# 1. Inleiding

- Inleiding: +- 200 woorden met aanleiding, achtergrondinformatie en doelstel-  
ling. Bronnen (PDF) kunnen in een aparte folder met verwijzing

Genexpressie is de expressie van een fenotype door transcriptie en translatie (Buccitelli & Selbach, 2020). Transcriptomics is de studie van het transcriptoom wat al het RNA in een cel of weefsel omvat wat iets over de genexpressie in een organisme zegt. Hiermee kunnen de effecten en oorzaken van verschillende ziektebeelden worden onderzocht. (Khodadadian et al., 2020).

Reumatoïde artritis (RA) is een chronische auto-immuunziekte waarbij systematische synovitis (ontsteking van synovium) en bot- en gewrichtsafbraak optreedt. 5 op de 1000 volwassenen heeft RA waarvan 5-20% van de patiënten slecht op anti-reumatische medicatie reageert. RA ontstaat onder anderen door genetische factoren waardoor auto-immuun tolerantie verminderd. De complexiteit van het ziekteverloop en de onbekende oorzaak van RA zorgen ervoor dat het moeilijk behandelbaar is. (Suwa et al., 2023)

De precieze oorzaak van RA is nog niet bekend, hoewel het duidelijk is dat genetische factoren een rol spelen. Door de oorzaak van RA in beeld te krijgen kunnen gerichtere therapieën en preventie worden ontwikkeld. In dit onderzoek wordt er m.b.v transcriptomics de expressie van genen in RA en normale personen vergeleken om een beter beeld van de pathogenese te krijgen.

2. Methode

- Methode: +- 200 woorden met methode, flowschema. Zie leerdoelen voor mi-  
nimale inhoud. Scripts, data etc. kunnen in een aparte folder met verwijzing

Er werden 4 samples van personen zonder RA (ACPA negatief) en 4 samples van personen met RA (diagnose van >12 maanden, ACPA positief) verkregen uit een synoviumbiopt. Informatie over personen is te vinden in tabel…. In…. Sequencing werd uitgevoerd waarna transcriptomics analyse werd uitgevoerd in R. Het referentiegenoom werd geïndexeerd met behulp van het menselijke referentiegenoom uit het NCBI-file: GCF\_000001405.40\_GRCh38.p14\_genomic.fna, en de packages BiocManager (Morgan & Ramos, 2024,v1.30.25) en Rsubread (Shi, Liao, & Smyth, 2024,v2.20.0). Monsters werden gemapt tegen het geïndexeerde referentiegenoom waaruit BAM-files ontstonden. BAM-files werden gesorteerd en geïndexeerd m.b.v Rsamtools (Morgan et al., 2024,v2.22.0). M.b.v readr (Wickham et al, 2024,v2.1.5), dplyr (Wickham et al., 2023, v1.1.4), Rsamtools en Rsubread en het annotation NCBI file GCF\_000001405.25\_GRCh37.p13\_genomic.gtf.gz werd een countmatrix gemaakt. Statistiek werd uitgevoerd op de count matrix file: count\_matrix.txt. Een DESeq2-analyse werd uitgevoerd met DESeq2 (Love et al., 2024, v1.46.0). Resultaten werden gevisualiseerd in een vulcano plot m.b.v EnhancedVolcano (Blighe et al., 2024, v1.24.0) en ggplot2 (Wickham et al., 2024*,* v3.5.2). Een Gene Ontology (GO)-verrijkingsanalyse werd m.b.v goseq (Young, Davidson, & Marini*, 2024,* v1.58.0), geneLenDataBase (Young, Davidson, & Marini, 2024*,* v 1.42.0) en org.Dm.eg.db (Carlson, 2023*,* v 3.20.0). De 10 meest significante resultaten werden gevisualiseerd. Een KEGG pathway analyse werd uitgevoerd m.b.v KEGGREST (Tenenbaum et al., 2024, v1.46.0) en de resultaten van de GO-analyse waarbij de pathway ‘rheumatoide arthritis’ KEGG ID: hsa05323 uit GO term ‘immune system process’ werd geanalyseerd m.b.v pathview (Luo, 2024*,* v1.46.0).

3. Resultaten  
- Resultaten: +- 200 woorden, inclusief correcte verwijzingen

Er werd een transcriptomics analyse in R uitgevoerd waarbij de een DESeq, GO en KEGG-analyses werden toegepast. Een DESeq analyse werd uitgevoerd om het aantal differentiële significante up- en downgereguleerde genen te bepalen. Resultaten zijn weergegeven in figuur… De meest statistische significante genen die upgereguleerd waren in reumapatiënten waren SRGN, BCL2A1 en downgeregulleerde genen waren ANKRD30BL, MT-ND6, SLC9A3R2, ZNF598.

Een GO analse werd uitgevoerd om differentiële significante pathways te bepalen. GO-analyse resultaten werden gevisualiseerd in figuur …. Uit de analyse bleek dat de pathway ‘immune system process’ veel differentiele significante genen bevatte. Verder onderzoek naar de pathway werd gedaan met de KEGG analyse, omdat deze pathwat relevant is in verband met reuma. Ondedeel uit de gekozen pathway, de ‘rheumatoide arthiritis’ pathway, werd gevisualiseerd in een KEGG pathway, resultaten zijn weergegeven in figuur … Verschillende genen in de pathway zijn differentieel in expressie. Genen die zorgen voor Angiogenesis, infiltratie van inflammentoire cellen, ontsteking van synoviale pannus, gewrichts en botafbraak waren sterk upgereguleerd.

Tutorials

(Bioinformatics Core Shared Training, 2020)

(Bioinformatics Consulting Group, n.d.)

4.Conclusie  
- Conclusie: +- 200 woorden, inclusief aanbevelingen en onderzoek in context  
plaatsen.

RA is een auto-immuunziekte waarbij het immuunsysteem uit balans is. Dit was in de resultaten terug te zien. De DEseq analyse toonde upgereguleerde genen **SRGN**, betrokken bij inflammentoire processen (Y. Chen et al., 2020), en **BCL2A1,** eenanti-apotptotisch eiwit (Gao et al., 2023). Genen met pro-inflammatoire eigenschappen zijn upgereguleerd in reumapatiënten. Downgereguleerde genen waren **MT-ND6,** betrokken bij het electronen transport in de mitochondriën (National Center for Biotechnology Information, 2024). **SLC9A3R2,** zorgt voor negatieve regulatie van endothele proliferatie (Arntz et al., 2024). **ZNF598,** een ribosomal kwaliteitscontrole eiwit (Oikawa et al., 2023). Genen met een functie om balans te behouden zijn downgereguleerd.

In het synoviale weefsel zijn dendritische cellen met MHC klasse 2 verhoogd en hebben verhoogde receptoren, dit leidt tot presentatie van lichaamseigenantigenen aan self reactive th1 cellen (Wehr et al., 2019). Deze zorgen voor upgeregulatie van pro-inflammatoire cytokines zoals TNF-gamma, IL1β, IL-6 die zorgen voor ontstekingen in inflamation van de synoveale pannus. Ook over activatie van synovial fibroblasten en B lymphocyten vindt plaats(Rahimi-Khorashad et al., 2023). Downregulatie van TGFbeta is ongebruikelijk. Synaviale fibroblasten over activeren osteoclasts waardoor V-ATPase is upregulated wat leid tot botafbraak (Kovács et al., 2022). Angiogenesis, een proces waarbij nieuwe bloedvaten worden gevormd, wordt bevorderd (Khodadust et al., 2022). CXCL en CCL, chemokines die de lymfocyten aantrekken naar de bloedvaten, zijn sterk upgereguleerd (X. Chen et al., 2020) (Zhu et al., 2021).

In RA zijn pro-inflammatoire genen, cytokines en cellen upregulated waardoor er chronische ontstekingen in het synoviale pannus ontstaan.

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Afbeelding met tekst, software, Computerpictogram, Multimediasoftware

Door AI gegenereerde inhoud is mogelijk onjuist.

**Upregulation of osteoclasts**

Imbalancein thefinely regulated osteoblast-osteoclast system plays a central role in the pathogenesis of several metabolic bone and systemic inflammatory diseases. Due to the complex interactions of the bone and the immune system, inflammation may accelerate/ increase bone resorption via osteoclast activation. Local osteoclast activation is promoted by proinflammatory cytokines released during inflammation (TNF-a, IL-1, IL-6, IL-17, IL-23),byRANK-Lproduced by lymphocytes and fibroblasts, and by direct cell-cell interactions (12, 13). The production of IL-17 by Th17 lymphocytes is elevated in certain inflammatory conditions, which has a synergic effect with IL-1 and TNF-a(14). Consequently, osteoclasts and synovial fibroblasts in the joint can be activated. Therefore, inflammation is associated with local and systemic bone loss in inflammatory arthropathies e.g. rheumatoid arthritis (RA) or psoriatic arthritis (PsA).

**V-atpase**

The increased osteoclast-specific secretion is supported by the increased expression of vacuolar-type H+ -ATPase (V-ATPase), sodium/hydrogen exchanger 9B2 (a Na+ /H+ antiporter) (39, 40), cathepsin K and B (proteolytic enzymes) (41, 42), and lysosomal acid phosphatase (43). Integrin alpha-V crossing the cell membrane and anchored to the bone surface is essential, with which osteoclasts come into close contact with bone and promote the formation of the resorption lacunae (44). These results show, that the proteins with increased expression in osteoclasts detected in our experiments fit well with the previously reported characteristics of these cells.

**This is not how it should be**

Both RANK-L and M-CSF are essential for the differentiation, activation, and survival of osteoclasts. The production of IL-17 by Th17 lymphocytes is elevated in certain inflammatory conditions, which has a synergic effect with IL-1 and TNF-a

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**upregulation Th17**

Th17 cells and Treg cells, each with specific functions and gene expression, develop from the same naive CD4+ T cells, but under different cytokine environment (3). Typical proinflammatory Th17 cells, through the induction of proinflammatory cytokines, lead to autoimmune-derived tissue inflammation and joint damage. Furthermore, the activity of Th17 cells, as well as other effector T cells, is suppressed by Treg cell

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Upregulation of receptors

T lymphocytes play an essential role in RA and their activation not only requires recognition of specific antigenic peptides by the T-cell receptor (TCR), but also the co-stimulatory signals provided by accessory surface molecules on T cells (3). CD28, a co-stimulatory receptor, is consecutively expressed on resting Tcells. CTLA-4, a co-inhibitory receptor, is a member of the CD28 family expressed on the surface of T cells soon after its activation (3, 4). CD80 and CD86 (B7 molecules), mainly expressed on B cells, monocytes/macrophages, and dendritic cells, are the ligands of CD28 and CTLA-4, which are upregulated when activated. The binding of the ligands and CD28 promotes the activation of a TCR-stimulated T cell, while the binding of CTLA-4 causes the inhibition of T cell activation (5). CTLA-4 has higher affinity with CD80-CD86 than CD28, although it is homologous to CD28 (6). In a continuous immune response, the expression of CTLA-4 is upregulated to inhibit the proliferation of T-cell and reduce interleukin (IL)-2 production. For B7 molecules, the main function is to augment and sustain T-cell responses by interacting with CD28, while they can also provide inhibitory signals when binding with CTLA-4 (7). The immunological mechanism of T cell activation is illustrated in Figure 1. Lack of CTLA-4 will therefore cause severe lymphoproliferation and harmful destruction of multiorgan tissues, indicating that it plays an vital role in negative regulatory functions (8). Hence, changes in CTLA4 and CD86 genes have the potential to increase the immune response of autoreactive T-cells to self-antigens (9). In mouse experiments, Ewing et al. (10) confirmed that T-cell co-stimulation by connecting with CD28 and its negative regulator CTLA-4 played an important role in accelerating atherosclerosis development.

Veranderingen in CTLA4- en CD86-genen hebben dus de potentie om de immuunrespons van autoreactieve T-cellen op zelfantigenen te verhogen

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**Upregulation of dendritic cells**

Dendritic cells (DCs) are the key professional antigen-presenting cells (APCs) for T cell priming. They also play a major role in immune tolerance. DCs discriminate between self- and non-self-antigens on the basis of associated innate immune activating or suppressive signals, after which DC antigen uptake and presentation promotes T cell activation or regulation. DCs and T cells collaborate in the pathogenesis of RA, particularly through the presentation of antigen that triggers the differentiation of autoreactive T cells, as well as innate immune effector functions.

Based on human and rodent model evidence, we propose a working model of RA, where autoantigen-specific CD4+ T cells, including follicular helper T cells (Tfh), are primed by major histocompatibility complex (MHC) class II+ DCs exposed to environmental inflammatory factors that enhance their maturation, as well as generation and presentation of neoepitopes. Autoreactive and potentially cross-reactive Tfh propagate autoimmune arthritis through activation of B cells with genetic tolerance defects, followed by germinal centre formation and affinity maturation and glycosylation of autoantibodies, which contribute to the effector phase of the disease through innate mechanisms. Although partially regulated, the autoimmune response persists due to ongoing stimulation of autoreactive T cell clones by a variety of synovial MHC class II+ APCs and draining lymph node (dLN) DCs (Fig. 1). This paper reviews the evidence for the contribution of DCs and T cells to this model.

In RA, DCs are thought to drive the activation of self-peptide-reactive inflammatory T cells, Tfh and consequently B cells for stimulating autoantibodies

Hoewel gedeeltelijk gereguleerd, blijft de autoimmuunrespons bestaan door voortdurende stimulatie van autoreactieve T celklonen door een verscheidenheid aan synoviale MHC klasse II+ APC's

RA voor, waarin auto-antigeen-specifieke CD4+ T-cellen, waaronder folliculaire helper T-cellen (Tfh), worden geactiveerd door het major histocompatibility complex (MHC) klasse II+ DC's die worden blootgesteld aan ontstekingsfactoren in de omgeving die hun maturatie versterken,

In RA, CD4+ T cells stimulate macrophages, synovial fibroblasts, and chondrocytes to yield proinflammatory cytokines (mainly interleukin (IL)1β, IL-6, and tumor necrosis factor alpha (TNFA)) and activate B lymphocytes. They also stimulate osteoclastogenesis in the bone. Ultimately, these cells lead to joint destruction and progression in RA. 4 In RA, Th1 cells and their cytokines, especially interferon-gamma (IFNG), effectively induce and perpetuate chronic inflammation and tissue destruction.

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**downregulated**

***MT-ND6***

Enables NADH dehydrogenase (ubiquinone) activity. Involved in mitochondrial electron transport, NADH to ubiquinone and mitochondrial respiratory chain complex I assembly. <https://www.ncbi.nlm.nih.gov/gtr/genes/4541/>

**SLC9A3R2**

SLC9A3R1, and SLC9A3R2 are related to inflammation and arthritis strengthens our findings that these proteins reflect the arthritic process. SLC9A3R2 is a negative regulator of endothelial proliferation (33), and SLC9A3R1 is involved in the positive regulation of NF-kB in vascular inflammation and in the IL-6 production in human smooth muscle cells (34). SLC9A3R1 and SLC9A3R2 connect plasma membrane proteins with members of the ezrin/moesin/radixin family, thereby helping to link them to the actin cytoskeleton and to regulate their surface expression. This may also explain the high degree of discordance between PGA-VAS and physician global assessment of RA (35), as, for example, muscle function is not included in the clinical assessment of RA <file:///C:/Users/Yaelf/Downloads/fmed-10-1247778.pdf>

**ZNF598**

Increased ribosome stalling raises the chance of ribosome collision, which triggers various ribosome quality control (RQC) pathways. This involves the activation of the ribosome-associated E3 ligase ZNF598, which ubiquitinates small subunit proteins at the stalled ribosomes and recruits other RQC factors to dissociate the aberrant translation intermediates on the transcripts ([53](https://pmc.ncbi.nlm.nih.gov/articles/PMC11466505/#R53), [54](https://pmc.ncbi.nlm.nih.gov/articles/PMC11466505/#R54)). If not resolved, the prolonged stalling will induce global stress response signaling pathways <https://pmc.ncbi.nlm.nih.gov/articles/PMC11466505/>

**Uprgulated**

**SRGN**

SRGN encodes the proteoglycan protein, and is mainly expressed in hematopoietic cells. Many studies have confirmed that SRGN promotes tumor invasion and metastasis in colorectal cancer, non-small cell lung cancers, multiple myeloma, nasopharyngeal carcinoma, and breast cancer (Li et al., 2011; Korpetinou et al., 2013; Purushothaman and Toole, 2014; Guo et al., 2017; Xu et al., 2018). SRGN is also involved in inflammatory processes through the regulation of numerous inflammatory mediators such as TNF-α, and activating the NF-κB signaling pathway (Zernichow et al., 2006; Korpetinou et al., 2014; Scuruchi et al., 2019). These processes caused by the combination of SRGN and CD44 receptor, could promote inflammation

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**BCL2A1**

BCL2A1 is a member of the BCL-2 family of anti-apoptotic proteins and one of the less well-studied anti-apoptotic BCL2 proteins 🡪 stoppen apoptose waardoor schadelike cellen langer leven

<https://pmc.ncbi.nlm.nih.gov/articles/PMC10637801/pdf/aging-15-205149.pdf>

While various anti-rheumatic drugs have been established in recent years, 5 - 20% of RA patients have poor responses to those medications (6). The pathogenesis of RA is thought to involve a reduced tolerance of the autoimmune system resulting from genetic and environmental. The role of dendritic cells and their immunometabolism in rheumatoid arthritis. Front. Immunol. 14:1161148. doi: 10.3389/fimmu.2023.1161148 COPYRIGHT © 2023 Suwa, Nagafuchi, Yamada and Fujio. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms. TYPE Mini Review PUBLISHED 12 May 2023 DOI 10.3389/fimmu.2023.1161148 backgrounds (7, 8). Synovitis in RA is induced by complex interaction of various cell types, including T and B lymphocytes involved in adaptive immunity, myeloid cells involved mainly in innate immunity, osteoclasts and synovial fibroblasts directly responsible for joint destruction. The complexity of disease pathogenesis is a primary cause of the difficulties in treatment (9– 11). Dendritic cells (DCs), a subtype of the myeloid lineage, could be related to the clinical treatment response in RA. Recently, we have reported that the proportion of precursor DCs (pre-DCs) in innate immunity, osteoclasts and synovial fibroblasts directly responsible for joint destruction. <file:///C:/Users/Yaelf/Downloads/fimmu-14-1161148.pdf>