

54. Visser, K.; Van Der Heijde, D. Optimal dosage and route of administration of methotrexate in rheumatoid arthritis: A systematic review of the literature. Ann. Rheum. Dis. 2009, 68, 1094–1099.

55. Mack, D.R.; Young, R.; Kaufman, S.S.; Ramey, L.; Vanderhoof, J.A. Methotrexate in patients with crohn's disease after 6-mercaptopurine. J. Pediatr. 1998, 132, 830–835.

56. Widemann, B.C.; Hetherington, M.L.; Murphy, R.F.; Balis, F.M.; Adamson, P.C. Carboxypeptidase-G2 rescue in a patient with high dose methotrexate-induced nephrotoxicity. Cancer 1995, 76, 521–526.

57. Buchen, S.; Ngampolo, D.; Melton, R.G.; Hasan, C.; Zoubek, A.; Henze, G.; Bode, U.; Fleischhack, G. Carboxypeptidase G2 rescue in patients with methotrexate intoxication and renal failure. Br. J. Cancer 2005, 92, 480–487.

58. Gorostegui, M.; Martínez, E.; Llort, A.; Gros, L.; Dapena, J.L.; Hidalgo, E.; Oliveras, M.; Díaz de Heredia, C.; Bastida, P.; Sánchez de Toledo, J. Carboxipeptidasa G2 (CPDG2) en el rescate de la nefrotoxicidad inducida por metotrexato a altas dosis (MTXHD). An. Pediatr. 2007, 66, 434.

59. Ramsey, L.B.; Balis, F.M.; O'Brien, M.M.; Schmiegelow, K.; Pauley, J.L.; Bleyer, A.; Widemann, B.C.; Askenazi, D.; Bergeron, S.; Shirali, A.; et al. Consensus guideline for use of glucarpidase in patients with high-dose methotrexate induced acute kidney injury and delayed methotrexate clearance. Oncologist 2018, 23, 52–61.

60. Levy, C.C.; Goldman, P. The enzymatic hydrolysis of methotrexate and folic acid. J. Biol. Chem. 1967, 242, 2933–2938.

61. Seideman, P.; Beck, O.; Eksborg, S.; Wennberg, M. The pharmacokinetics of methotrexate and its 7-hydroxymetabolite in patients with rheumatoid arthritis. Br. J. Clin. Pharmacol. 1993, 35, 409–412.

62. Grim, J.; Chladek, J.; Martinkova, J. Pharmacokinetics and pharmacodynamics of methotrexate in non-neoplastic diseases. Clin. Pharmacokinet. 2003, 42, 139–151.

63. Treon, S.P.; Chabner, B.A. Concepts in use of high-dose methotrexate therapy. Clin. Chem. 1996, 42, 1322–1329.

64. Alexander, J.L.; Wilson, I.D.; Teare, J.; Marchesi, J.R.; Nicholson, J.K.; Kinross, J.M. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat. Rev. Gastroenterol. Hepatol. 2017, 14, 356–365.

65. Nayak, R.R.; Stapleton-Gray, K.; O'Loughlin, C.; Fischbach, M.; Turnbaugh, P.J. Methotrexate is an antibacterial drug metabolized by human gut bacteria. ACR Meeting Abstracts. Arthritis Rheumatol. 2017, 69. Available online: https://acrabstracts.org/abstract/methotrexate-is-an-antibacterial-drug-metabolized-by-human-gut-bacteria-2/ (accessed on 22 October 2020).

66. Zhou, B.; Xia, X.; Wang, P.; Chen, S.; Yu, C.; Huang, R.; Zhang, R.; Wang, Y.; Lu, L.; Yuan, F.; et al. Induction and amelioration of methotrexate-induced gastrointestinal toxicity are related to immune response and gut microbiota. EBioMedicine 2018, 33, 122–133.

67. Tang, D.; Zeng, T.; Wang, Y.; Cui, H.; Wu, J.; Zou, B.; Tao, Z.; Zhang, L.; Garside, G.B.; Tao, S. Dietary restriction increases protective gut bacteria to rescue lethal methotrexate-induced intestinal toxicity. Gut Microbes 2020, 12, 1714401.

68. Smolen, J.S.; Landewé, R.; Breedveld, F.C.; Buch, M.; Burmester, G.; Dougados, M.; Emery, P.; Gaujoux-Viala, C.; Gossec, L.; Nam, J.; et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann. Rheum. Dis. 2014, 73, 492–509.

69. Azadkhan, A.; Truelove, S.; Aronson, J. The disposition and metabolism of sulphasalazine (salicylazosulphapyridine) in man. Br. J. Clin. Pharmacol. 1982, 13, 523–528.

70. Deloménie, C.; Fouix, S.; Longuemaux, S.; Brahimi, N.; Picard, B.; Denamur, E.; Dupret, J.M. Identification and functional characterization of arylamine N-acetyltransferases in eubacteria: Evidence for highly selective acetylation of 5-aminosalicylic acid. J. Bacteriol. 2001, 183, 3417–3427.

71. Bishop, J.B.; Witt, K.L.; Gulati, D.K.; MacGregor, J.T. Evaluation of the mutagenicity of the anti-inflammatory drug salicylazosulfapyridine (SASP). Mutagenesis 1990, 5, 549–554.

72. Das, K.M.; Eastwood, M.A.; McManus, J.P.; Sircus, W. Adverse reactions during salicylazosulfapyridine therapy and the relation with drug metabolism and acetylator phenotype. N. Engl. J. Med. 1973, 289, 491–495.

73. Pullar, T.; Capell, H.A. Variables affecting efficacy and toxicity of sulphasalazine in rheumatoid arthritis. A review. Drugs 1986, 32, 54–57.

74. Peppercorn, M.A.; Goldman, P. The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J. Pharmacol. Exp. Ther. 1972, 181, 555–562.

75. Rafii, F.; Franklin, W.; Cerniglia, C.E. Azoreductase activity of anaerobic bacteria isolated from human intestinal microflora. Appl. Environ. Microbiol. 1990, 7, 2146–2151.

76. Rafii, F.; Cerniglia, C.E. Reduction of azo dyes and nitroaromatic compounds by bacterial enzymes from the human intestinal tract. Environ. Health Perspect. 1995, 103, 17–19.

77. Fox, R.I. Mechanism of action of leflunomide in rheumatoid arthritis. J. Rheumatol. Suppl. 1998, 53, 20–26.

78. Breedveld, F.C.; Dayer, J.M. Leflunomide: Mode of action in the treatment of rheumatoid arthritis. Ann. Rheum. Dis. 2000, 59, 841–849.

79. Beynen, A.C.; Van der Molen, A.J.; Geelen, M.J. Inhibition of hepatic cholesterol biosynthesis by chloroquine. Lipids 1981, 16, 472–474.

80. Kyburz, D.; Brentano, F.; Gay, S. Mode of action of hydroxychloroquine in RA-evidence of an inhibitory effect on toll-like receptor signaling. Nat. Clin. Pract. Rheumatol. 2006, 2, 458–459.
81. Jang, C.H.; Choi, J.H.; Byun, M.S.; Jue, D.M. Chloroquine inhibits production of TNF-α, IL-1β and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. Rheumatology 2006, 45, 703–710.
82. Silva, J.C.; Mariz, H.A.; Rocha, L.F., Jr.; Oliveira, P.S.; Dantas, A.T.; Duarte, A.L.; Pitta, I.; Galindo, S.L.; Pitta, M.G. Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients. Clinics 2013, 68, 766–771.
83. Shi, N.; Zahng, S.; Silverman, G.; Li, M.; Cai, J.; Niu, H. Protective effect of hydroxychloroquine on rheumatoid arthritis-associated atherosclerosis. Anim. Models Exp. Med. 2019, 2, 98–106.
84. Wang, B.; He, Y.; Tang, J.; Ou, Q.; Lin, J. Alteration of the gut microbiota in tumor necrosis factor-α antagonist-treated collagen-induced arthritis mice. Int. J. Rheum. Dis. 2020, 23, 472–479.