**Abstract.** Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation of the joints. Generally, this disease is suffered by elderly patients. However, it is possible to occur at a young age, such as juvenile rheumatoid arthritis (JRA), the most common type of arthritis in children and adolescents. Conventional therapies given to patients with rheumatoid arthritis to modulate the immune response, including disease-modifying anti-rheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs), have limited therapeutic effects in RA patients. Long-term use of these drugs can cause side effects and resistance to therapy. In recent years mesenchymal stem cells (MSCs) are highly recommended therapy. They are considered promising because MSCs are potential immunomodulators that can differentiate into various cell types and stimulate tissue repair. These cells also have strong chemotactic abilities because they can migrate to damaged tissues and act as an anti-inflammatory. Therefore, MSC is potentially suitable for autoimmune therapy, plus there has been a lot of research evidence (clinical trials) stating that there are no toxicity and side effects in the long term. One type of MSC based on its tissue source is umbilical cord mesenchymal stem cells, which are believed to be the best among other types in terms of non-invasive isolation procedure, superior biological characteristics such as low immunogenicity, better separation efficiency, differentiation, and self-renewal ability [1]. This review discusses a UC-MSC-based therapeutic approach in children, adults, and the elderly focusing on published clinical data and clinical trials for the treatment of RA that are currently ongoing.

**Key words:** UC-MSC, rheumatoid arthritis, juvenile idiopathic arthritis, safety, efficacy

**INTRODUCTION**

Rheumatoid arthritis (RA) is the most common cause of chronic inflammatory disease characterized by symmetrical peripheral polyarthritis of the joints. RA manifestation can be present in various extra-articular organs because it is systemic. Some cases show a progressive disease course that leads to disability and even death.

Worldwide, RA occurs in 0.5-1% of the adult population. During the last decade, the incidence of RA has decreased while the prevalence has remained the same [2]. In Indonesia, the prevalence varies from 0.3-0.6% in different areas [3].

More women than men experience RA. The ratio reaches 2:3:1, while Latin America and African countries show ratio of 6-8:1. The pathomechanism of synovial joint inflammation is a combination of genetic, environmental, and immunological factors, which can lead to immune system dysregulation and the destruction of self-tolerance mechanisms. However, what initiates the interference of this condition with the immune system, in terms of genetic or environmental factors, is still not fully understood [2].

In addition, no therapy is approved that cures RA until now. The principle of RA therapy is only to control inflammation as quickly as possible to improve the condition. Therefore, corticosteroids are needed to overcome inflammation with a rapid onset. In addition, disease-modifying anti-rheumatic drugs (DMARDs) will be given simultaneously. DMARDs are divided into non-biologic (methotrexate) and biologic (TNF inhibitor & IL-6). Biological DMARDs are usually used, when there are no satisfactory results from non-biological DMARDs. Then non-steroidal anti-inflammatory drugs (NSAIDs) can be given to control pain and stiffness in the joints [4].

However, conventional therapies mentioned above have limited therapeutic effects in RA patients. Long-term use of these drugs can cause side effects and resistance to therapy. In recent years mesenchymal stem cell (MSC) has become highly recommended therapy. It is considered promising because there has been a lot of research evidence (clinical trials) stating that there are no toxicity and side effects in the long term [5].

MSCs are stem cells that can differentiate into endoderm, mesoderm, and ectoderm cells [6]. MSCs are potential immunomodulators to suppress and regulate the immune response that can proliferate and differentiate into various types of cells and stim-
ulate tissue repair with their self-renewal capacity [7, 8]. These cells also have strong chemotactic abilities because they can migrate to damaged tissues and act as anti-inflammatory [7]. Therefore, MSC is suitable for autoimmune therapy [7, 8]. MSC has several types based on its source. Clinical studies show that umbilical cord mesenchymal stem cell (UC-MSC) occupies the highest prevalence compared to MSC from other tissue sources, used in nearly 50% of active studies [5]. Thus, the authors assess the need for writings that comprehensively discuss the potential of Umbilical Cord-Mesenchymal Stem Cell (UC-MSC) for Rheumatoid Arthritis (RA) therapy through research sources carried out and synthesize secondary data using a systematic study method [9].

**METHOD**

The research design used was a Systematic Review study, following the PRISMA 2020 guidelines (Preferred Reporting Items for Systematic reviews and Meta-Analyses). Literature search strategy using the PICO method: P = RA patients, I = UC-MSC therapy, C = placebo, O = safety and efficacy.

In outcome, the variables used to assess safety were adverse events, laboratory tests, and investigations, while for efficacy, namely clinical improvement, dose reduction, recurrence, efficacy levels, immunological evaluation, as well as several evaluation indices such as disease activity score-28 (DAS28), visual analog scale (VAS), health assessment questionnaire (HAQ), European league against rheumatism (EULAR), and American College of Rheumatology 20/50/70 criteria (ACR 20/50/70).

**Eligibility Criteria**

This systematic review uses research data from clinical trials, both RCT and non-RCT, and observational studies. Other requirements to fulfill the eligibility test are patients with a diagnosis of rheumatoid arthritis (RA)/juvenile rheumatoid arthritis (JRA) of all ages according to the ARA (American Rheumatism Association) or ACR criteria. The intervention group used UC-MSC (umbilical cord mesenchymal stem cells)/UCB-MSC (umbilical cord blood-derived mesenchymal stem cells) therapy either as monotherapy or in combination. The presence or absence of a control group does not matter. The control group could use a placebo, conventional treatment, or UC-MSC monotherapy in the combined UC-MSC intervention group. The conventional treatment uses DMARDs (Disease-modifying antirheumatic drugs).

Exclusion criteria: (1) treatment was not focused on RA, (2) mesenchymal stem cell (MSC) therapy or MSC types other than UC-MSC, (3) journals not accessible to full-text, (4) journals in foreign languages or other than English, (5) journal publishing period more than 10 years, (6) journal in the form of a review (narrative or systematic), (7) research subjects are experimental animals.

**Search Strategy**

This study uses a strategy to search the literature published internationally. These sources were published through online journals and online databases, including SAGE journal, PubMed, Hindawi, NEJM, Science direct, MDPI (cells), and Google Scholar with the key words: "umbilical cord mesenchymal stem cells", "rheumatoid arthritis", and "juvenile rheumatoid arthritis". The search strings used include (umbilical cord mesenchymal stem cell OR umbilical cord-derived mesenchymal stem cell OR UCMSC OR UC-MSC) AND (rheumatoid arthritis OR juvenile idiopathic arthritis OR juvenile rheumatoid arthritis). The search is restricted to journals published after 2010 and using English and using the title/abstract filter.

**Selection Process**

After obtaining literature from identifying various databases according to the search strategy above, the authors independently screened appropriate titles and abstracts and excluded duplications. Next, the authors read the entire text to select eligibility according to the inclusion and exclusion criteria.

**Assessment of Methodological Quality**

Journal quality assessment is carried out after selecting journals with inclusion and exclusion criteria to critically assess a study, reduce potential bias by researchers, analyze research designs, and ensure that research results are transparent and reproducible [10]. Journal quality assessment uses the Joanna Briggs Institute (JBI) Critical Appraisal Tools checklist. JBI is an institution that provides evidence-based health services. The JBI Critical Appraisal Checklist consists of 13 questions that can be answered with “yes,” “no,” “not clear,” or “not applicable.” From the total answers, the quality of the journals can be classified as follows: (1) high: meets > 80% of JBI criteria, (2) Medium: meets 50-80% of JBI criteria, (3) low: > 50% of JBI criteria.

**Data Extraction**

Data extraction was carried out independently by the author (Ferdiana L) and supervised by supervisors (Yudhi N and Boenga N). Data extraction variables consisted of registration, location, design, type of MSC, sample size, allocation of therapy administration and
duration, patient characteristics (gender, age, duration of RA, patient inclusion criteria, previous poor treatment response, and co-therapy), and results.

Results

Research Selection

In identification, 250 journals were initially obtained through online journal search and online databases. After filtering by time, 26 journals were excluded because publications were more than 10 years old. Furthermore, 224 journals were screened. From the results of the screening, 178 were excluded because they did not meet the criteria, and 8 were excluded because of duplication. There are 38 journals left for the eligibility test, then 31 journals were excluded until produce 7 inclusion journals.

[Fig. 1. PRISMA flowchart diagram on research selection
Source: (Primary Data, 2021)]

Research Characteristics

The designs used were four non-RCTs and two RCTs, while one journal was a combined cohort and RCT. The type of MSC source taken was mostly hUC-MSC (human umbilical cord-derived mesenchymal stem cell). Only one journal used hUCB-MSC (human umbilical cord blood-derived mesenchymal stem cells). The journals were published between 2013 and 2020. The number of samples used in the study was between 9 and 172 patients, but this number in several journals could not be used for final data because some patients lost follow-up.

Not all journals have a control group. The treatment in the intervention group and control group and the duration of therapy also varied in each study. The duration of therapy ranges from 30 minutes to months, but not all journals mention the duration of therapy. Meanwhile, follow-up takes from 3 months to 3 years. The majority of studies stated that the gender criteria were dominated by women, which was between 80-100%. Five of the six journals that included age criteria had an average (mean) adult age sample, between 42-57.4 years, while one journal with a sample age range of 2-15 years. Of the seven journals, five stated there was previous poor medication. All journals have co-therapy, including DMARDs and corticosteroids, both for maintenance, taper off, or anti-allergic. Other characteristics, namely the average duration of RA, administration of therapy in the intervention group and control group

Journal Quality Assessment

Based on the assessment result, from a total of 7 journals that met the inclusion criteria and did not include the exclusion criteria four journals were found to be with a non-RCT design, two journals with an RCT design, and one journal with an RCT+ cohort design. The four journals with non-RCT designs are included in the high category. Besides that, the journals with a combined RCT and cohort study design also meet the high category for the Checklist for Cohort Studies. Meanwhile, 3 RCT journals, including one of them combined RCTs and cohorts, are in the medium category. According to these results, all included journals in this study can be used for systematic reviews.

Safety Evaluation

Parameters to assess safety include adverse events, either in mild, moderate, or severe levels and those that do not include the degree of severity. In addition, hematology/serum, other organ or systemic functions, and a decrease in co-therapy dose were assessed.

In three journals, there were patients with mild adverse events such as chills or fever who recovered within a few hours without intervention, while one journal reported mild adverse events of leukocytopenia and mild transaminases elevation in some patients. Another journal stated that moderate adverse events were moderate leukocytopenia. Four of the seven journals showed no severe adverse events in patients, while the rest did not explain this. Other adverse events that were not reported by severity were gastrointestinal disturbances and joint pain 60 minutes after the transfusion administration.
However, for joint pain, the study report stated that it was not the result of therapy.

Furthermore, the hematological or serum findings were all within normal limits. Although there were changes or abnormalities, overall, they did not occur significantly, so that they did not affect the patient's general condition. Parameters used to assess hematology/serum in this study were: hemoglobin, albumin, total serum protein, total serum globulin, platelets, WBC, MCV, and uric acid. The majority of journals describing these criteria stated an increase in hemoglobin, albumin, and serum total protein, while total serum globulin, platelets, and WBC decreased. Only one journal described an increase in MCV, and one journal reported a minor increase in uric acid.

Assessing the function of other organs showed no decline in organ function. Liver and kidney functions were all in good condition. Several journals state that urine analysis, radiography, and ECG of the chest and digestive organs are in good condition. Systemically, two journals stated no severe infection and no GvHD.

**Efficacy Evaluation**

The parameters used to assess the efficacy were: symptom improvement, a decrease in co-therapy dose, improvement in markers of the immune system associated with RA/JIA, evaluation indices, recurrence, and efficacy level.

From seven journals, only one did not describe improvement in symptoms, while the other six reported improvements in symptoms such as sleep, diet, physical activity, and joint pain and swelling. One journal with a JIA sample stated that two patients who were initially unable to walk now can walk to school independently even with rapid growth and development, and two patients with necrosis did not show aggregation.

A total of five journals did not explain whether or not there was a decrease in the dose of co-therapy, but the other two journals contained a gradual reduction in the dose of NSAIDs and prednisone.

In immunological evaluation, almost all journals showed a decrease in CRP, ESR, RF, anti-CCP markers. Other markers that decreased in several journals include TNF alpha, IL-6, IL-8, and CD4+IL-17A+ Th17. Meanwhile, the marker that increased in most journals was CD4+ CD25+ Foxp3+ T reg.

Several evaluation indices were used to assess the results in this study, namely DAS28, DAS28-ESR, low disease activity (DAS28-ESR < 3.2), VAS, HAQ, EULAR, and ACR. On the DAS28 criteria, all journals stated a decrease in score. One journal uses the VAS criteria, and there is a decrease in scores on that criterion. The HAQ score showed a decrease in all journals except for one that did not explain these criteria. The EULAR criteria were only described in one journal, and a 53.4% score was obtained. While the ACR parameters were used in three journals, two journals used the ACR20/50/70 criteria, and one used ACR20/50.

Relapse was not described in five journals, whereas only two patients relapsed in two journals. Only three journals describe the level of efficacy and the results of the efficacy level range from 49% to 93.3%.

**DISCUSSION**

Rheumatoid arthritis is a systemic autoimmune disease of unknown etiology, characterized by chronic inflammation and infiltration of immune cells in the synovial membrane [11]. There is no treatment that cure RA at this time. Conventional treatments only reduce symptoms and have side effects [12, 13]. Currently, MSC therapy has become a highly recommended therapy for autoimmune diseases [14] mesenchymal stem cell (MSC). In this systematic review, the results of MSC therapy with the UC-MSC type have been obtained.

**Safety of UC-MSC in RA therapy**

UC-MSC therapy shows promising results regarding safety. Of the seven research journals, only a few received adverse events (AE), the majority had only mild symptoms, and no severe symptoms were found. These symptoms include fever that improves within a few hours without intervention and the presence of mild-moderate leukocytopenia. According to the journal, in the study by Park et al. (2018), joint pain 60 minutes after transfusion was not caused by UC-MSC therapy, possibly the pathophysiology of RA itself. Hematologic findings, organ, and systemic functions were all within normal limits. It means that UC-MSC therapy in terms of safety is quite good, as shown in Fig. 2.

Systemic increases or decreases in components and functions in blood, urine, and organs can be seen from the comparison of the pre-therapy and follow-up examinations after therapy. The length of time and the number of follow-ups varied from 3 months to 3 years, with visits ranging from 1 to 6 times. In each visit, changes in the results can be found increasing or decreasing. The majority had significant changes on the first examination. Then on further examinations, the changes were not significant or stable.
In the research of Xu et al. (2020) and Yang et al. (2018), it was stated that there was no Graft versus Host Disease (GvHD). This was due to the role of UC-MSC. GvHD is a systemic disorder that occurs when immune cells in donor/transplant tissues recognize the host as foreign and attack the recipient’s cells [15] when the first bone marrow transplantation was performed by Thomas et al., hematopoietic stem cell transplantation (HSCT). This condition is caused by the proliferation of T cells, while MSCs have low immune reactivity so that these properties are needed, so that host cells do not attack donor cells. GvHD is common in allogeneic hematopoietic stem cell transplantation or bone marrow transplantation. Therefore, therapy with UC-MSC is more suitable than BM-MSC. One of the reasons is that UC-MSC expresses MHC lower than BM-MSC, so it is less likely that APCs will activate T cells [16].

Research by Wang et al. (2013) stated an increase in liver function. It could be due to the therapeutic effect of UC-MSC. UC-MSC can heal liver injury and liver fibrosis, differentiate into albumin-producing hepatocytes, raise serum albumin levels, and increase endogenous cell survival rates [16]. Then resulted in elevated serum albumin levels and liver conditions in good function even experienced an increase in the research of Wang et al. (2013).

**Efficacy of UC-MSC in RA therapy**

This study also proved that UC-MSC therapy effectively treats Rheumatoid arthritis (Fig. 3). Several research journals state that combination therapy is more effective than UC-MSC monotherapy, such as UC-MSC + LG and recombinant UC-MSC + IFN-γ. It can be seen from evaluation indices such as DAS28, VAS, and HAQ, which show a decrease in all studies in inclusive journals.

**DAS28 evaluation index**

Disease activity score in 28 joints (DAS28) is a scoring system to evaluate disease activity and response to therapy in rheumatoid arthritis [17]. Patients with a DAS28 score < 2.6 showed remission; a score between 2.6 and 3.2 indicates low disease activity; a score between 3.2 and 5.1 indicates mod-
erate disease activity; and a score of > 5.1 indicates a high activity (high disease activity) [9].

Based on regular follow-up of patients, the DAS28 score showed a significant decrease in the first post-treatment evaluation. On the next evaluation, most of the patients were stabilized or decreased not significantly. For example, in the research of Wang et al (2013) with P < 0.01; Qi & Gao (2020) with P < 0.05; Yang et al. (2018) with P < 0.05; Park et al. (2018); and Wang et al. (2015) and Xu et al. (2020). Wang et al. (2015) succeeded in reducing eight moderate disease activity (MDA) patients and two high disease activity (HDA) patients to all low disease activity (LDA) patients. Xu et al. 2020 succeeded in reducing the score of patients with mean HDA to only a few HDA, mostly MDA or LDA, and a small percentage in remission.

Only Wang et al. (2013) stated that there was a significant decrease between the pre-therapy evaluation and post-therapy evaluation (P < 0.001), as well as a significant decrease between the first and second evaluation after therapy (P < 0.05). The explanation above can be seen in Fig. 3.

Two studies provided repeated UC-MSC therapy in RA patients (Fig. 4). Based on the amount of therapy given, Wang et al. (2013) showed a significant decrease after the first and second treatments. However, Wang et al. (2015) showed a significant decrease only after the first treatment evaluation, while after the second treatment showed insignificant results.

Clinical Improvement

The clinical symptoms most showed a very rapid improvement and remission. It is related to the characteristics of UC-MSC, which has a higher proliferative ability, more stable doubling time (DT), faster self-renewal ability, lower immunogenicity, and more feasible results compared to MSC from other sources such as AD-MSC and BM-MSC [16].

Dose Reduction

The research of Yang et al. (2018) and Park et al. (2018) said there was a dose reduction for other co-therapy drugs; this indicates the success of UC-MSC as monotherapy and reduces the source of long-term side effects commonly caused by conventional drugs such as DMARDs, corticosteroids, and NSAIDs [18]. No adverse events were found in the tapering-off process [19, 20].

Recurrence

Evaluation results do not always show stable results, sometimes fluctuating at several follow-ups. If the increase or decrease in results shows signs of relapse, additional UC-MSC therapy is needed to treat the relapse [20]. Only a small proportion experienced recurrence in this study, around 6-8%.

Fig. 4. Efficacy of UC-MSC therapy in RA using the DAS28 score in repeated therapy
Source: (Primary Data, 2021)
# Represents a significant reduction between pre-therapy and post-therapy evaluations; & describes a significant reduction between after the first treatment vs. after the second treatment.
Immuno-logical evaluation

The contact between immunocompetent cells and MSCs is multi-layered and complex. MSC immunosuppressive effects may occur because MSCs modulate the immune response at different levels [8].

MSCs can downregulate Th17 cells and upregulate the immunosuppressive potential of T-regulatory cells using their therapeutic potential, then restore immune function by triggering immune modulation, inducing tolerance, and reducing pro-inflammatory cytokine secretion [8, 21]. MSCs also regulate the innate and adaptive immune systems by inducing CD4+CD25+FoxP3+ Treg Cells [20]. In evaluating the immune system, inflammatory mediators such as TNF-α and IL-1β and adaptive immune cells such as CD4+ T cells were also found; these molecules play a role in summoning MSCs.

a. mechanism of action of mesenchymal stem cells

If there is tissue injury, there will be immune cells and inflammatory cells that play a role. These immune cells are triggered by apoptotic cells, necrotic cells, and microvascular damage, so that immune cells such as macrophages and neutrophils and adaptive immune cells such as CD4+ T cells, CD8+ T cells, and B cells will be activated. Not only that, inflammatory mediators such as TNF-α, IL-1β, free radicals, chemokines, and leukotrienes will also be produced by phagocytes to respond to cell damage. Combining inflammatory molecules and immune cells with fibroblasts and endothelial cells will change the microenvironment conditions so that MSC mobilization and differentiation occur [22].

Once the MSC enters the microenvironment of the injured tissue, it stimulates the secretion of growth factors such as EGF (epidermal growth factor), VEGF (vascular endothelial growth factor), HGF (hepatocyte growth factor), and etc., through several stimulant factors, including TNF-α, IL-1, IFN-γ, and toxins from infectious agents. These growth factors trigger the development of fibroblasts, endothelial cells, and progenitor tissue cells that will repair the tissue [22]. In addition, VEGF-A results in the production of a soluble factor that has been shown to have immunosuppressive action, otherwise known as IDO (indoleamine 2,3-dioxygenase) [8]. In the study of Qi & Gao (2020), there was an increase in HGF. It aligns with the MSC mechanism because HGF has a role in tissue repair as vasculogenesis and intrinsic neural cell regeneration [22].

Immuno-logical evaluation of combined UC-MSC therapy

Qi & Gao (2020) research stated that the combination of Lugua polypeptides (LG) and UC-MSC is very effective as a therapy or to reduce side effects. LG can support the UC-MSC mechanism and increase the effectiveness of UC-MSC significantly through upregulation of the immune system. Cervus and Cumis polypeptide (Lugua polypeptide/LG) is a new treatment from Traditional Chinese Medicine (TCM), a combination of sika deer bone extract and musk-melon seeds. This drug is not a curative treatment for RA, but the mechanism of the drug can be used to overcome the course of RA disease. Polypeptides in LG will stimulate UC-MSC to reduce pro-inflammatory factors in the internal environment and increase immune regulation and regeneration of UC-MSC [23].

a. mechanisms of immunosuppression exerted by UC-MSC

Meanwhile, the research of Xu et al. (2020) stated that the efficacy of UC-MSC was more significant when combined with recombinant IFN-γ; this was due to the mechanism of IFN-γ itself. MSC has the ability to reduce the immune system. However, MSCs are not directly able to suppress the immune system. The inactivation of this ability is caused by the anti-IFN-γ receptor, which blocks the immunosuppressive effect on MSCs [20]. So, the role of IFN-γ is needed here, IFN-γ will be combined with pro-inflammatory cytokines such as TNF-α, and IL-1β causing MSC to obtain very high immunosuppressive factors, then all of these factors will work together and unite near MSC to form a microenvironment that will strengthen and create a potent immunosuppressive effect. These immunosuppressive factors include a large number of chemokines and expression of adhesion molecules such as CCR5 ligands, CXCR3 ligands), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) [22].

Suggestion: Future research is expected to emphasize safety by determining the correct dose for repeated administration; future research is expected to focus on multicenter clinical trials with sufficient sample sizes, appropriate selection criteria for RA patients, and more extended follow-up periods to obtain a valid efficacy evaluation; future studies are expected to use blinded clinical trials in RCT designs to avoid bias.

Conclusion

UC-MSC has good safety and efficacy for RA. Of the 532 patients who received UC-MSC therapy, as many as 92% of patients had no symptoms or signs of side effects after transfusion, clinical findings, laboratory and supporting examinations were within normal limits, and some even experienced improvements in organ function. All patients did not experi-
ence the signs of GvHD, which is common in stem cell transplantation; this shows that UC-MSC has an advantage over other types such as BM-MSC. Most RA patients using UC-MSC therapy experienced rapid clinical and immunologic improvement. UC-MSC therapy is suitable for long-term therapy, seen from a follow-up of up to 3 years. Most of them do not show recurrence. This therapy is also effective in replacing conventional treatment, and its effectiveness is more significant when combined with LG and recombinant IFN-γ.

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Author’s contributions
YN: the idea, conceptualization, investigation, analysis the data, editing the original draft. FLM: conceptualization, investigation, resource and data curation. BN and CF: curation of analysis, writing-editing, and supervision.

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Consent for publication
We, the undersigned, give our consent for the publication of identifiable details, which can include photograph(s) and/or videos and/or case history and/or details within the text (“Material”) to be published in Rheumatology (Bulgarian) as it written in the attached cover letter.

Competing interests
All authors declare that they have no competing interests.

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