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**DYNAMIC AUTOMATED SYNOVIAL IMAGING (DASI) FOR  
DIFFERENTIATING BETWEEN RHEUMATOID AND PSORIATIC  
ARTHRITIS: AUTOMATED VERSUS MANUAL INTERPRETATION IN  
CONTRAST-ENHANCED ULTRASOUND**

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# Abstract

**Introduction:** Rheumatoid (RA) and psoriatic arthritis (PsA) are common diseases affecting about 1% of population and are characterized by chronic joint inflammation. Although both have peculiar features such as the presence of specific autoantibodies, in the case of RA, or involvement of skin and nails, in the case of PsA, they show many similarities. Joint distribution, clinical and radiological manifestations may be so identical -especially early in the beginning of disease- that differentiation gets impossible except for hard to gain biopsy specimens showing distinct vascularization patterns for both diseases. Among all forms of arthritides, RA has the worst outcome. Early identification and treatment is considered imperative. Synovitis in RA is consistent with inflammation, synovial hyperplasia and neovascularization, that correlates with disease activity, aggressiveness and joint destruction. Synovitis is in RA the primary event, in PsA secondary to inflammation of entheses, capsules and other perisynovial structures. In RA inflammatory vessels are homogeneously distributed in synovia and show linear and branching architecture. In PsA vessel distribution is more inhomogeneous in synovial and perisynovial regions and are more tortuous and bushy. Contrast enhanced ultrasonography (CEUS) has been proven to be a very sensitive method in assessing inflammatory neovascularization equivalent to magnetic resonance imaging. The possibility to discriminate RA from PsA with the help of vascularization patterns detected non-invasively by CEUS has not yet been tested.

**Material and methods:** 107 outclinic patients presenting arthritis of finger joints were recruited, 56 with defined RA and 51 with defined PsA. The most active joint was chosen for CEUS exams. The hands were water-immersed and steady probe was used to increase image quality. While contrast bolus injection, contrast tune imaging with low mechanical index was used for image acquisition. CEUS images were validated manually by radiologists for both CEUS grade and presumptive diagnosis of RA or PsA considering histopathological differences. RA was assumed to present with a more homogenous and synovial enhancement and faster time of contrast appearance due to linear and branching vessel architecture, whereas PsA with inhomogeneous enhancement both in synovial and perisynovial region representing entheses and capsules, and later contrast appearance due to tortuous, bushy vessels. Further contrast kinetics were analyzed by ad hoc automated analysis software including a new developed pixel-based and a region-based procedure similar to available commercialized systems. Contrast kinetics of each image pixel was described by a gamma curve  $f(t)=A(t-t_0)^a \times e^{-(t-t_0)/b}$ , and 98 flow parameters in synovial and perisynovial tissue were derived and analyzed. 37 of these parameters proved to be significantly different ( $p < 0.05$ ) between RA and PsA populations. A linear discriminant classifier was trained to identify the transformation optimizing

the linear separability of the two groups, and each patient was assigned to RA or PsA with a Bayesian classification algorithm providing the a posteriori probability to belong to the RA or PsA group. The diagnostic power of the identified vascularization pattern was tested by means of leave-one-out analysis. Correlations between flow parameters and clinical data were calculated.

**Results:** Accuracy of automated pixel-based CEUS analysis to discriminate RA from PsA was 0.93 in training and 0.83 in test conditions, by adding data about rheumatoid factor and anti-cyclic citrullinated peptides accuracy was enhanced to 0.99 and 0.93 respectively. Accuracies of manual (0.69) and region-based automated analysis (0.61) were definitively lower. The best flow parameters for the construction of vascularization pattern discriminating between RA and PsA were mean synovial raise time (faster in RA), mean synovial raise constant (lower in RA), time to synovial peak (faster in RA), mean synovial peak value (higher in RA), synovial active regions (more numerous in RA), mean dimension of synovial active regions (greater in RA), synovial and perisynovial blood volume (both greater in RA), synovia/perisynovia blood volume and flow (all higher in RA). No correlations were identified between flow parameter and clinical data.

**Conclusion:** Dynamic automated synovial imaging (DASI) is consistent with a new tool to study directly vascularization patterns in synovitis, which was possible only by histologic specimens up to now. DASI is highly effective to differentiate RA from PsA by identifying distinct vascularization patterns.

# Riassunto

**Introduzione:** L'artrite reumatoide (AR) e l'artrite psoriasica (APs) sono delle affezioni comuni, che colpiscono l'1% della popolazione generale, e sono caratterizzate dalla infiammazione articolare cronica. Anche se presentano delle peculiarità distintive, quali la presenza di autoanticorpi, nel caso dell'AR, e il coinvolgimento della pelle e delle unghie, nel caso dell'APs, entrambe le forme di artrite presentano molti elementi in comune. La distribuzione articolare e le manifestazioni cliniche e radiologiche possono essere identiche, soprattutto nelle fasi iniziali della malattia, cosicché la distinzione diventa impossibile, se non attraverso lo studio istologico della membrana sinoviale, che presenta un tipo di vascolarizzazione specifica per le due artriti, ma però non è facilmente reperibile. Tra le varie artriti l'AR ha la prognosi più sfavorevole e pertanto una diagnosi e terapia precoci sono imperative. La sinovite reumatoide consiste di infiammazione, iperplasia sinoviale e neovascolarizzazione, che correla con l'attività di malattia, aggressività e distruzione articolare. Nell'AR la sinovite è l'evento primitivo, invece nella APs è secondaria all'infiammazione delle entesi, capsule e altre strutture peri-articolari. Nell'AR i vasi infiammatori di morfologia retta e arborescente sono omogeneamente disposti nella sinovia. Nell'APs invece sono tortuosi e "a cespuglio" e la loro distribuzione è disomogenea nella sinovia e peri-sinovia. L'ecografia con mezzo di contrasto (contrast-enhanced ultrasound, CEUS) è una metodica per lo studio vascolare, soprattutto della neovascolarizzazione infiammatoria, molto sensibile raggiungendo il livello della risonanza magnetica. La possibilità di discriminare con la CEUS l'AR dalla APs attraverso l'analisi non-invasiva degli specifici tipi di vascolarizzazione non è ancora stata studiata.

**Materiali and metodi:** 107 pazienti afferenti alla nostra unità di Reumatologia ed affetti da artrite delle mani sono stati reclutati. 56 erano stati diagnosticati come AR e 51 come APs. L'articolazione più attiva riferita dal paziente è stata scelta per l'esame CEUS. Le mani sono state immerse in acqua usando una sonda fissa per migliorare la qualità di immagine. Durante l'iniezione di contrasto le immagini sono state acquisite tramite la modalità per contrasto, che hanno in utilizzo indici meccanici bassi. Le immagini CEUS sono state validate manualmente dai radiologici sia per il grado CEUS che per la presunta diagnosi di artrite tenendo conto delle differenze istologiche note. Si assumeva che l'AR presentasse un rinforzo contrastografico sinoviale più omogeneo e un tempo di arrivo del contrasto più breve dato la morfologia retta e arborescente dei vasi, mentre l'APs con un rinforzo disomogeneo sia nella zona sinoviale che in quella peri-sinoviale comprendenti le entesi e capsule, e un arrivo del contrasto tardivo per i vasi tortuosi e "a cespuglio".

Inoltre le cinetiche del contrasto sono state analizzate da un sistema analitico automatizzato programmato ad hoc per questo studio. Le analisi hanno compreso una procedura a base di singoli pixel, sviluppata ex novo, e a base di intere regioni, analoga a sistemi attualmente commercializzati. La cinetica dei singoli pixel è stata descritta tramite una curva  $f(t)=A(t-t_0)^a \times e^{-(t-t_0)/b}$ , e 98 parametri di flusso diversi sono stati identificati ed analizzati nella zona sinoviale e peri-sinoviale. 37 di questi parametri presentavano differenze significative tra pazienti con AR e APs ( $p<0.05$ ). Un classificatore lineare discriminatore è stato allenato per identificare le trasformazioni necessarie per ottimizzare la separabilità lineare dei due gruppi. Ciascun paziente è stato assegnato al gruppo AR o APs attraverso un algoritmo di classificazione bayesiana per determinare la probabilità a posteriori di appartenere ad un gruppo invece che all'altro. La potenza diagnostica del prototipo di vascolarizzazione così creato è stata verificata tramite un'analisi "leave-one-out". Infine correlazioni tra parametri di flusso CEUS e dati clinici sono stati ricercati.

**Risultati:** L'accuratezza nel discriminare pazienti con AR da quelli con APs è 0.93 per l'analisi CEUS automatizzata a base di pixel nella fase di allenamento e 0.83 nella fase di verifica. Aggiungendo i dati su fattore reumatoide ed anticorpi contro peptidi citrullinati ciclici l'accuratezza aumentava al 0.99 e 0.93 relativamente nella fase di allenamento e verifica. L'accuratezza dell'analisi CEUS manuale (0.69) e dell'analisi automatizzata a base di regioni (0.61) erano definitivamente più basse. I parametri di flusso contrastografico più importanti nella creazione del prototipo di vascolarizzazione discriminante tra AR e APs erano la velocità media di incremento sinoviale (più veloce in AR), la costante di incremento sinoviale (più basso in AR), il tempo al picco sinoviale (più veloce in AR), il valore medio del picco (più alto in AR), il numero delle regioni sinoviali attive (più numerose in AR), la dimensione media della regioni sinoviali attive (più grande in AR), il volume sanguigno sinoviale e peri-sinoviale (più ampio in AR), il volume e flusso sanguigno sinovia/peri-sinovia. Non vi erano correlazioni tra parametri di flusso CEUS e dati clinici.

**Conclusioni:** Lo studio di immagini dinamico e automatizzato della sinovia (dynamic automated synovial imaging, DASIS) costituisce un nuovo strumento per lo studio diretto della vascolarizzazione in corso di artrite, che finora era solo possibile tramite la biopsia invasiva. DASIS è altamente efficace nel discriminare l'AR dall'APs attraverso l'identificazione di un prototipo di vascolarizzazione distinto.

# Introduction

Arthritis is a modern socioeconomic emergency due to high prevalence -exceeding largely cardiovascular and pulmonary diseases- and potentially destructive process compromising irreversibly personal independence and charging institutional resources [1,2]. The prevalence of self-reported and doctor diagnosed arthritis is projected to increase in the US from actual 47.8 million in 2005 to nearly 67 million by 2030 (25% of the adult population). And by 2030, 25 million (9.3% of the adult population) are projected to report arthritis attributable activity limitations [3].

## 1. Arthritis

Arthritis is not a single disease - it is a term that covers over 100 medical conditions. Osteoarthritis (OA) is the most common form of arthritis and generally affects elderly patients. It is a degenerative disease of articular cartilage and subchondral bone exacerbated by other factors such as weight and trauma and secondary synovial inflammation.

Primary inflammation of synovial tissue (synovitis) is on the other hand a common final pathway in different inflammatory arthritides. Inflammatory arthritis may be caused by crystals (gouty arthritis), microbes (septic arthritis) or persistent immune reaction to microbial components (reactive arthritis). Finally autoimmune pathway is on the base of a consistent number and forms of inflammatory arthritis.

The 2 major and clinically most important primary inflammatory rheumatic diseases which affect small hand and feet joints are rheumatoid arthritis (RA) and psoriatic arthritis (PsA). The most important initial histopathological feature of RA is synovitis followed by chronic proliferative granulomatous pannus-tissue, which is associated with cartilage and bone destruction. Early inflammatory changes in RA also develop synchronously within the subchondral bone marrow. Enthesitis is the hallmark of seronegative spondyloarthritis (SpA) including PSA, and is often seen as one of the first radiological manifestations of the diseases. As a rule inflammation within the synovial joints, histologically similar to RA, is not so pronounced. Consequently destructive changes within the synovial joints are much less with the exception of PsA in which pronounced bone destruction may develop (arthritis mutilans). Considerable overlapping in clinical and morphological manifestation of RA and PsA may be present. For evaluation of hand and feet joints and surrounding soft tissue structures in RA and PsA different imaging modalities are used, which include conventional radiography (CR), ultrasound (US), radionuclide techniques and magnetic resonance imaging (MRI). Appreciation of typical combinations of various inflammatory features

within the small hand and feet joints and surrounding anatomic structures, as well as evaluation of their distribution, extension and intensity helps in making differential diagnosis.

## **2. Normal anatomy and pathology of the synovial joints and the entheses**

Peripheral small hand and feet joints are anatomically complex diarthrodial (or synovial) joints, which enable great range of movements between the bones. Histologically they are composed of 4 major structures, the synovium, the synovial fluid, the cartilage and the capsule with its ligaments. The hallmark of RA is synovitis. Since synovium is one of the most important structural components of the peripheral hand and feet joints abundant inflammatory changes within these joints are present in RA [4].

In SpA including PsA the hallmark of the diseases is enthesitis, an inflammatory enthesopathy at the insertions of the joint capsules, ligaments, tendons and aponeuroses [5]. Non-synovial fibrocartilaginous joints enable only limited range of movements and are mainly adapted for transmission of biomechanical forces. Entheses are fibrocartilaginous structures and are the sites exposed to repetitive biomechanical stressing. Excessive compressive and shearing forces can be transmitted for example from the muscle through the tendon to the bone. The anatomy and physiology of the entheses have not been studied extensively until recently. There are proves that the extracellular composition of the entheses includes a matrix synthesized by chondrocytes similar to those present in cartilage [6]. Concerning the synovial hand and feet joints entheses do not represent prominent anatomical structures and are limited to the small peripheral area, where the joint capsule inserts into the cortex of the bone shaft. Additional sites of enthesal insertions outside of the small joints are attachments of the collateral ligaments and paraarticular tendons. These are the sites where typical primary inflammatory changes occur at or near peripheral synovial joints in PsA. Synovial inflammation (synovitis) in PsA is supposed to be secondary to primary enthesitis. However overlapping of features typical for RA and those typical for SpA may be seen in both entities. The best example is PsA in which prominent synovial proliferation, morphologically indistinguishable from RA can be present within the hand and feet joints, the so-called simil-rheumatoid PsA.

## **3. Rheumatoid Arthritis**

### **3.1. Impact of rheumatoid arthritis**

RA is a chronic autoimmune inflammatory disease affecting 1% of general population, and is characterized by erosive polyarthritis leading to joint destruction, disability and handicap. As consequence the majority of RA patients incur work loss, quality of life impairment and premature death. The accumulated economic burden resulting from RA is enormous. The total cost of RA in 2006 was estimated at €45 billion in Europe and €42 billion in the United States, or close to €100 billion in the countries covered [7]. The precise evaluation of the activity (inflammation) and severity (destruction) of RA is of paramount importance to assess disease prognosis and progression, and treatment efficacy.

### **3.2. Pathogenesis of rheumatoid arthritis**

RA involves a complex interplay among genotype, environmental triggers, and chance [8]. Twin studies implicate genetic factors [9]. Genome analyses confirm that immune regulatory factors underlie the disease [10]. The long-established association with HLA DRB1 locus has been confirmed in seropositive patients [11]. Alleles that contain a common amino acid motif (QKRAA) in the HLA-DRB1 region, termed the shared epitope, confer particular susceptibility. These findings suggest that some predisposing T-cell repertoire selection, antigen presentation, or alteration in peptide affinity has a role in promoting autoreactive adaptive immune responses. Other possible explanations for the link between RA and the shared epitope include molecular mimicry of the shared epitope by microbial proteins, increased T-cell senescence induced by shared epitope-containing HLA molecules, and a potential proinflammatory signaling function that is unrelated to the role of the shared epitope in antigen recognition [12,13].

Other immune regulator genes responsible for immune regulation such as nuclear factor  $\kappa$ B (NF- $\kappa$ B)- dependent signaling (REL, TNFAIP3, TRAF1, BLK, CCL21, FCGR2A, PADI4, PRDM1, TNFRSF14), T-cell stimulation, activation, and functional differentiation (PTPN22, AFF3, CD28, CD40, CTLA4, IL2RA, IL-2, IL-21, PRKCQ, STAT4, TAGAP, CTLA4) are implicated in RA.

Gene-environment interactions such as smoking and other forms of bronchial stress increase the risk of RA among persons with susceptibility HLA-DR4 alleles [14].

Environmental stressors such as smoke promote posttranslational modifications, through peptidyl arginine deiminase, type IV (PADI4), that result in quantitative or qualitative alteration in citrullination of mucosal proteins. Loss of tolerance to such neopeptides elicits an autoimmune response and anti-cyclic citrullinated peptide (CCP) production. Infectious agents (Epstein-Barr

virus, Cytomegalovirus, Proteus species, Escherichia coli, Porphyromonas gingivalis in periodontal disease, oral and intestinal microbiota) and their products (heat-shock proteins) have long been linked with RA by molecular mimicry [15]. The formation of immune complexes during infection may trigger the induction of rheumatoid factor (RF), a high-affinity autoantibody against the Fc portion of immunoglobulin, which has long served as a diagnostic marker of RA arthritis and is implicated in its pathogenesis.

The greater risk of RA among women and the sometimes observed onset associated with adverse life events are explainable by links between the hypothalamic–pituitary–adrenal axis and cytokine production [16].

Traditionally, RA has been considered a Th-1 cell mediated disorder, and therefore is thought to be driven by a population of T cells producing inflammatory cytokines and chemokines [17].

Antigen-activated CD4<sup>+</sup> T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokines IL-1, IL-6 and TNF $\alpha$  and to secrete matrix metalloproteinases through cell-surface signaling as well as through the release of soluble mediators. IL-1, IL-6 and TNF $\alpha$  are the key cytokines that drive inflammation in RA. Activated CD4<sup>+</sup> T cells also stimulate B cells, through cell surface contact and through the binding of CD40-CD40L, to produce immunoglobulins, including rheumatoid factor. The precise pathogenic role of RF is unknown, but it may involve the activation of complement through the formation of immune complexes. Activated CD4<sup>+</sup> T cells express osteoprotegerin ligands that stimulate osteoclastogenesis. Activated macrophages, lymphocytes, and fibroblasts, as well as their products, can also stimulate angiogenesis, which explains the increased vascularity found in the synovium of patients with RA. Endothelial cells in the synovium are activated and express adhesion molecules that promote the recruitment of inflammatory cells into the joint. This process is enhanced by the release of chemokines, i.e. IL-8.

Cell-cell contact is necessary both during the inductive phase of T cell activation and the effector phase, in which T cells indirectly mediate autoantibody production, joint inflammation and bone resorption [18]. In the inductive phase, TCR binding to antigen/MHC on antigen presenting cells (APCs) is a critical first step for T-cell activation. APCs are dendritic cells (DCs), activated macrophages, B cells and activated fibroblast-like synoviocytes (FLS). However, the nature of accessory signals received from APCs and of local cytokine environment during TCR stimulation determines the type of T-cell response and governs disease progression. Costimulation of T cells occurs through ligation of CD28 with CD80/CD86 on APCs. Once activated, T cells upregulate expression of CTLA-4, an inhibitory receptor that has a higher affinity for CD80/CD86 than CD28, in order to modulate activation [19,20]. Inducible costimulator (ICOS) is highly expressed on

activated T cells found in patients with RA. The ligand for ICOS is expressed on professional APCs in synovial tissue [21,22]. The integrin lymphocyte function associated antigen (LFA)-1 (CD11a) is expressed on activated T cells and binds to intercellular adhesion molecule-1 (ICAM-1) found on the surface of many cell types. LFA-1 is important for lymphocyte homing into inflamed tissues through binding to blood vessel walls and subsequent extravasation. However, it also mediates cellular contact between T cells and APCs during antigen presentation [23].

The effector functions of arthritogenic T cells are carried out in the synovial lining and intra-articular space of the joints. Upon activation, T cells upregulate surface expression of CD40L, which stimulates APCs through interaction with CD40. In B cells, ligation of CD40 in combination with cytokine activation stimulates antibody synthesis and isotype switching. Ligation of CD40 also induces expression of costimulatory and adhesion molecules as well as production of proinflammatory cytokines, including IL-6, IL-8, MIP-1 (macrophage inflammatory protein-1), TNF $\alpha$ , and IL-12, by APCs [24,25]. These cytokines are known to participate in joint inflammation and act reciprocally on T cells to drive production of other cytokines and surface molecules involved in the effector phase of joint inflammation. Non-specifically cytokine activated T cells contribute to joint pathology. Another important effector function of synovial T cells involves upregulation of receptor activator of NF- $\kappa$ B ligand (RANKL) on the cell surface. T cells activate synovial monocytes to differentiate into osteoclasts, that mediate bone destruction.

Recent evidence deriving from mouse models has questioned the role of Th1 cells in RA and identified a new Th subset, Th-17 cells. Th17 cells develop in the presence of tumor growth factor (TGF)- $\beta$ , IL-6 and IL-23 and are characterized by production of the highly inflammatory cytokine IL-17. IL-17 induces expression of IL-1, IL-6, IL-8, prostaglandin E2 and granulocyte colony stimulating factor (CSF) and acts synergistically with TNF $\alpha$ . IL-17 receptor is expressed ubiquitously. IL-17 directly and indirectly augments both inflammatory mediator production and joint destruction. IL-17 induces cartilage destruction and bone erosion by upregulation of RANKL. Many studies have built a strong case that IL-17 is a key suspect in the pathogenesis of RA: it is overexpressed in RA synovium and blood, it induces and synergizes with many inflammatory mediators important in joint pathology, and it is both necessary and sufficient for joint inflammation in animal models [26,27].

T regulatory cells (Treg) have become a major focus of immunologic research in the past decade due to their participation in controlling effector T cell functions in vitro and to their potential for regulating autoimmune inflammatory responses in vivo [28]. Treg cells produce high levels of TGF- $\beta$  and IL-10 [29]. Treg cells suppress immunological responses in multiple ways, which may involve negative signals produced by inhibitory surface molecules, cytotoxic killing,

downregulation of APC function, and induction of other regulatory cells. In RA, Treg cells have a defect in suppression of TNF- $\alpha$  and IFN- $\gamma$  production from CD4<sup>+</sup> T cells or monocytes, even though they can suppress the proliferation of effector T cells [30,31]. In other studies, it has been shown that effector T cells from peripheral blood of RA patients were resistant to Treg mediated suppression [32]. Treg cells express TNF $\alpha$  receptor 2, and signaling through this receptor by TNF- $\alpha$  results in inhibition of suppressive function and decreased Foxp3 expression [33]. Treatment of RA patients with anti-TNF- $\alpha$  antibody leads to in vivo expansion of Treg cells, increased Foxp3 expression, and restoration of cytokine suppressive function. Interestingly, healthy individuals respond to the arthritis-associated autoantigen, HCgp39 by producing IL-10 whereas patients with RA tend to produce proinflammatory cytokines. An important difference between healthy people and patients with RA is the ability to expand Treg cells specific for autoantigens [34].

NK cells subsets are widely distributed within the rheumatoid synovial membrane and could constitute a significant cytokine source. NK-cell activation by cytokines, including IL-12, IL-15, IL-18 leads to increased NK cell cytotoxic activity and release of cytokines, such as TNF $\alpha$  and IFN $\gamma$  [35].

In addition to autoantibody production, and thereby immune-complex formation, the B cell lineage contributes to pathogenesis of RA. B cells produce cytokines and chemokines. B cell derived cytokines regulate the activation of follicular DCs and lymphoid neogenesis. The lymphocyte infiltrate in the synovium comprises various patterns of structural organization: cells can be diffusely distributed, loosely aggregated or form ordered structures that contain germinal centres (occurring in <20% of individuals affected with RA). Their presence predisposes to poorer outcome. DCs in synovial membranes that contain ectopic germinal centres produce high levels of the B cell survival factor APRIL (a proliferation inducing ligand). Macrophages and synovial fibroblasts in most synovial tissues can produce BAFF (B cell activating factor). Both stimulate proliferation and activation of B cells [36]. Cytokines and chemokines are directly implicated in this lymphocyte organization. CXCL13 and CCL21 promote the formation of synovial germinal centres. Germinal-centre formation in the synovium also requires the expression of lymphotoxin (LT) $\beta$ , at least by B cells. The role of B cells in the joints as APCs is also important, as B-cell depletion prevents the formation of ectopic germinal centres and the optimal activation of T cells [37].

Macrophages as member of innate immunity are considered an important source of synovial pro-inflammatory cytokines. Activation of and subsequent cytokine production by macrophages (and synovial fibroblasts) in the synovium is likely to occur through pattern-recognition receptors (PRRs), such as Toll-like receptor (TLR)2, TLR3, TLR4 and TLR6, which recognize various microbial products, as well as putative endogenous ligands, including heatshock proteins and

fibronectin [38]. TLR expression is established in chronic disease and as such could serve not only to initiate but also to perpetuate disease. Cytokine expression in human synovial cultures is promoted through pathways that depend on the TLR signaling adaptor proteins MyD88 and TIRAP. Synovial monocytes can also be activated to produce cytokines by immune complexes through their cell-surface FcγRs. Finally, the synthesis of proteases, such as Mast cell tryptase and trypsin, by neutrophils and mast cells, regulates macrophage cytokine release by PAR2.

Of the innumerable cytokines, that are released by synovial macrophages, TNFα is clearly of primary importance in the pathogenesis of RA [39]. TNFα induces leukocyte and endothelial-cell activation, synovial-fibroblast activation and survival, pain-receptor sensitization and angiogenesis, which together represent key pathological features of RA. Further essential macrophage derived cytokines are IL-1, IL-6, IL-15, IL-18 and IL-32.

Other innate immune effector cells implicated in RA pathogenesis are neutrophils present in high numbers in synovial fluid and synovial membrane. They synthesize a wide variety of cytokines, including TNFα, IL-1, IL-6, IL-15, IL-18 and BAFF, and therefore could support a range of pathological events. Neutrophils are activated by immune complexes, complement components and cytokines to release chemokines, prostaglandins, reactive oxygen and nitrogen intermediates and proteolytic enzymes, and therefore also probably contribute significantly to the general hypoxic and destructive milieu in inflamed joints [40]. Mast cells are similarly implicated at the crossroad of innate and adaptive synovial immunity. They are widely distributed in RA synovial tissue and express various proteases and pro-inflammatory cytokines [41].

One important feature of rheumatoid synovitis is the relative expression deficiency of several regulatory cytokines, receptors and tissue enzyme inhibitors, thereby contributing to the imbalance between pro-inflammatory and anti-inflammatory actors.

Inflammation and bone erosion are closely linked [42,43]. Normal physiological processes ensure a balance between bone formation and bone resorption to maintain skeletal homeostasis. This balance is perturbed in RA in favor of bone resorption. Bone resorption depends on osteoclasts. Mice with differentiation defects in the osteoclast lineage develop inflammatory arthritis that is not associated with bone erosion. In RA, osteoclasts at the interface between synovial tissue and articular bone induce bone resorption, which in turn permits invasion by cells of the synovial membrane and results in pannus formation. This process depends on the influx of osteoclasts precursors into inflamed synovial tissue and the differentiation of these cells into mature osteoclasts. Macrophage CSF (M-CSF) and RANKL are essential for the differentiation of osteoclasts from their precursor cells, and a lack of either molecule is sufficient to block osteoclast formation completely. M-CSF is expressed by synovial mesenchymal cells and to a lesser extent by T cells. TNF induces the

production of M-CSF by synovial fluid cells, as does IL-7, which promotes M-CSF production by Th1 cells. The action of M-CSF on monocytes is essential for osteoclastogenesis, but alone is insufficient to induce their final differentiation [44,45]

RANKL, a member of the TNF superfamily, is expressed by mesenchymal cells, such as synovial fibroblasts, and activated synovial T cells. In rheumatoid synovial tissue, RANKL expression is up-regulated and constitutes an important prerequisite for osteoclast differentiation. RANKL expression is regulated by inflammatory cytokines, such as TNF $\alpha$ , IL-1, IL-6 and IL-17, but is also influenced by non-cytokine inflammatory mediators such as prostaglandin E2. RANKL induces the final differentiation of osteoclasts and their bone resorbing activity. The interaction of RANKL with its receptor RANK is modulated by osteoprotegerin (OPG), a soluble decoy receptor, which is expressed by mesenchymal cells in the RA synovium [46]. In RA, an imbalance between OPG and RANKL expression promotes RANKL induced bone loss.

Other cytokines in the inflammatory milieu also contribute to the destructive process. TNF $\alpha$  is a potent driver of osteoclast formation, acting either additively with RANKL or directly through TNF $\alpha$  receptor I. TNF $\alpha$  also mobilizes CD11b<sup>+</sup> osteoclast precursors from the bone marrow [47]. IL-1 $\beta$  induces RANKL expression. Moreover, IL-1 $\beta$  is a key component of TNF-mediated osteoclastogenesis. IL-17 induces RANKL, TNF $\alpha$  and IL-1 expression by synovial fibroblasts to support osteoclast formation. Th17 cell inducing cytokines, including IL-23, TGF $\beta$  and IL-6, therefore, probably act on osteoclasts, too. RANKL expression is a key link in osteoimmunology, providing an explanation to why immune activation is linked to bone loss.

Bone formation, which is the 'physiological' counterpart response to increased bone resorption is mediated by osteoblasts and is virtually absent in RA. TNF $\alpha$  inhibits osteoblast differentiation and function. TNF $\alpha$  upregulates secretory molecules, which acts as WNT protein inhibitors and blocks bone and cartilage formation [48].

Synovial fibroblasts concur in joint damage by producing inflammatory signals. In rheumatoid joints, synovial fibroblasts exhibit anchorage independent growth, loss of contact inhibition and increased proliferation, and play a central role in chronic synovitis and pannus formation, which depends from cadherin-11 [49]. Synovial hyperplasia is induced by exposure to cytokines, such as fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and TGF $\beta$ , together with the induction of expression of oncogenes, such as RAS and MYC, and survival proteins, such as heat-shock protein 70 (HSP70). Matrix degrading enzymes are produced by synovial fibroblasts, neutrophils and mast cells, which are closely located to articular cartilage, and chondrocytes themselves. Such enzymes released constitute enzymatic milieu responsible for the local migratory activity of pannus and articular cartilage invasion [50]. Synovial fibroblasts promote further T-cell

and B-cell migration, activation and survival. In turn, B cells promote synovial-fibroblast activation through IgG binding to Fc $\gamma$ R on synovial fibroblasts. Together these data indicate a central role for synovial fibroblasts in integrating the inflammatory and destructive phases of inflammatory arthritis.

The production of vascular endothelial growth factor (VEGF), FGF, oncostatin M and IL-18 by synovial fibroblasts promotes angiogenesis typical of the rheumatoid synovium. This profuse angiogenetic activity is in turn a prerequisite for inflammation and destruction. Inhibition of angiogenesis suppresses synovitis [51].

### **3.3. Clinics of rheumatoid arthritis**

Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia (“swelling”), autoantibody production (RF and anti-CCP), cartilage and bone destruction (“deformity”), and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders.

The typical case of RA begins insidiously, with the slow development of signs and symptoms over weeks to months. Often the patient first notices stiffness in one or more joints, usually accompanied by pain on movement and by tenderness in the joint. The number of joints involved is highly variable, but almost always the process is eventually polyarticular, involving five or more joints, especially small joints of hands and feet. RA is an additive polyarthritis, with the sequential addition of involved joints. Occasionally, patients experience an explosive polyarticular onset. Other not as usual manifestations are polymyalgia-like onset, systemic onset and oligo-/monoarticular forms. The joints involved most often are the proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints of the hands, the wrists (particularly at the ulnar-styloid articulation), shoulders, elbows, knees, ankles, and metatarsophalangeal (MTP) joints. The distal interphalangeal (DIP) joints are generally spared as the spine except the atlanto-axial articulation is. Morning stiffness, persisting more than one hour but often lasting several hours, may be a feature of any inflammatory arthritis but is especially characteristic of RA. Its duration is a useful gauge of the inflammatory activity of the disease. Symmetric joint swelling, although not invariable, is characteristic of RA. Fusiform swelling of the PIP joints of the hands is a common early finding. MCP, wrists, elbows, knees, ankles and MTP are other joints commonly affected where swelling is easily detected. Redness of affected joints is not a prominent feature of RA. Occasionally inflamed joints will feel warm to the touch. Pain on passive motion is the most sensitive test for joint inflammation. Inflammation may limit the range of motion of the joint. As the pathology progresses the inflammatory activity leads to tendon displacement or rupturing and to erosions with destruction of the joint surface, which impairs range of movement and leads to deformity and joint ankylosis.

The fingers may suffer from almost any deformity depending on which joints are most involved. Most characteristic deformities are ulnar deviation, “Z”-thumb, boutonniere and swan neck deformity. Nonspecific systemic symptoms primarily fatigue, malaise, anorexia, weight loss and depression, may concur or precede other symptoms of the disease by weeks to months. Patients complain of severe fatigue 4 to 6 hours after wakening. Fever occasionally occurs and is almost always low grade [52].

Extra-articular manifestations other than anemia are clinically evident in about 15-25% of individuals with RA [53]. The rheumatoid nodule, which is often subcutaneous, is the skin feature most characteristic of RA. The initial pathologic process in nodule formation is unknown but may be essentially the same as the synovitis, since similar structural features occur in both. The nodule has a central area of fibrinoid necrosis that may be fissured and which corresponds to the fibrin-rich necrotic material found in and around an affected synovial space. Surrounding the necrosis is a layer of palisading macrophages and fibroblasts, corresponding to the intimal layer in synovium and a cuff of connective tissue containing clusters of lymphocytes and plasma cells, corresponding to the subintimal zone in synovitis. The typical rheumatoid nodule may be a few millimeters to a few centimeters in diameter and is usually found over bony prominences, such as the olecranon, the calcaneal tuberosity, the MCP joints, or other areas that sustain repeated mechanical stress. Nodules present in 20% of patients are associated with a positive RF titer and severe erosive arthritis. Rarely, these can occur in internal organs or at diverse sites on the body. Vasculitic skin manifestations include nailfold microinfarcts, livedo reticularis, purpura, ischemia, ulcers or erythema nodosum. Palmar erythema and diffuse thinning and skin fragility may be present.

There are several pulmonary manifestations of RA, including pleurisy with or without effusion, intrapulmonary nodules, rheumatoid pneumoconiosis (Caplan's syndrome), diffuse interstitial fibrosis, and rarely BOOP, pneumothorax and pulmonary hypertension. Cardiac manifestations are pericarditis as the most common, further disturbances of atrioventricular conduction, endocarditis with resulting valvulopathies and rarely myocarditis.

Renal amyloidosis can occur as a consequence of chronic inflammation. RA may affect the kidney directly through vasculopathy or mesangial glomerulonephritis, but this is less well documented. Hepatic involvement in RA is essentially asymptomatic. Activation and cytokine production by Kupffer cells can be so marked that the increase in hepatocyte activity may result in palpable nodular hyperplasia of the liver. Intestinal vasculitis is a very rare manifestation of RA.

Keratoconjunctivitis sicca is the most common ocular manifestation of RA due to secondary Sjögren syndrome. When severe, dryness of the cornea can lead to keratitis and loss of vision.

Episcleritis occurs occasionally and is manifested by mild pain and intense redness of the affected eye. Scleritis and scleromalacia are rare but may result in perforation, infection and visual loss.

Peripheral neuropathy and mononeuritis multiplex are due to rheumatoid vasculitis. The most common problem is carpal tunnel syndrome caused by compression of the median nerve by swelling around the wrist. Atlanto-axial subluxation can occur, owing to erosion of the odontoid process and/or rupture of transverse ligament. Clumsiness is initially experienced, but without due care this can progress to quadriplegia.

Felty's syndrome is nowadays a rare complication of RA and is characterized by splenomegaly, and leukopenia - predominantly granulocytopenia.

Local osteoporosis occurs in RA around inflamed joints. It is postulated to be caused by inflammatory cytokines. General osteoporosis results from immobility, systemic cytokine effects, local cytokine release in bone marrow and corticosteroid therapy.

Atherosclerosis is accelerated in RA by effect of systemic inflammation. The major cause of mortality in RA is cardio- and cerebrovascular disease. The incidence correlates with C-reactive protein (CRP) levels [54].

RA patients with active disease are at higher risk to develop non-Hodgkin lymphoma (NHL) of B cells and bronchial carcinoma.

### **3.4. Diagnosis of rheumatoid arthritis**

Despite the typical clinical finding of polyarthritis of the small hand and foot joints bloods exams can reveal increase of CRP, erythrocyte sedimentation rate (ESR) and other acute phase proteins. Normocytic normochromic anemia may be present due to inflammation driven iron sequestration in reticuloendothelial system, hepcidin induced reduction of intestinal iron absorption due to inhibition of blood stem cells by inflammatory cytokines. Leukocytosis and thrombocytosis may occur. Immunological alterations which may be present include the positivity for RF, anti-citrullinated protein antibodies (ACPA), anti-nuclear antibodies (ANA) and hypergammaglobulinemia. The most common tests for ACPA are the anti-CCP and the antibodies against mutated citrullinated Vimentin [55].

Diagnosis of RA is established following the American College of Rheumatology (ACR) classification criteria of 1987 or newer ACR/European League against Rheumatism (EULAR) classification criteria of 2010 [56,57]. The latter criteria promote earlier diagnosis and start of therapy. In cases not completely fulfilling criteria evaluation of radiographic erosions may ensure diagnosis of RA [58].

ACR criteria 1987 for the classification of RA:

- Morning stiffness of >1 hour for at least 6 weeks
- Arthritis and soft-tissue swelling of 3 joint groups, present for at least 6 weeks
- Arthritis of hand joints (MCP, PIP, wrist), present for at least 6 weeks
- Symmetric arthritis, present for at least 6 weeks
- Rheumatoid nodules
- Positivity of RF/anti-CCP
- Radiological changes as joint erosions and/or periarticular osteoporosis

At least four criteria have to be met for classification as RA.

ACR/EULAR criteria 2010 for the classification of RA:

A Joint involvement	Score
• 1 large joint	0
• 2-10 large joints	1
• 1-3 small joints	2
• 4-10 small joints	3
• >10 joints (at least 1 small joint)	5
B Serology	
• Negative APCA and RF	0
• Low-positive APCA or RF	1
• High-positive APCA or RF	2
C Acute phase reactants	
• Normal CRP and ESR	0
• Abnormal CRP or ESR	1
D Duration of symptoms	
• < 6 weeks	0
• => 6 weeks	1

At least 6 points have to be met for classification as RA.

### **3.5. Outcome of rheumatoid arthritis**

During the mid-1980s, it became apparent that most patients seen in rheumatology clinics with symptoms and signs of RA for longer than 3–6 months rarely experienced spontaneous remission, and most had a progressive disease [59,60]. It was recognized that short-term drug efficacy was not translated into long-term effectiveness as most patients seen in the 1980s were found to experience radiographic progression, severe functional declines, work disability, and premature mortality, and many patients required joint surgery [61-64]. The long-term severity of RA was recognized in longitudinal studies of clinical cohorts that indicated continuous radiographic progression over follow-ups in excess of 20 years. These reports led to calls for early and aggressive use of disease-modifying anti-rheumatic drugs (DMARDs) including aggressive strategies to prevent severe long-term outcomes of RA [65-67].

Joint damage occurs early in the course of RA. Radiographs are a suitable outcome measure in patients with RA. They reflect the history of the joint pathology and provide a permanent record necessary for serial evaluation of the disease. The extent of damage in the joints of the hands and wrists gives a good overall indication of both the extent of overall joint damage at a given time and the rate of progression of damage. Several authors have shown in cohort studies of patients with early RA that MTP are eroded earlier and show more damage [68]. 30% of patients have radiographic evidence of bony erosions at the time of diagnosis, and this proportion increases to 60% by two and to 70% by 3 years despite conventional treatment [69,70]. Patients with more than one erosion, when first seen, are more likely to have severe radiographic damage at 3 years. Positivity for RF and/or ACPA, elevated erythrocyte sedimentation rate (ESR), non-response to therapy and delayed treatment influence further negatively radiographic progression. Unfortunately, bony erosions and deformities are largely irreversible and represent a cumulative process of inflammation over time [71]. Initiation of therapy with DMARDs within three months after the diagnosis of RA is crucial; a delay of as little as three months in the introduction of these medications results in substantially more radiographic damage at five years. Therefore, early diagnosis, although challenging, is critical [72-76]. It is commonplace for patients to have erosions when they are first seen. Patients with very early RA, who were seen within 3 months of their first symptoms, had erosions present at their first assessment in 13% of cases, and after 12 months of follow-up, this had increased to 28% [77]. The call for prompt therapeutic intervention even earlier to ameliorate patients' response and outcome has led to the recommendation to investigate patients with polyarthritis lasting for more than 6 weeks for the suspect of RA.

RA has a major impact in many areas of individuals' lives, not just those traditionally considered to be the domain of medical intervention. The most important problems are persistent pain and loss of

function, that result in disability and impaired quality of life. Pain is a dominant concern of patients with RA, and its persistence is a highly negative consequence of disease. Pain scores correlate with patients' global assessment of disease and morning stiffness far more than from radiographic or other clinical variables such as the number of tender and swollen joints. Clinical experience suggests that at least part of the pain of early RA appears to be related to depression. However, patients may also suffer other symptoms, such as fatigue, and adverse effects from therapy. Fatigue measured is a dominant factor in determining the quality of life and psychosocial aspects of daily functioning.

Loss of functional capacity is a major determinant of morbidity and a predictor of mortality of patients with RA [78-82]. In term of disability the long-term prognosis of RA is poor: 80% of affected patients are disabled after 20 years [83]. Disability in RA is measured using disease-specific measures, especially the Health Assessment Questionnaire (HAQ). RA patients have considerable disability before they start treatment. Therapy with symptomatic agents and DMARDs initially improves synovitis and hence associated disability. The reversible component is that related to joint pain, stiffness, and swelling due to inflammation, or associated symptoms such as depression. Thereafter the disability rises again slowly as joint damage and other disease manifestations progress in a manner that no longer respond to therapy. Indeed, in early RA, disability, measured by HAQ is correlated with disease activity, whereas correlation with joint damage increases with time. Responsiveness in HAQ scores is inversely associated with mean disease duration in RA. Thus, HAQ comprises mainly a reversible activity related component and an irreversible one due to joint damage. Disease activity can be best assessed using composite indices; joint damage is usually measured by the Total Sharp Score (TSS) and its derivatives. Both the TSS and the change in TSS (progression rate) are significant determinants of the HAQ score. It has been estimated that 0,1 HAQ points correspond to 10 TSS points [84]. However, disability is also related to other factors, like age, psychosocial aspects or comorbidities. Physical disability is worsening with increasing level of comorbidity, irrespective of disease activity [85-87].

RA has many consequences, not only for the individual but also for their friends and family and for the whole of society. The impact of RA on society includes high medical and indirect costs, reduced ability to work, and the need for additional help and support from family and friends. Particular concern has raised about work disability. It has been estimated that about one-third of people with RA leave employment prematurely, and work disability involves patients with early RA as well as those with longstanding disease. Work disability often starts soon after diagnosis and subsequently continues at a steady rate. Estimates of the prevalence of work disability among people with RA are as high as 62%, with permanent disability ranging from 31 to 42% [88].

In addition, RA patients have approximately twice the mortality rate of the general population, with cardio- and cerebrovascular diseases accounting for the excess of death. RA patients have a 2- to 3-fold increased risk of myocardial infarction, a 2-fold increased risk of congestive heart failure, a 2-fold increased risk of sudden death, and a 1.7-fold increased risk of strokes. Serious infections (leading to hospital admission, intravenous antibiotics or death) are major contributor to increased mortality, with the risk estimated to be two-to-three times that of the general population. Further contributors to increased morbidity and mortality are gastrointestinal, pulmonary and renal disease due to RA or its medication and excess in malignancy incidence especially of lymphoma and bronchial carcinoma, whom are correlated to disease activity [89]. In summary, life expectancy of RA patients is reduced by an average of 3 to 18 years [90].

### **3.6. Distinguishing features of rheumatoid arthritis**

- Th1 immune reaction associated with HLA DRB1 and driven by CD4+ T-cells
- Woman are more often affected.
- There is a predilection for the synovial joints, at the beginning typically of MCP, MTP and PIP joints of the hands and the feet with possible affection of all synovial joints in chronic disease. As a rule DIP are not affected.
- Inflammatory changes are multifocal and symmetrical with centripetal progression during the course of disease.
- Synovitis and/or Tenosynovitis can be assessed clinically and by CR, computer tomography, bone scintigraphy, PET, US, MRI.
- From the beginning of the disease destructive, “minus” changes predominate – juxtaarticular demineralization, cartilage and bone erosions. Therefore RA in contrary to peripheral PsA is called “minus” or “atrophic” arthritis.
- Productive changes – periostosis, osteosclerosis, bone ankylosis, enthesiophytes (typical for SpA) are absent or modest.
- Affection of the fibrocartilaginous joint is rare and modest.
- RF and APCA are positive. Anemia may be present. CRP and ESR are very often elevated.
- Rheumatic nodules are present in seropositive from. Systemic and organ involvement is possible.
- The most important initial histopathological feature of RA is synovitis followed by chronic proliferative granulomatous pannus-tissue, which is associated with cartilage and bone

destruction. Early inflammatory changes in RA also develop synchronously within the subchondral bone marrow.

## **4. Psoriatic Arthritis**

### **4.1. Impact of psoriatic arthritis**

PsA has been defined as an inflammatory arthritis, usually seronegative, associated with psoriasis (PsO). It emerged as a clinical entity separate from RA following the discovery of the RF in 1948 [91]. Prevalence of PsO has been estimated between 2% and 3%, the estimated prevalence of inflammatory arthritis among patients with psoriasis has varied widely from 6% to 42%. A recent study from Sweden suggests that PsA occurs in 30% of patients with psoriasis [92]. Similarly a study of patients attending a psoriasis clinic identified 31% as having PsA. The prevalence of PsA in the general population should be close to 1%. PsA occurs just as frequently in both sexes. The impact of the disease in patients with PsA appears to be similar to that of patients with RA.

### **4.2. Pathogenesis of psoriatic arthritis**

Compared with most other rheumatic diseases, heredity plays a particularly strong role in the development of PsA. About 15% of the relatives of an index patient with PsA will also have PsA, and an additional 30% to 45% will have PsO. Accordingly, the presence of either PsO or PsA in a family member of a patient suspected of having PsA provides support for the diagnosis. Identification of the genes responsible for this high degree of familial aggregation remains an ongoing process but, among the identified genes, the HLA genes in the MHC are of primary importance in the development of PsA. The patterns of inheritance of PsO and PsA are those of a genetically complex multigenic disease [93].

In contrast to most other autoimmune diseases in which susceptibility is specified by HLA-DR or other class II MHC genes, in PsA it is the class I genes, notably alleles at the HLA-B and HLA-C loci, that are involved [94]. These include the HLA-C allele Cw\*0602, which is the major determinant of susceptibility to PsO, and the HLA-B alleles B\*27 and B\*39, and possibly some additional alleles HLA-DRB1\*04 alleles encoding the shared epitope are strongly associated with susceptibility to RA, but not with PsA. Furthermore, they showed that HLA-Cw\*06 and HLA-DRB1\*07 were indeed associated with patients with PsA having type I (onset before age 40 years) but not type II PsO (onset after age 40 years). Ongoing analyses indicate that these several MHC alleles operate independently in specifying the disease phenotype of PsA. This suggests that there could be two genetic pathways to PsA. One is through the function of the HLA-B alleles B\*27 and

B\*39, and another is through the function of haplotypes containing the HLA-C allele Cw\* 0602 (Psors1). Evidence is emerging that these two forms of PsA that share the PsO phenotype are subtly different. It appears that the Cw\*0602 alleles confer a phenotype with more severe skin disease and, on average, a long interval ( $\geq 10$  years) between the appearance of PsO and the development of the musculoskeletal features of PsA. In those with B\*27 or B\*39, the musculoskeletal component appears more synchronously with the cutaneous component, and PsA is more likely than in the presence of Cw\*0602.

The presence of susceptibility genes in an individual defines the first preclinical stage of the development of PsA. The explanation for the association of the HLA alleles B\*27 and B\*39, and Cw\*0602 with PsA susceptibility is that the molecules encoded by these alleles recognize self-peptides derived from proteins found in enthesal and synovial sites. T-cell clones specific for these self-peptides would be inappropriately activated, perhaps by dendritic cells, and the activated state perpetuated by the continual supply of self-peptides. The T-cell repertoire that is developed on the individual's self-peptides and self-MHC is poised for autoreactivity, but remains quiescent until triggered. An overbalance of stimulatory signals by infections, smoke or trauma may be responsible for engagement of the CD8+ T cells through NK receptors or other innate immune signal receptors. Once triggered the immune process results in the development of the two main features of PsA: the inflammatory infiltrate of CD8+ T-cells and accessory cells into the entheses and synovium, and the response of the synovial and enthesal tissues to the products and consequences of the inflammatory infiltrate.

### **4.3. Clinics of psoriatic arthritis**

Wright identified five clinical patterns among patients with PsA: distal predominant pattern (5%), oligoarticular asymmetrical (70%), polyarticular RA-like (15%), spondylitis (5%), and arthritis mutilans (5%); the majority of PsA patients having oligoarthritis [95]. These patterns are likely more relevant at disease onset as patterns likely change and patients may tend to develop the polyarticular pattern over time.

The specific clinical features include the common involvement of DIP joints in PsA. The joint distribution tends to occur in a ray pattern in PsA so that all the joints of a single digit are more likely to get affected than the same joints on both sides, which is typical of RA. This may explain the tendency to asymmetry that occurs even in the polyarticular disease in PsA. The concomitant extensive tendon involvement rises the picture of sausage digits. The degree of erythema over affected joints and lower level of tenderness are also typical features of PsA. The deformities that may result from PsA lead to shortening of digits because of severe joint or bone lysis, with the most

severe form being the telescoping of digits. Bony fusion of joints may also occur in PsA. These changes are seen in radiographs as the classic pencil in cup and ankylosis, respectively. Marginal erosions alternated with areas of bone proliferation and periosteal appositions are also typical radiographic aspects [96].

Typical feature of PsA is the involvement of entheses and of axial skeleton (sacroileitis and spondylitis, fusion of sacroiliac joints and syndesmophytes), often in an asymmetric manner. Therefore PsA is classified with SpA. Spondylitis is present in up to 40% of patients, extra-articular features may occur: mucous membrane lesions, uveitis, urethritis, diarrhea, aortic root dilatation and association with HLA-B27 [97].

Although by definition, all patients with PsA must have psoriasis, the arthritis may precede or occur without PsO. Nail lesions occur in about 40–45% of patients with PsO uncomplicated by arthritis and about 87% of patients with PsA.

Rheumatoid nodules are absent. RF is detected in only about 13% of patients with PsA. Major organ involvement is rare, and metabolic syndrome often associated.

#### **4.4. Diagnosis of psoriatic arthritis**

Diagnosis of PsA is established by CASPAR (CLASSification criteria for Psoriatic Arthritis) criteria and other criteria systems [98,99]

CASPAR criteria: A patient must have inflammatory articular disease (joint, spine, or enthesal) with  $\geq 3$  points based on points assigned to each feature

Criteria	Score
Presence of psoriasis	2
Personal or family history of psoriasis	1
Dactylitis	1
Juxtaarticular new bone formation	1
Rheumatoid factor negativity	1
Nail dystrophy	1

#### **4.5. Outcome of psoriatic arthritis**

PsA is suggested to be less severe than that seen in RA. However, about 20% of the patients develop a very destructive disabling form of arthritis. A recent study of early onset PsA showed that within two years of onset, 47% of patients demonstrated at least one erosion.<sup>36</sup> This rate is in keeping with previous observations that 67% of patients seen in PsA clinics had evidence of erosive disease [100]. Identified predictors for future radiographic damage include polyarthritis, nail disease, high use of steroids, female gender, established damage, HLA-B27 in the presence of HLA-DR7, HLA-B39 and HLADQw3 in the absence of HLA-DR7 [101]. In a study each actively inflamed joint (tender and/or swollen) resulted in a 4% risk of increased damage at the next visit. Thus, if a patient had 20 actively inflamed joints, there was an 80% chance of progression of damage from the beginning to the end of the study [102]. A low ESR and HLA-B22 were noted to be protective.

One study showed patients with PsA had similar radiological damage as patients with RA, suggesting that the disease may be just as destructive radiologically. Another study suggested that the radiological changes are not quite as severe [103,104].

Patients with PsA have reduced quality of life and functional capacity compared with PsO patients or healthy controls. Thus, the severity of the disease has an impact on the functional status and quality of life of patients with PsA. The Medical Outcome Survey Short Form 36 has also been validated in PsA and has shown significant differences between patients with PsA and the general population. The severity of PsA is reflected also in increased mortality. Patients with PsA are at an increased risk for death with a mortality ratio of 1.62. The causes of death are similar to those noted in the general population, with cardiovascular causes being the commonest. The risk for premature death is related to previously active and severe disease, the level of medication, the presence of erosive disease, and a high sedimentation rate at presentation to clinic [104].

#### **4.6. Distinguishing features of psoriatic arthritis**

- Th2 or Th0 immune reaction mediated by CD8+ T-cells associated with MHC I molecules
- PsA occurs just as frequently in both sexes.
- There is a predilection for fibrocartilaginous articulations (entheses, sacroiliac joints, discovertebral junction etc.)
- Inflammatory changes are frequently distally and asymmetrical in distribution.
- Enthesitis is the hallmark of PsA and SpA and the first radiological manifestations of the diseases. It can be detected by assessed clinically and by CR, computer tomography, bone scintigraphy, PET, US, MRI.

- From an early stage of the diseases PsA is characterized by bone productive changes: periosteal apposition, osteosclerosis, bony ankylosis, ossification of ligaments, tendons, aponeuroses, articular capsules. Therefore in contrary to RA –which is termed “minus” or “atrophic” arthritis- seronegative peripheral arthritis represents so-called “plus” arthritis. Productive changes dominate the end stage of the disease which is characterized by metaplastic transition of the fibrous tissue to bone with pronounced joint ankylosis.
- An important diagnostic feature of PsA and SpA is simultaneous existence of all reactive bone - joint capabilities. From the beginning of the disease there is a combination of destructive changes (such as juxtaarticular demineralization and erosions) as well as productive signs (which include periosteal apposition, osteosclerosis and bone ankylosis).
- Specific antibodies are not known. CRP and ESR are often within normal range.
- PsO, nail dystrophy and other SpA features are often present. Organ involvement is rare.
- Inflammation within the synovial joints, histologically similar to RA, is not so pronounced. Consequently destructive changes within the synovial joints are much less with the exception of PsA in which pronounced bone destruction may develop (arthritis mutilans).

## 5. Histopathology in Arthritis

Synovitis is a major characteristic of chronic inflammatory joint diseases of autoimmune origin, such as RA and SpA. It can also occur as a secondary inflammatory symptom in OA, which is primarily induced by biomechanical stress on cartilage and subchondral bone.

Studies in RA indicate that the synovial membrane has a dominant role in the joint inflammation and destruction, as suggested by the changes in synovial histology: (a) thickening of the synovial lining layer, as a result of infiltration by CD68+ cells and both proliferation and reduced apoptosis of type B synoviocytes; (b) neovascularization of the sublining layer; (c) infiltration of the sublining with T and B lymphocytes, plasma cells, and macrophages; and (d) alteration of the adhesion molecule expression, including the expression of  $\alpha V$  integrins which may have a role in both neovascularization and pannus formation [105].

These observations suggest that the synovial membrane is both the primary site of inflammation, triggered by autoreactive T cells and macrophages, and the main effector organ, as the hyperplastic “aggressive” pannus leads to cartilage and bone erosion. The normal synovium contains mesenchymal-derived FLSs and resident macrophages. In RA, the membrane lining is expanded, and FLSs assume a semiautonomous phenotype characterized by anchorage independence, loss of contact inhibition, and the expression of high levels of disease relevant cytokines and chemokines,

adhesion molecules, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs). FLSs thereby contribute directly to local cartilage destruction and the chronicity of synovial inflammation, and they promote a permissive microenvironment that sustains T-cell and B-cell survival and adaptive immune organization [106]. The molecular mechanisms that sustain synovial hyperplasia are incompletely understood. The increased proliferative capacity of FLSs is not explanatory. A more likely possibility is altered resistance to apoptosis, which is mediated by diverse pathways, including mutations of the tumor-suppressor gene p53; expression of stress proteins (e.g., heat-shock protein 70), which foster the survival of FLSs; and modulation of the function of the endoplasmic reticulum by synoviolin, an E3 ubiquitin ligase that regulates the balance of cell proliferation and apoptosis [107,108]. Synoviolin negatively regulates p53 expression and its biologic functions. In addition, cytokine-induced activation of the NF- $\kappa$ B pathway in FLSs favors survival after ligation of TNF- $\alpha$  receptor. Methylation and acetylation of cell-cycle regulatory genes and expression of microRNAs may be critical factors [109]. Synovial hyperplasia could also reflect increased influx of mesenchymal cells. In a mouse model of arthritis with severe combined immunodeficiency, FLSs were shown to migrate and thereby promote articular involvement [110]. A crucial advance has been the elucidation of the molecular pathways that sustain integral membrane structure in rheumatoid arthritis. Cadherin-11 and  $\beta$ -catenin mediate FLS-homotypic interactions that are essential for membrane formation and for subsequent inflammation [111].

Inflammatory synovitis is characterized by increase in volume of the synovium due to hypertrophy and multiplying of the synoviocytes, by influx of inflammatory cells, such as the lymphocytes, plasma cells and macrophages into the synovium, by edema and fibrosis of the synovial matrix and by increased vascularity. A hyperplastic synovium is the major contributor to cartilage damage in RA. Loss of the normally protective effects of synovium (reduced expression of lubricin) alter the protein-binding characteristics of the cartilage surface, promoting FLS adhesion and invasion. FLS synthesis of MMPs (particularly MMP-1, 3, 8, 13, 14, and 16) promotes disassembly of the type II collagen network and collagenous cartilage matrix, a process that alters glycosaminoglycan content and water retention and leads directly to biomechanical dysfunction. Other matrix enzymes (e.g., ADAMTS 5) degrade aggrecan and thus further diminish cartilage integrity. Endogenous enzyme inhibitors, such as TIMPs, fail to reverse this destructive cascade [112]. Moreover, articular cartilage itself has limited regenerative potential. During inflammation (IL-1 and IL-17A) chondrocytes undergo progressive deprivation and apoptosis. These processes ultimately lead to the destruction of the surface cartilage and the radiographic appearance of joint-space narrowing. Bone erosion occurs rapidly (affecting 80% of patients within 1 year after diagnosis). Synovial cytokines,

particularly M-CSF and RANKL promote osteoclast differentiation and invasion of the periosteal surface adjacent to articular cartilage. TNF $\alpha$  and IL-1, 6 and 17 amplify osteoclast differentiation and activation. Osteoclasts have the acidic enzymatic machinery necessary to destroy mineralized tissues, including mineralized cartilage and subchondral bone [113,114] Resorption pits are filled by inflammatory tissue. Breach of cortical bone permits synovial access to the bone marrow, which causes inflammation of the bone, in which T-cell and B-cell aggregates gradually replace marrow fat. It is established that inflammation can also start directly in bone marrow and precede erosions [115]. Eroded periarticular bone shows little evidence of repair in RA, unlike bone in other inflammatory arthropathies. Cytokine-induced mediators (DKK-1) inhibit WNT system and therefore the differentiation of mesenchymal precursors into chondroblasts and osteoblasts [116].

Synovial histopathology in PsA is generally characterized by neovascularization, and inflammatory infiltration with predominantly mononuclear cells (T lymphocytes, B lymphocytes and plasma cells, and macrophages), although PMCs can also be detected. Mild to moderate synovial lining hyperplasia is observed in a considerable percentage of cases [117].

The macro- and microscopic features of synovitis seem to be similar in different arthropathies, and have been defined as nonspecific synovitis [118]. However, some studies compared peripheral synovitis and histopathological characteristics of PsA with those of ankylosing spondylitis (AS)/undifferentiated SpA and RA, and compared the synovium of oligoarticular versus polyarticular PsA [105,119]. The histological analysis included examination of the lining layer thickness, vascularity, cellular infiltration, lymphoid aggregates, plasma cells, neutrophils, T cell subsets, E-selectin, ICAM-1, vascular cell adhesion molecule-1, S100A12, intracellular citrullinated proteins and MHC–human cartilage (HC) gp39 peptide. complexes.

Comparing SpA (PsA, AS and SpA) with RA, vascularization, neutrophils and CD163+ macrophage counts were greater in SpA, whereas lining layer thickness and the number of CD83+ dendritic cells were greater in RA. At microscopic level there was further an increased maximal lining thickness in late RA compared to early RA, whereas all other parameters were the same in both disease stages. On synovial fibroblasts in RA compared to SpA a strongly significant decrease of  $\alpha$ V $\alpha$ 3 in the synovial lining layer and increase of  $\alpha$ V $\alpha$ 5 in the sublining layer is found. As engagement of  $\alpha$ V integrins regulates proliferation, migration, and collagenase expression of a variety of cell types, this differential integrin expression may have an important role in the aggressive growth of the synovial pannus in RA.

CD3+, CD4+ and CD20+ lymphocytes and plasma cells were overrepresented in RA compared with SpA, the CD4/CD8 ratio higher in RA. Th1 cytokine pattern of expression is characteristic of RA whereas in SpA, a Th2 or Th0 cytokine pattern is more frequent. For both diseases synovial

lining, vascularization degree and cellular infiltrations were dependent from local disease activity and effusion. CRP and ESR correlated only weakly with local inflammation in RA, whereas no correlation was found with CRP and ESR in PSA/SpA and with other disease activity parameters such as counts of swollen and tender joints both in RA and PsA/SpA [105]. In RA, 44% of samples exhibited positive staining for intracellular citrullinated proteins and 46% for MHC–HC gp39 peptide complexes, whereas no staining for these markers was observed in PsA and SpA samples. No influences of disease modifying anti-rheumatic drug (DMARDs) and/or corticosteroid treatment was noted [120-123].

Focusing on PsA, no significant differences were observed between PsA and SpA, and between oligoarticular and polyarticular PsA. Both PsA groups can be differentiated from RA on the basis of these same synovial features, suggesting that peripheral synovitis in PsA belongs to the SpA concept.

## **6. Vascularization in Arthritis**

Neovascularization, or growth of new blood vessels from preexisting vessels of the synovium, is the hallmarks of synovitis. The formation of new blood vessels appears to be an essential pathogenic step for synovitis. Microscopical examinations of synovial biopsies show, that development of microvessels is the earliest sign of RA. This so called angiogenesis correlates with disease activity, and therefore several imaging modalities attempt to visualize those early vascular changes [124,125]. Studies in experimental models of arthritis even suggest that destruction of bone and cartilage may be more closely linked to angiogenesis than to pannus swelling. VEGF concentrations are elevated in RA and are known to correlate with disease activity and radiographic progression. Synovial tissues expressing VEGF show a significantly higher microvascular density than those not expressing it [126]. Synovial vascularization correlates with disease activity and aggressiveness [127].

The development of rheumatologic arthroscopy and microinvasively obtained tissue samples represent a significant step forward in investigating joint disease, especially the macro- and microscopic vascular changes characteristic of synovitis. Histopathological assessment of synovial vascular changes in chronic arthritis is of diagnostic and pathogenetic value [128].

Studies found distinct vascular patterns of synovium for PsA, SpA, reactive arthritis (ReA) and RA. RA patients had predominantly straight, branching vessels, whereas patients with PsA, SpA and ReA had predominantly tortuous, bushy vessels. Specific vascular growth factors may be involved [129]. Whereas vascularization degree was higher in PSA/SpA than RA, expression of adhesion

molecules on vessel walls did not differ between RA and SpA [105,119]. The blood vessels were preferentially seen in the superficial layers just beneath the synovial lining layer in both diseases. Whereas in RA, activity of synovial proliferation was confined to the areas surrounding cartilage, in PsA it was distributed more inhomogeneously in various parts of the joint. Nevertheless the vascularization was the most distinctive and reliable synovial feature [105,128-132]. Vascular patterns are not modified by disease duration or DMARD treatment [128].

## **7. Vascular Imaging in Arthritis**

Overall, prompt diagnosis of arthritis, correct determination of prognosis and careful monitoring of disease activity is essential to reach modern treatment targets [133]. Specifically, imaging is helpful in clinical routine practice to early detect patients with mild clinical and laboratory inflammatory signs, but also to determine an aggressive course of disease, leading to a high degree of damage and destruction of joints and tendons, and to monitor response to treatment. Vascular imaging for angiogenesis may be more sensitive than clinical assessment of disease activity.

Radiography is the traditional gold standard in assessing joint damage of RA patients and one of the ACR criteria published in 1987 [56]. CR is able to visualize the late signs of preceding disease activity but there is evidence for MRI and US being highly sensitive for early inflammatory and destructive changes in RA joints [134].

MRI is known to be more sensitive than conventional clinical examination and radiography for detecting inflammatory and destructive joint changes in RA [135]. The exact assessment of the severity of active synovitis is an important issue because MRI studies have shown that the amount of pannus correlates with the aggressiveness of the disease [136]. Contrast-enhanced (CE-) MRI can distinguish between simple joint effusion and synovial proliferation. Synovial membrane enhancement in CE-MRI correlates strongly with histopathological cell infiltrates, blood vessel density and fractional area [137,138].

Power Doppler ultrasound (PDUS) has demonstrated its utility in clinical practice for the assessment of disease activity by semiquantitative measurement of synovial vascularization [139]. PDUS correlates with histological findings in RA, proving its validity for qualitative evaluation of RA [140]. However, PDUS is limited in its ability to detect slow flow present in synovial neoangiogenesis. The use of contrast-enhanced US (CEUS) has ameliorated the study of synovial perfusion at microvascular level reaching performance of CE-MRI [141]. Recent studies demonstrated that CEUS is even superior to CE-MRI in the detection of synovitis [142-144].

CE-ultrasound (CEUS) has drastically increased the sensitivity of PDUS enabling a better detection and quantification of inflammation, and consequently a more accurate distinction between inactive fibrous synovial proliferation and active synovitis, also in asymptomatic joints [145,146]. Further overall thickness measurements related to active synovitis were significantly improved by administering contrast agents [147].

With arthroscopy as reference, CEUS was found to be more useful than the unenhanced method in the recognition of increased vascularity of synovial villi [148]. The microvessel density measured by CEUS correlates exactly with angiographic and histologic findings [149-152]. Computer-aided validation and automated outlining of synovial boundaries enhance utility yield of CEUS in basal und follow-up assessment [153,154]. Therefore, CEUS appears particularly suitable to investigate possible distinct patterns of synovial vascularization in different forms of arthritis as demonstrated from histopathological studies.

# Aims of the study

To assess performance of automated versus manual classification system in differentiating RA from PsA arthritis by CEUS derived synovial vascularization patterns. To correlate clinical data and CEUS flow parameters.

# Material and methods

## Patients and clinical validation

107 outclinic patients presenting with clinically active arthritis of finger joints were recruited from our Rheumatology Unit, University of Padova. 56 patients were affected from RA according to the classification criteria of 1987 or 2010 depending from disease duration [56,57]. 51 PsA patients were enrolled defined by CASPAR criteria [98]. 19 patients showed polyarticular, 11 oligoarticular, 3 classic, 5 spondylitis subtype and 13 patients polyarticular disease with major erosions (mutilans). Patients' demographic data, disease duration and medical therapy were collected. Patients were validated clinically by DAS28, measurement of CRP and ESR and detection of RF and anti-CCP. All patient were asked for their informed consent.

## CEUS examination

The most active joint was chosen for CEUS exams after collection of informed consent. As shown in Figure 1 the hands were water-immersed and steady probe was used to increase image quality as yet described [146]. CEUS was performed with US device (MyLab25; Esaote) equipped with Contrast tuned Imaging (CnTI; Esaote), using a low mechanical index. The mechanical index and acoustic pressure were set at 0.1 and 30 kPa, respectively. The contrast agent consisted of microbubbles filled by sulfur hexafluoride (SonoVue; Bracco International, Princeton, NJ). A 4.8 ml bolus of contrast agent was injected into a peripheral vein of the opposite arm, followed by the injection of 20 ml saline solution. The selected joint was scanned in CnTI mode. The recording of the dynamic phases began simultaneously with the bolus injection and continued for 2 minutes. Sonograms were transferred to a PC workstation and both the anatomical B-mode image and the CnTI cineloop video were digitally stored for subsequent quantitative analysis or manual review.

## CEUS manual analysis

Two in arthritis experienced radiologists unaware of patients' history manually assessed grade of synovial contrast using a semiquantitative three-point scale (0–2), as recommended by the IACUS

study group [147], where grade 0 indicates no visible synovial contrast enhancement, grade 1 a detectable enhancement but less than in the periarticular tissues, and grade 2 an enhancement definitely stronger than in the periarticular structures (Figure 2).

Presumptive diagnosis of RA or PsA was expressed by radiologists considering typical histopathological features mentioned in the chapters before. Figure 3 shows exemplary CEUS studies for RA and PsA. RA is assumed to present with a more homogenous and synovial enhancement and faster time of contrast appearance due to linear and branching vessel architecture, whereas PsA with inhomogeneous enhancement both in synovial and perisynovial region representing entheses and capsules, and later contrast appearance due to tortuous, bushy vessels. After period of open training radiologists evaluated manually images for consistence with RA or not. Interobserver agreement was tested for manual CEUS grade and diagnosis. Additionally, radiologists outlined the boundaries of the synovial tissue on the gray-scale US images of each patient so that the subsequent analysis could be performed on the specific region of interest represented by the synovia, and on the region laying within 1 mm from the synovial boundaries (perisynovia), as it is shown on figure 4.

### **Contrast kinetics model for automated CEUS analysis**

In the general case of perfusion problems, we can consider a bolus of non-diffusible tracer given at time  $t = 0$  in a feeding vessel to a volume of interest: The individual particles of the tracer follow possibly different paths through the volume and their transit times thus have a distribution characteristic of the flow and the vascular structure. In the specific case of CEUS experiment, there is no diffusion of particles outside the vascular bed, and no perfusion of any tissue, so that we can reliably assume that each point in which some tracer (contrast dye) is detected corresponds to a vessel. Since there is no diffusion process involved, the kinetics of the tracer can be described by a Gamma-variate function as shown in figure 5 [155].

$$c(t) = \begin{cases} b & t < t_0 \\ b + a \cdot (t - t_0)^\alpha \cdot e^{-\frac{t-t_0}{\beta}} & t \geq t_0 \end{cases}$$

where  $t_0$  represents the contrast arrival time in the region of interest, and  $\alpha$  and  $\beta$  are two parameters that modulate the raise and washout of the dye from the vascular bed, whereas  $a$  accomodates the model for different peak intensity levels and  $b$  for different background (or baseline) intensity.

## CEUS automated analysis

Each examination is composed by an image  $I_{gs}$  obtained with the gray-scale US, onto which the synovia has been manually identified, and by a video  $I_{CEUS}^{(t)}$  imaging the kinetics of the contrast medium. In order to provide a reliable analysis, it is needed that both the US head and the patient's joint do not move during the acquisition of  $I_{CEUS}^{(t)}$ , and that the anatomical information gathered from  $I_{gs}$  can be perfectly superimposed to  $I_{CEUS}^{(t)}$ . Since these conditions are hardly met in clinical settings, it is therefore necessary to register the gray-scale  $I_{gs}$  image to each frame of the harmonic images  $I_{CEUS}^{(t)}$ . In order to register the two different acquisition. By exploiting the high reflectivity in both modalities of the superficial tissues of the joint and of the bones, we firstly identified them on  $I_{gs}$  by segmenting all pixels with intensity greater than the 95% percentile of the image intensities. With the same strategy we segment the pixels on each frame of  $I_{CEUS}^{(t)}$ , and then we estimate the displacement between  $I_{gs}$  and  $I_{CEUS}^{(0)}$ , and then between each couple of subsequent frames  $I_{CEUS}^{(i)}$  and  $I_{CEUS}^{(i+1)}$ , using the maximum of the phase-correlation. A representative result of the registration of the synovial region onto the CEUS data in an early and late enhancement phase is shown in figure 6.

Moreover, in order to reduce the computational requirement of identifying the five parameters  $[t_0, a, b, \alpha, \beta]$  describing the model of Eq. 1 for each pixel in the synovial regions, we chose to estimate the baseline intensity  $b$  as the mean intensity of the first 25 frames corresponding to the first 1s of the harmonic imaging, usually free from contrast signal .

Given the  $N$  points  $s_j = (x_j, y_j)$  placed within the manually outlined synovial and perisynovial region, we obtain the corresponding  $N$  parameter estimates  $\hat{p}(j) = [\hat{t}_0(j), \hat{a}(j), \hat{\alpha}(j), \hat{\beta}(j)]$ , by means of a non-linear least squares fitting of the parametric perfusion model  $c(t; p)$  to the data  $I_{CEUS}^{(t)}(s_j)$ .

From these parameters, we can derive a set of additional model characteristics as the peak value ( $c_{max}(j) = \max_t c(t, \hat{p}_j)$ ), the time of peak ( $t_{max}(j) = \operatorname{argmax}_t c(t, \hat{p}_j)$ ) the raise time (time  $t_{raise}(j)$  from the appearance  $t_0(j)$  to reach half the peak), or the washout time (the time  $t_{wash}(j)$  from  $t_{max}(j)$  that allows an intensity decrease of half the peak value).

Pixel-based procedure:

$$\bar{p}_{pixel} = \frac{1}{N} \sum_i \hat{p}_{pixel}(i)$$

Region-based procedure:

$$c_{region}(t) = \frac{1}{N} \sum_i I_{CEUS}^{(t)}(x_i, y_i)$$

Hence, for each video, at least mean value, the standard deviation, the 25<sup>th</sup> and 75<sup>th</sup> percentiles are computed for each model parameter and derived curve characteristic, so that 98 perfusion features are obtained (40 for the synovial perfusion and 37 for the perisynovial perfusion, 5 ratios synovial/perisynovial perfusion from pixel-based analysis, 16 perfusion features from region-based analysis). Pixel-based kinetics analyzed for synovia were time to appearance (t0), time to raise (traise), raise constant (a), 50% of raise (t1), time to peak (tmax), t2 (50% of washout), washout constant (b), time to washout (twash), amplification factor (A). For these parameters mean, std, 25 and 75 percentile were considered. Further synovial factors were peak value (ymax), peak from data, number of active clusters and pixels, mean and max cluster area, number of synovial pixels, mean, standard and total dye over time, blood flow (RBF) and volume (RBV). Pixel-derived kinetics analyzed for perisynovia were time to appearance (t0), time to raise (traise), raise constant (a), 50% of raise (t1), time to peak (tmax), t2 (50% of washout), washout constant (b), time to washout (twash), amplification factor (A). For these parameters mean, std, 25 and 75 percentile were considered. Further synovial factors were peak value (ymax), peak from data, number of active clusters and pixels, mean and max cluster area, number of synovial pixels, mean, standard and total dye over time, blood flow (RBF) and volume (RBV). Ratios synovia/perisynovia were expressed for mean, standard and total dye and RBF and RBV. For region-based kinetics analyzed were t0, traise, a, t1, tmax, t2, b, twash, A, values of peak, peak from data, peak-base, signal intensity (SI), mean dye, RBF and RBV.

In addition CEUS flow parameters, both pixel-based and region-based, were analyzed for correlations with DAS28, CRP, ESR, CEUS grade and diagnosis of radiologists.

## **CEUS automated classification**

### LDA classifier

We trained a simple linear discriminant classifier (LDA) [156-158], that estimate the hyperplane that provide the best separation of the two classes of interest described by their features (kinetic parameters, serological and antibodies data ...).

Given a certain number of classes with supposedly different characteristics, LDA is a method for linearly mapping the high dimensional characteristics vector in a lower dimensional space, which maximize the separation between classes, supposing their distribution normal. LDA is based on the

maximization of a function  $J$  that is an indicator of the class separation. The most common of such measures involves the evaluation of the intra-class scatter matrix  $S_w$  and the inter-class scatter matrix  $S_b$ . Given  $N$  patients,  $M$  classes and a  $1 \times F$  vector  $x$  of  $F$  features each one belonging to one and only one class, the intra-class scatter matrix is:

$$\Sigma_w = \sum_{i=1}^M P_i \Sigma_i$$

with  $P_i$  the a priori probability of the  $i$ th class, and  $\Sigma_i$  its covariance matrix.

The inter-class scatter matrix  $S_b$  is:

$$\Sigma_b = \sum_{i=1}^M P_i (\mu_i - \mu_0)(\mu_i - \mu_0)^T$$

with  $\mu_i$  the mean feature vector of the  $i$ th class,  $\mu_0$  the global (weighted) mean.

Thus, the class separation measure  $J$  maximized by LDA is:

$$J = \text{trace}(S_w^{-1} S_b)$$

The  $m \times m$  matrix  $S_w^{-1} S_b$  has rank  $M-1$ : a linear transformation mapping the original  $F$ -dimensional features space into a new  $(M-1)$ -dimensional space, can therefore yields the same value for  $J$  while obtaining a lower dimensionality.

In the specific case, since the classes of interest are 2 (rheumatoid and non-rheumatoid arthritis),  $M=2$  and the LDA transformation maps the  $F$  dimensional feature vectors into a scalar number, to which a simple threshold can be applied to diagnose RA or non-RA arthritis.

Given the vector  $f_{ij}$  of the features belonging to patient  $j$  classified as class  $i$ , the transformed scalar feature is obtained through the linear transformation:

$$y_{ij} = C f_{ij}$$

And the final classification is obtained applying a threshold value  $\vartheta$  to  $y_{ij}$ :

$$\text{diagnosis} = \begin{cases} \text{RA} & \text{if } y_{ij} \geq \vartheta \\ \text{non - RA} & \text{if } y_{ij} < \vartheta \end{cases}$$

The threshold is chosen to minimize the classification error of the available data.

### Feature selection

In order to reduce the computational complexity and to discard non-informative features, the classifier has been wrapped with a greedy sequential forward feature selection [159], using as target score the accuracy of the classifier, that is defined as the fraction of samples (patients) that are correctly classified.

By this means, the list of features used to estimate the LDA classifier is iteratively increased adding the single features that provides the greatest increase in the class-separability measure  $J$ .

### Cross-validation

In order to test the robustness of the classifier, and to provide an estimate of its performance on new data, a leave-one-out cross validation has been performed. The  $N$  data are divided into a training set composed by  $N-1$  samples (patients) and the testing set composed by the remaining sample. The LDA transformation matrix  $C$  and the optimal threshold  $\vartheta$  are estimated on the training set and then applied to the feature vector  $f_{\text{test}}$  in the testing set. The procedure is repeated  $N$  times, using as testing set a different patient and the remaining  $N-1$  as training set.

Mean (test) performance metrics as specificity, sensitivity and accuracy can be then estimated.

### Performance metrics

The classifier is evaluated measuring its ability to classify RA patients as such (sensitivity), to classify non-RA patients as such (specificity), overall correct classification (accuracy), to correctly predict that RA-classified patients are actually RA (positive predictive value), and that non-RA-classified patients are actually non-RA (negative predictive value).

Formally, defining as true positives (TP) the RA-patients classified as RA, the true negatives (TN) as the non-RA patients classified as non-RA, the false positives (FP) as the non-RA patients classified as RA, and finally as false negatives (FN) as the RA patients classified as non-RA, we have:

$$\begin{array}{ll} \text{sensitivity} & \frac{TP}{TP + FN} \\ \text{specificity} & \frac{TN}{TN + FP} \\ \text{accuracy} & \frac{TN + TP}{TN + FP + TP + FN} \\ \text{positive predictive value} & \frac{TP}{TP + FP} \\ \text{negative predictive value} & \frac{TN}{TN + FN} \end{array}$$

## Statistics

For statistical analysis of classification, features selection, cross-validation, performance metrics, Student T and Chi Square Test as appropriate Matlab® (Matrix Laboratory; Math Works, Natick, Massachusetts USA) was used.

# Results

## Patients

107 patients with active hand arthritis -56 with RA and 51 with PsA- were enrolled in the study and underwent CEUS examination. Patients' characteristics are summarized in table 1. DAS28, ESR and presence of autoantibodies were significantly higher in RA than PsA ( $p < 0.05$ ). CRP and ESR correlated with DAS28. Within PsA patients percentage of men were higher as expected for disease. Age and disease duration did not differ. Patients' treatment with steroids, DMARDs and biologics did not differ between RA and PsA.

In 69.2% of cases CEUS analyzed joint was MCF, in 18.7% wrist and in 12.1% IFP joint. No patient reported adverse event because of US contrast administration.

## Manual CEUS interpretation of CEUS

Interobserver agreement of radiologist for validation of CEUS grade ( $k = 0,98$ ) and diagnosis ( $k = 1$ ) was excellent (data not shown). 42.1% of overall patients were scored with CEUS grade 2, 33% with grade 1 and 24.8% with grade 0. Patients with CEUS grade 2 presented significantly higher DAS28 than CEUS grade 1 patients, but only for RA group. 72.9 % of patients were diagnosed by radiologists to have RA, 27.1% to have PsA. CEUS diagnosis correlated with CEUS grades as radiologists tended to diagnose RA in front of higher CEUS grades.

Diagnostic accuracy of manual validation by radiologists in differentiating RA from PsA was 0.69 (sensitivity 0.71, specificity 0.66, NPV 0.62, PPV 0.72) as shown in table 2. Integrating clinical parameters DAS28, CRP, ESR did not enhance accuracy (data not shown). By adding data about positivity of autoantibodies the diagnostic accuracy of radiologists could be increased to 0.89 (sensitivity 0.96, specificity 0.76, NPV 0.9, PPV 0.89) in training condition as shown in table 3, but not really in test conditions (Table 4) with virtual de novo patients (leave one out cross validation)

## **Automated interpretation of CEUS**

All CEUS exams except 3 were available for further computed automated software analysis. Within the 98 flow parameters derived from automated software analysis 35 resulted statistically significant between RA and PsA.

Classification accuracy using pixel-derived analysis was increasing by number of flow parameters included as shown in figure 7. The sum of 40 flow parameters constituted best constructed vascularization pattern to discriminate RA from PSA. Accuracy was 0.93 (sensitivity 0.91, specificity 0.94, NPV 0.90, PPV 0.94) during training. In Test situation with virtual de novo patients (leave one out cross validation) this would result in accuracy of 0.83 (sensitivity 0.84, specificity 0.77, NPV 0.80, PPV 0.81). Results are seen in table 5 and 6.

The integration of information about RF and anti-CCP increased diagnostic accuracy and decreased number of flow parameters needed to construct discriminating vascularization pattern (Figure 9).

With vascularization pattern using 28 flow parameters plus RF and anti-CCP accuracy resulted 0.99 (sensitivity 0.98, specificity 1.0, NPV 0.97, PPV 1.0) in training and 0.93 (sensitivity 0.90, specificity 0.94, NPV 0.87, PPV 0.96) in test conditions, respectively shown in table 7 and 8. When RF or anti-CCP were used singularly accuracy decreased, but was better for RF (0,76) than anti-CCP (0,63).

The best flow parameters for the construction of vascularization pattern discriminating between RA and PsA were mean synovial raise time (faster in RA), mean synovial raise constant (lower in RA), time to synovial peak (faster in RA), mean synovial peak value (higher in RA), synovial active regions (more numerous in RA), mean dimension of synovial active regions (greater in RA), synovial and perisynovial blood volume (both greater in RA), synovia/perisynovia blood volume, flow (all higher in RA).

A region-based analysis including 16 flow parameters as available in actually commercialized software packages showed no advances compared to manual analysis. Accuracy was of 0.73 (sensitivity 0.73, specificity 0.72, NPV 0.69, PPV 0.75) in differentiating RA from PsA in training and 0,61 (sensitivity 0.51, specificity 0.66, NPV 0.53, PPV 0.64) in test conditions as shown in table 9 and 10.

No correlations were detected between software derived kinetics and CRP, ESR and DAS28.

# Discussion

The 2 major and clinically most important primary inflammatory rheumatic diseases which affect small hand and feet joints are RA and PsA. RA and PsA are both common diseases in general population and have distinct pathologic, clinic, serologic and prognostic features.

RA is characterized by primary synovial inflammation and transformation in tumor-like pannus with highly destructive potential. Systemic inflammation, specific autoantibodies and erosions are often and early detectable by blood exams and different imaging modalities such as CR, US, MRI. On the other side PsA is associated with pathognomonic skin and nail disease. Primary site of inflammation are entheses and perisynovial structures. Specific autoantibodies up to now are not known, and systemic inflammation is often absent. Radiologic features characteristically include marginal erosions and bone regeneration and proliferation, absolutely absent in RA. RA compared to PsA is a destructive polyarthritis and requires early diagnosis and treatment including highly priced biologics to avoid irreversible invalidation. Nevertheless correct diagnosis is not as easy as supposed from this paradigmatic view of both disease entities. In particular at disease beginning difference may be more subtle. Further antibodies and systemic inflammation are not always present in RA. PsO analogously can be absent or appear years after or before arthritis or result only from family history. Last not least PsA can mimic RA. In fact PsA can present with or evolve to polyarthritis (simil-rheumatoid form) or show erosions (20% of cases) and important destructions (mutilans form).

The evolution of imaging techniques allowed better insights in anatomy and pathology of arthritis. CR is the traditional gold standard in assessing joint damage of RA, but works poorly in early RA. However, this technique has a number of limitations such as low contrast resolution, obscuring superimposition of projections and the use of ionizing radiation. Scintigraphy is highly sensitive for inflammatory hyperemia within the intraarticular and paraarticular soft tissues as well as for bone marrow hyperemia and increased osteoblastic activity, but specificity is low and requires at least combination with CR, because it is positive in etiologically quite different joint diseases [160,161]. An advantage of scintigraphy, even when compared with routine MRI (with exception of whole body MRI) is its ability to demonstrate multiple sites of inflammation.

MRI depicts soft tissue changes and damage to cartilage and bone earlier and better than CR does. It is an excellent tool to assess synovial swelling and volume. CE-MRI is used to identify vascularized synovial proliferation and erosions. Further advantage of MRI for RA diagnosis is the quantification of bone marrow edema as a forerunner of erosions, direct cartilage visualization as well as inflammatory activity of inflamed synovial membrane [162,163]. US is an important imaging

modality for evaluation of RA and PsA. US, especially with use of highly sensitive Doppler imaging has high soft tissue contrast resolution. Sensitivity of US for demonstration of early synovitis and tenosynovitis as well as for evaluation of synovial vascularization is comparable to MRI. It is also of sensitivity for presentation of peripheral bone erosions [164,165]. MRI and US exhibited high specificities in detecting bone erosions in RA, even in radiographically non-eroded joints, when CT was used as the reference method [166].

In parallel attempts to differentiate arthritis forms by specific imaging features were tried. Exemplary, the presence of MRI synovitis and erosion and bone scintigraphic pattern compatible with RA showed 100% specificity for a diagnosis of RA at 2 year follow-up in patients with unclassified arthritis after standard validation with clinic, biochemical and radiographic examinations [167]. Accuracy combining both techniques was therefore 84%. MRI synovitis and erosion alone resulted in specificity of 87% and accuracy of 80%. RA-pattern on scintigraphy had only specificity of 74% and accuracy of 71%.

Using CE-MRI of hands and feet in patients with PsA, inflammatory changes were studied to define several subgroups and to present some specific findings important for early differential diagnosis between PsA and RA. A major subgroup of PsA patients with peripheral arthritis presented with wide spread soft tissue involvement extending well beyond the joint capsule and was clearly different from RA. The abnormalities were predominately extra-articular comprising thickened collateral ligaments, enthesitis at the insertions of the collateral ligaments and joint capsules, spreading to the surrounding soft tissues. Joint inflammation was asymmetrical, single ray distribution of all the joints of a single digit and DIP joint involvement were frequent. Dactylitis consistent with combinations and degrees of arthritis, enthesitis at the insertions of the collateral ligaments and joint capsules, tenosynovitis and inflammation of the surrounding soft tissues was demonstrated frequently. MRI findings of predominately extra-articular inflammatory involvement in PsA in connection with increased bone density (periostitis, osteitis) on CR, cases with prevalence of productive changes, resulted prognostically more favorable. On the other side in more aggressive rheumatoid-like PsA subgroup, CE-MRI was not able to identify specific features. Both RA and rheumatoid-like PsA had a typical symmetrical distribution with affection of the PIP, MCP and MTP joints and sparing of the DIP joints. The hallmarks synovitis and subchondral bone marrow inflammation were present in both disease entities. Bare areas and subchondral parts of the joints resulted to be preferential targets. Hyperemia of the soft tissue structures and bone marrow were localized and not as intense, and inflammatory changes were confined within the joint capsule, involving both sides of the joint [168].

The areas of contrast enhancement by MRI were indistinguishable for PsA and RA in the joint synovial membrane and flexor tendons, whereas were significantly higher in PsA for the first and second extensor compartments, and for RA in the extensor carpi ulnaris region [169]. Erosions were statistically more frequent in patients with RA and periostitis in patients with PsA as demonstrated by CE-MRI, but no difference was found in the frequency of synovitis, although carpometacarpal MCF joints were affected more frequently in RA and PIP in PsA [170].

These studies confirm the feasibility to discriminate between RA and PsA especially using para-articular characteristics and bone features found in PsA and not in RA. On the other hand they showed lack of efficacy to differentiate between both disease entities by study of synovitis, although histopathological analyses clearly demonstrated distinct pathognomonic vascularization patterns between RA and PsA, both in its oligo- and polyarticular subgroup [119]. The explanation may be that contrast agents in MRI are not true intravascular agents, and are easily extravasated. They dependent from vascular perfusion but also vessel permeability resulting in leakage into the interstitial space [171]. Despite extremely high sensitivity, contrast agents used in CEUS persist in the vascular bed and do not leak into the extrasynovial compartments. CEUS can therefore provide a more accurate live picture of changes in the synovial vascularization [172].

In our study for the first time feasibility of studying not invasively different vascularization patterns as histopathological proven for RA and PsA was demonstrated. The innovation we propose consists of studying arthritis by dynamic automated synovial imaging (DASI). CEUS examinations of active finger joints were analyzed for both RA and PsA by ad hoc developed software analysis program and semiquantitatively by radiologists.

Clear differences in 98 contrast kinetics and therefore the possibility to construct a distinct vascularization pattern of RA and PsA were identified. The pixel-derived analysis resulted in high accuracy in diagnosing correctly RA and PsA: 0.93 in training and 0.83 in test conditions. The detection of distinct patterns was possible on one joint without ulterior data such as clinical data, joint involvement, DAS28, ERS and CRP. Adding data of autoantibodies enhanced accuracy to 0.99 in training and 0.93 in test conditions. The informations derive from a single joint rising some criticism. However, we recently demonstrated that joint selection based on the patients referred pain identifies joint with most synovitis in CE-MRI [173]. Distinction between RA and PsA could not be achieved with contrast study curves in dynamic CE-MRI, where RA and PsA both oligo and polyarticular appeared with similar patterns [174]. Evidently more precise analysis including more flow parameters are necessary. This may explain less accuracy of region-based analysis (0.61) as available in actually commercialized software packs (e.g. Qontrast®) or manual CEUS interpretation (0.69) regardless of autoantibody status.

Manual interpretation of CEUS images were based on results from histopathological studies. Blinded radiologists showed accuracy of 0.69 in diagnosing RA or PsA with near 100% interobserver agreement. RA is assumed to present with a more homogenous and synovial enhancement and faster time of contrast appearance due to linear and branching vessel architecture, whereas PsA with inhomogeneous enhancement both in synovial and perisynovial region representing entheses and capsules, and later contrast appearance due to tortuous, bushy vessels.

These assumptions and histopathological data are confirmed by pixel-based analysis. RA shows greater and more homogenous neovascularization in synovia (synovial mean peak, active regions, mean dimension of synovial active regions and blood volume) und perisynovia (perisynovial blood volume) than PsA. In PsA inflammation is concentrated in perisynovial regions (lower synovia/perisynovia blood flow and volume). In RA vessels are straight and less tortuous than in PsA (lower synovial raise, raise constant and time to synovial peak).

Disease activity (DAS28) was higher in RA versus PsA patients. This depends from more factors. First of all PsA contained mainly polyarticular but also oligoarticular subtypes. CRP and ESR were often negative or low in PsA. Validation of DAS28 spares hip, ankle and feet joints, very often affected in PsA. Tendonitis and dactylitis involvement frequent in PsA is tricky to insert in DAS28 joint count. DAS28 in general may not be adequate to measure disease activity in PsA except for simil-rheumatoid variant. On the other side PsA patients have been as frequently treated with steroids, DMARDs or biologics as RA patients as expression of similar disease severity. Patients measured by radiologists with CEUS grade 2 had higher DAS28 than those with grade 1. Radiologists tented to diagnose CEUS grad 2 more often to be RA. Probably signal intensity (SI) is a determinant factor in decisions of radiologists' eyes and correlates well with semi-quantative PD scores [153]. Nevertheless automated CEUS flow parameters contrary to MRI do not correlate with classical disease activity parameters such as DAS28, CRP and ESR [153,173,174]. They represent new data independent from disease activity.

## **Conclusion**

Development of DASI represents a new method to study vascularization in synovitis without the need of histopathological specimens. DASI gives insights in the vascular engine of joint inflammation and destruction. DASI is an effective tool to differentiate RA from PsA.

# Abbreviations

A = amplification factor

a = raise constant

ACR = American College of Rheumatology

ANA = anti-nuclear antibodies

APC = antigen presenting cell

APCA = anti-citrullinated protein antibodies

APRIL = a proliferation inducing ligand

BAFF = B cell activating factor

CASPAR = CIASsification criteria for Psoriatic Arthritis

CCP = cyclic citrullinated peptide

CD = cluster of differentiation

CE = contrast enhanced

CEUS = contrast-enhanced ultrasound

CnTI = contrast tuned imaging

CR = conventional radiology

CRP = C-reactive protein

CSF = colony stimulating factor

CTLA = cytotoxic T-lymphocyte antigen

DASI = dynamic automated synovial imaging

DCs = dendritic cells

DIP = distal interphalangeal

DKK = dickkopf

DMARD = disease modifying anti-rheumatic drugs

ESR = erythrocyte sedimentation rate

EULAR = European League against Rheumatism

FGF = fibroblast growth factor

FLS = fibroblast-like synoviocytes

gp = glycopeptide

HC = human cartilage

HLA = human leukocyte antigen

HSP = heat shock protein

ICAM = intercellular adhesion molecule

ICOS = inducible costimulator  
IL = interleukin  
INF = interferon  
LDA = linear discriminant classifier  
LFA = leucocyte function-associated antigen  
MCP = metacarpophalangeal  
MHC = major histocompatibility complex  
MIP = macrophage inflammatory protein  
MMP = matrix metalloproteinases  
MRI = magnetic Resonance Imaging  
MTF = metatarsophalangeal  
NF- $\kappa$ B = nuclear factor  $\kappa$ B  
NHL = non Hodgkin lymphoma  
NK = natural killer  
NPV = negative predictive value  
OA = osteoarthritis  
OPG = osteoprotegerin  
PADI4 = peptidyl arginine deiminase, type IV  
PDGF = platelet derived growth factor  
PDS = power Doppler ultrasound  
PIP = proximal interphalangeal  
PPV = positive predictive value  
PsA = psoriatic arthritis  
PsO = psoriasis  
RA = rheumatoid arthritis  
RANKL = receptor activator of NF- $\kappa$ B ligand  
RBF = blood flow  
RBV = blood volume  
ReA = reactive arthritis  
RF = rheumatoid factor  
SI = signal intensity  
SpA = spondyloarthritis  
Std = standard deviation  
T0 = time to appearance

TCR = T cell receptor

TGF = transforming growth factor

Th = T helper

TIMP = tissue inhibitors of metalloproteinases

TLR = toll like receptor

Tmax = time to peak

TNF = tumor necrosis factor

Traise = time to raise

Treg = T regulator

Twash = washout time

US = ultrasound

VEGF = vascular endothelial growth factor

y<sub>max</sub> = peak value

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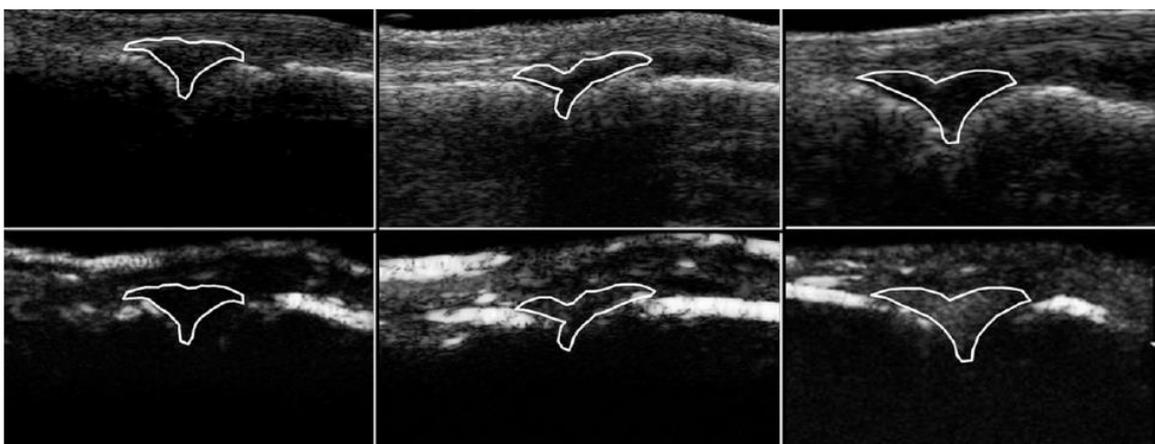
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## Tables and figures



**FIGURE 1.** Patient's hand immersed in a plastic tub filled with water at 28°C, with the transducer in the water and held firmly by a bracket 10 mm above the selected joint throughout the test.



**FIGURE 2.** Probe focused on synovial hyperplasia prior to administering contrast (upper boxes). After intravenous bolus injection, synovial contrast enhancement was graded as 0 (left lower box), 1 (middle lower box), or 2 (right lower box), according to the IACUS study group classification.

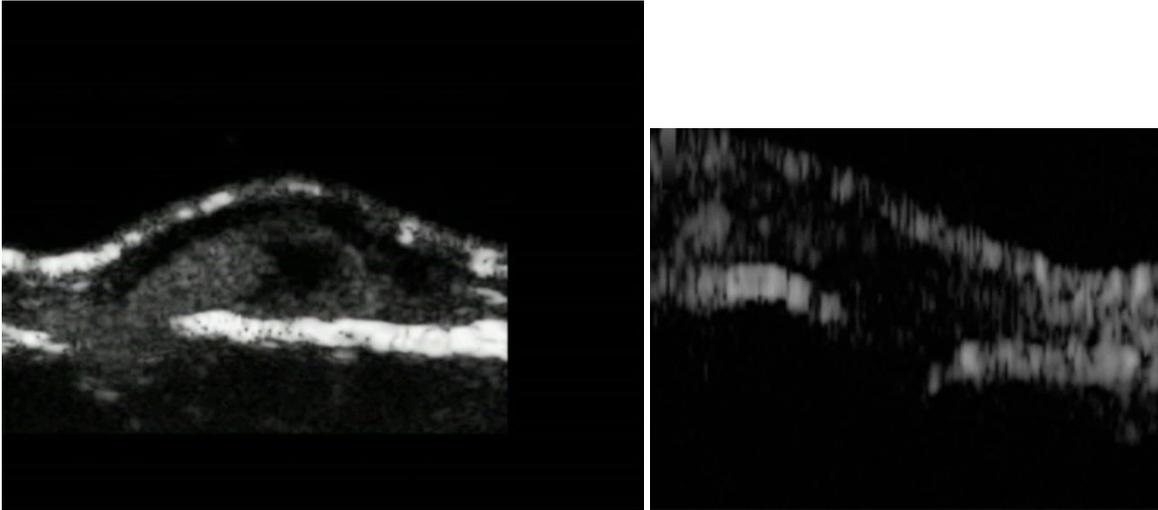


Figure 3: Distinct contrast enhancement in synovial and perisynovial region at peak value as seen in RA (left panel) and PsA (right panel) patient.

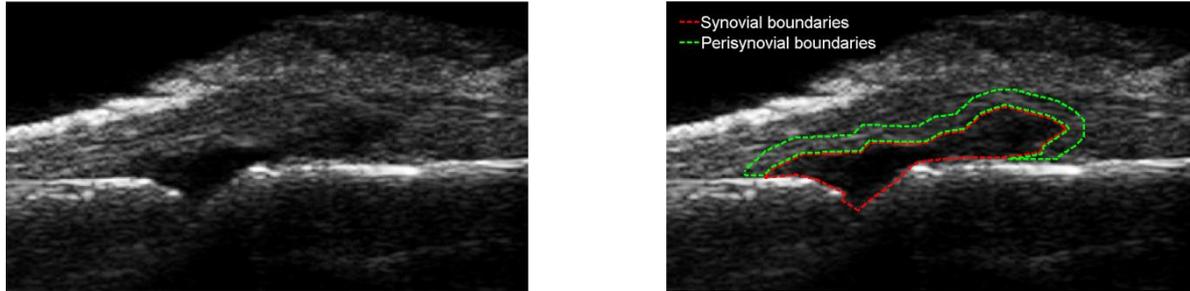


Figure 4: Gray scale B-mode image of a metacarpophalangeal joint (left panel), and the annotated synovial boundaries (red, right panel) with the perisynovial region outline (green, right panel).

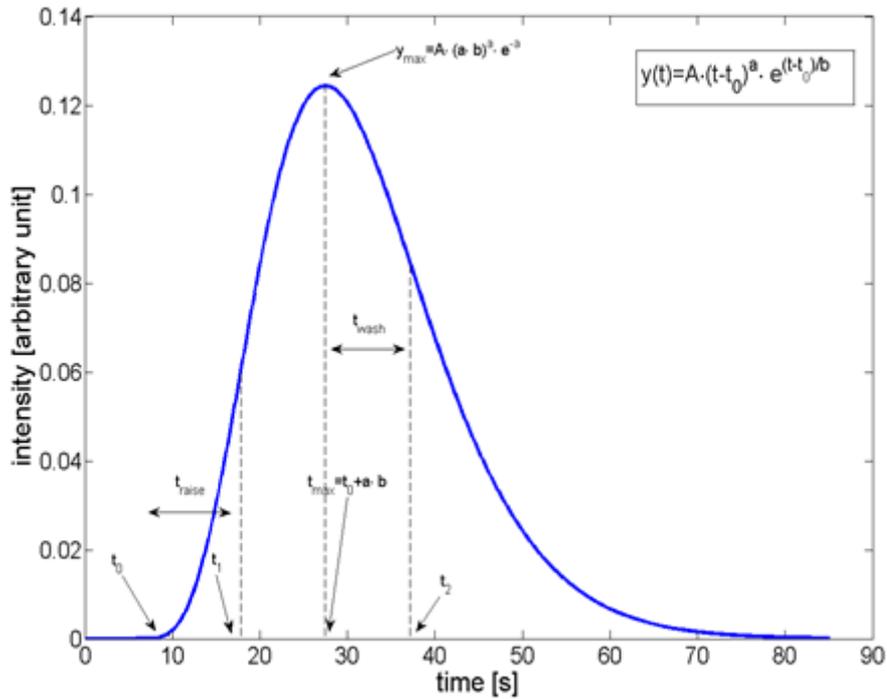


Figure 5:

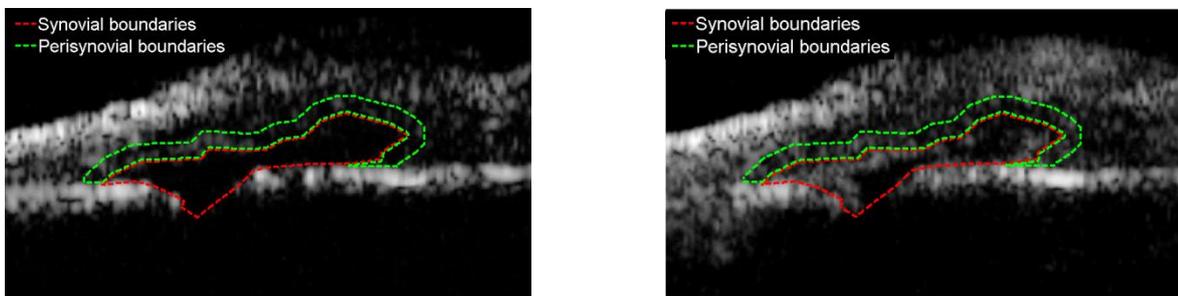


Figure 6: Registered synovial and perisynovial boundaries on different frames of the CEUS video. It is apparent the correct positioning both in the early enhancement phase (left panel) and in the late enhancement phase (right panel) of a representative PsA patient.

# Arthritis Classification on CEUS kinetics parameters

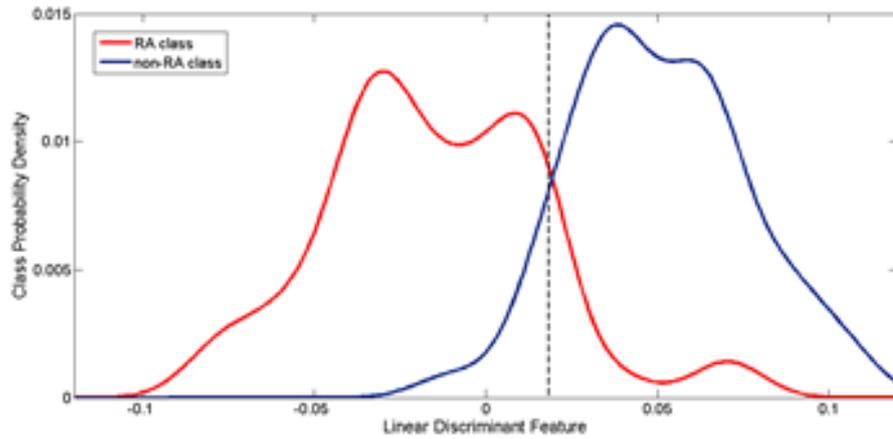


Figure 7: Significantly different parameters were used for linear discriminant analysis to identify the transformation optimizing the linear separability of the two groups, and each patient was assigned to RA or non-RA with a Bayesian classification algorithm providing the a posteriori probability to belong to the RA or non-RA group.

<b>Parameters</b>	<b>RA</b>	<b>PsA</b>	<b>p</b>
n	56	51	
Sex female (%)	91,1	70,4	< 0,05
Age (years)	55,5 ± 9,9	52,6 ± 12,3	ns
Disease duration (years)	11,1 ± 8,7	10,6 ± 6,8	ns
DAS28	4,98 ± 1,1	3,69 ± 1,4	< 0,05
CRP mg/l	18,7 ± 20,6	14,6 ± 26	ns
ESR mm/h	44,5 ± 27,3	28,1 ± 20	< 0,05
RF positive (%)	67,1	11,3	< 0,05
Anti-CCP positive (%)	52,2	9,4	< 0,05
No therapy (%)	11,9	3,8	ns
Steroid daily mg	4,7 ± 3,7	4 ± 4,2	ns
Steroid therapy (%)	79,1	64,1	ns
DMARDs therapy (%)	59,7	52,8	ns
Biologic therapy (%)	47,7	62,2	ns

Table 1.

		<b>CLINICAL DIAGNOSIS</b>				
			<b>RA</b>	<b>PSA</b>		
<b>MANUAL CEUS DIAGNOSIS</b>	<b>RA</b>	<b>48</b>	<b>20</b>	<b>0.74</b>	<b>PPV</b>	
	<b>PsA</b>	<b>21</b>	<b>18</b>	<b>0.62</b>	<b>NPV</b>	
		<b>0.71</b>	<b>0.66</b>	<b>0.69</b>		
		<b>Sensitivity</b>	<b>Specificity</b>			

Table 2: Diagnostic accuracy of radiologists on CEUS exams.

		CLINICAL DIAGNOSIS				
			RA	PSA		
MANUAL CEUS DIAGNOSIS +RF/anti-CCP	RA	44	6	0.89	PPV	
	PsA	2	19	0.9	NPV	
		0.96	0.76	0.89		
		Sensitivity	Specificity			

Table 3: Diagnostic accuracy of radiologists on CEUS exams plus RF and anti-CCP (training).

		CLINICAL DIAGNOSIS				
			RA	PSA		
MANUAL CEUS DIAGNOSIS RF/anti-CCP	RA	38	15	0.72	PPV	
	PsA	15	15	0.50	NPV	
		0.72	0.50	0.67		
		Sensitivity	Specificity			

Table 4: Diagnostic accuracy of radiologists on CEUS exams plus RF and anti-CCP (test).

## Kinetic Parameters (training) classification accuracy

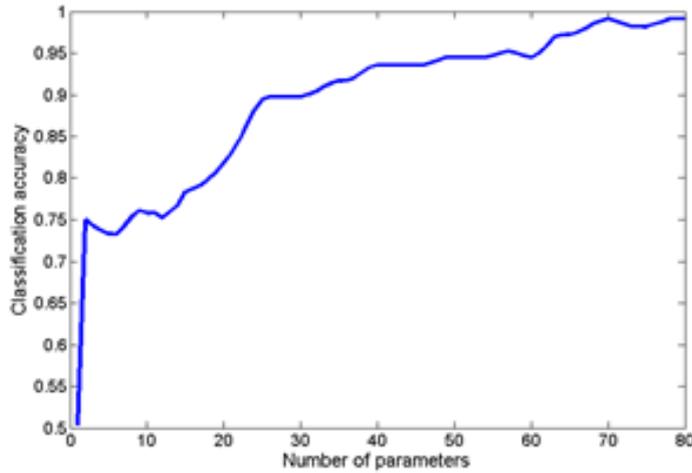


Figure 8: Diagnostic accuracy of pixel-derived automated analysis is increasing with number of parameters considered.

		CLINICAL DIAGNOSIS			
		RA	PSA		
PIXEL-BASED AUTOMATED CEUS DIAGNOSIS	RA	51	3	0.94	PPV
	PsA	5	45	0.90	NPV
		0.91	0.94	0.93	
		Sensitivity	Specificity		

Table 5: Diagnostic accuracy of pixel-based automated CEUS analysis using 40 flow parameters (training).

		CLINICAL DIAGNOSIS				
			RA	PSA		
PIXEL-BASED AUTOMATED CEUS DIAGNOSIS	RA	47	11	0.81	PPV	
	PsA	9	37	0.80	NPV	
		0.84	0.77	0.83		
		Sensitivity	Specificity			

Table 6: Diagnostic accuracy of pixel-based automated CEUS analysis using 40 flow parameters (test).

## Classification using kinetic parameters and RF + antiCCP

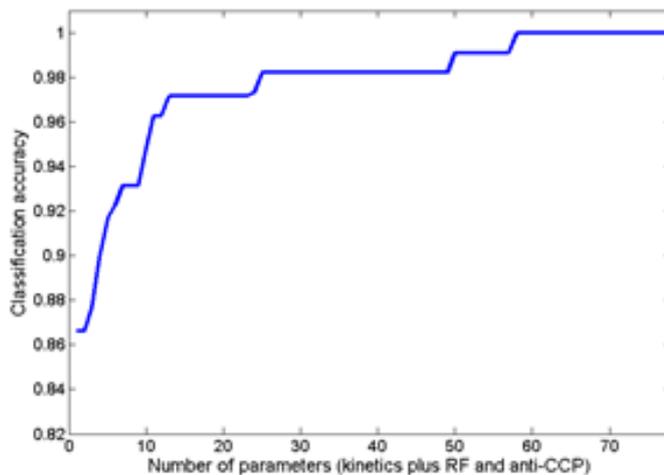


Figure 9: Diagnostic accuracy of pixel-derived automated analysis is increasing with number of parameters considered. Number of parameters needed is decreased by integrating RF and anti-CCP.

		CLINICAL DIAGNOSIS				
			RA	PSA		
PIXEL-BASED AUTOMATED CEUS DIAGNOSIS + FR/anti-CCP	RA	47	1	1.0	PPV	
	PsA	1	35	0.97	NPV	
		0.98	1.0	0.99		
		Sensitivity	Specificity			

Table 7: Diagnostic accuracy of pixel-based automated CEUS analysis using 28 flow parameters plus FR and anti-CCP (training).

		CLINICAL DIAGNOSIS				
			RA	PSA		
PIXEL-BASED AUTOMATED CEUS DIAGNOSIS +RF/anti-CCP	RA	43	2	0.96	PPV	
	PsA	5	33	0.87	NPV	
		0.90	0.94	0.93		
		Sensitivity	Specificity			

Table 8: Diagnostic accuracy of pixel-based automated CEUS analysis using 28 flow parameters plus FR and anti-CCP (test).

		CLINICAL DIAGNOSIS				
			RA	PSA		
REGION-BASED AUTOMATED CEUS DIAGNOSIS	RA	40	13	0.75	PPV	
	PsA	15	34	0.69	NPV	
		0.73	0.72	0.73		
		Sensitivity	Specificity			

Table 9: Diagnostic accuracy of region-based automated CEUS (training).

		CLINICAL DIAGNOSIS				
			RA	PSA		
REGION-BASED AUTOMATED CEUS DIAGNOSIS	RA	29	18	0.64	PPV	
	PsA	28	31	0.53	NPV	
		0.51	0.66	0.61		
		Sensitivity	Specificity			

Table 10: Diagnostic accuracy of region-based automated CEUS (test).