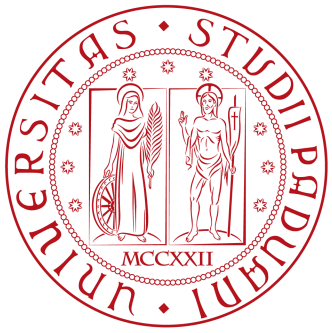

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Tesi di Laurea

The impact of SARS-CoV-2 infection and vaccination on inflammatory arthritis: a cohort study

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A tutti i ragazzi e le ragazze che non hanno avuto le forze per arrivare alla fine,
portati via dalla pressione delle aspettative sociali,
e a chi crede di non farcela.

Contents

Acronyms

aCCP Anti-Cyclic Citrullinated Peptide antibodies

ACE2 Angiotensin Converting Enzyme 2

AEFI Adverse Events Following Immunization

AFOP Acute Fibrinous and Organizational Pneumonia

AIFA Agenzia Italiana del FArmaco

ARDS Acute Respiratory Distress Syndrome

AS Ankylosing Spondylitis

ASDAS Ankylosing Spondylitis Disease Activity Score

ATR1 Angiotensin Type-1 Receptor

BASDAI Bath Ankylosing Spondylitis Disease Activity Index

BASFI Bath Ankylosing Spondylitis Functional Index

BASMI Bath Ankylosing Spondylitis Metrology Index

bDMARDs Biological Disease-Modifying AntiRheumatic Drugs

BMI Body Mass Index

CAM Cell Adhesion Molecules

CDAI Clinical Disease Activity Index

CDC Centers for Disease Control and prevention

CLS Capillary Leak Syndrome

COPD Chronic Obstructive Pulmonary Disease

COVID-19 COronaVIrus Disease-2019

COXIB Cyclo-OXygenase type II selective InhiBitors

CRP C-Reactive Protein

CRS Cytokine Release Syndrome

csDMARDs Conventional synthetic Disease-Modifying AntiRheumatic Drugs

CT Computed Tomography

DAD Diffuse Alveolar Damage

DAMPs Damage Associated Molecular Patterns

DAPSA Disease Activity index for PSoriatic Arthritis

DAS28-CRP Disease Activity Score-28 for rheumatoid arthritis with CRP

DIC Disseminated Intravascular Coagulation

DIP Distal InterPhalangeal joints

EMA European Medicines Agency

En-SpA Enteropathic SpondyloArthritis

ESR Erythrocyte Sedimentation Rate

FDA Food and Drug Administration

GBS Guillain-Barré Syndrome

GM-CSF Granulocyte-Macrophage Colonies Stimulant Factor

HAQ Health Assessment Questionnaire

HLA Human Leukocyte Antigen

IA Inflammatory Arthritis

IFN InterFeroN

IL InterLeukin

IP-10 Interferon inducible Protein 10

ISS Istituto Superiore di Sanità

JAK Janus Kinase

JAKi Janus Kinase Inhibitor

JSpA Juvenile SpondyloArthritis

LDH Lactate DeHydrogenase

LEI Leeds Enthesitis Index

MASES Maastricht Ankylosing Spondylitis Enthesitis Score

MCP MetaCarpoPhalangeal joints

MDA Minimal Disease Activity

MERS Middle Eastern Respiratory Syndrome

MIS-A Multi-system Inflammatory Syndrome in Adults

MIS-C Multi-system Inflammatory Syndrome in Children

MR Magnetic Resonance

MTP MetaTarsoPhalangeal joints

MTX MethoTreXate

NET Neutrophil Extracellular Traps

NIH National Institute of Health

NO Nitric Oxide

NSAIDs Non-Steroidal Anti-Inflammatory Drugs

PAMPs Pathogen Associated Molecular Patterns

PASI Psoriasis Area and Severity Index

PGA Patient Global Assessment

PGE2 ProstaGlandin E2

PIP Proximal InterPhalangeal joints

PsA Psoriatic Arthritis

RAAS Renin-Angiotensin-Aldosterone System

ReA Reactive Arthritis

RF Rheumatoid Factor

ROS Reactive Oxygen Species

RT-PCR Real-Time reverse transcriptase-Polymerase Chain Reaction

RTX RiTuXimab

SARS-CoV-2 Severe Acute Respiratory Syndrome COronaVirus 2

SDAI Simple Disease Activity Index

SpA SpondyloArthritis

SSZ SulfaSalaZine

STAT Signal Transducer and Activator of Transcription proteins

TGF Transformed Growth Factor

TNF Tumor Necrosis Factor

tsDMARDs Targeted synthetic Disease-Modifying AntiRheumatic Drugs

US UltraSonography

USpA Undifferentiated SpondyloArthritis

VAS Visual Analog Scale

VEGF Vascular Endothelial Growth Factor

VOC Variants Of Concern

VOI Variants Of Interest

VTT Vaccine-induced Thrombosis with Thrombocytopenia syndrome

VUM Variants Under Monitoring

WHO World Health Organization

ABSTRACT

Objectives To investigate the effects of SARS-CoV-2 infection, as well as short- (within 48 hours) and long-term (within 30 days) adverse events (AEs) of SARS-CoV-2 vaccines, including arthritis flares in a large cohort of patients with inflammatory arthritis (IA).

Methods A retrospective cohort study comprising 362 patients: 94 (26%) rheumatoid arthritis, 158 (43.6%) psoriatic arthritis and 110 (30.4%) ankylosing spondylitis; and 165 healthy controls (HC) to ascertain the prevalence and severity of SARS-CoV-2 infection in patients with IA, the rate of AEs associated with SARS-CoV-2 vaccines and disease flares within a month of the vaccination. All patients provided informed consent and data about SARS-CoV-2 infection and/or vaccination status.

Results One-hundred-seventeen (32.3%) patients and 39 (23.6%) HC were affected by SARS-CoV-2 infection. Forty (34.2%) patients experienced an IA flare within one month of infection, of whom 3 (7.5%) needed to switch therapy. The prevalence of SARS-CoV-2 infection, disease severity, and hospitalization rate were not significantly different. At least one shot of SARS-CoV-2 vaccine was administered in 331 (91.4%) patients and 147 (89.1%) HC. Within 48 hours, 102 (30.8%) patients developed vaccine-related AEs; 52 (15.7%) patients with >1 vaccine dose experienced an IA flare-up, of whom 12 (23.1%) needed to switch therapy.

Conclusions A significantly higher rate of IA flare was observed among patients who contracted SARS-CoV-2 infection vs. those without infection. Patients with IA experienced flares after SARS-CoV-2 vaccination, though it was not statistically significant.

RIASSUNTO

Obiettivi Studiare gli effetti dell'infezione da SARS-CoV-2, così come gli eventi avversi (EA) a breve (entro 48 ore) e a lungo termine (entro 30 giorni) dei vaccini anti-SARS-CoV-2, comprese le riacutizzazioni (flare) di malattia, in una vasta coorte di pazienti con artriti infiammatorie (AI).

Materiali e metodi Uno studio retrospettivo di coorte comprendente 362 pazienti: 94 (26%) affetti da artrite reumatoide (AR), 158 (43,6%) da artrite psoriasica (APs) e 110 (30,4%) da spondilite anchilosante (SA); e 165 controlli sani (CS) per accertare la prevalenza e la severità dell'infezione da SARS-CoV-2 in pazienti con AI, il tasso di EA associato ai vaccini anti-SARS-CoV-2 e di flare di malattia entro un mese dalla vaccinazione. Tutti i pazienti hanno fornito il consenso informato e i dati relativi all'infezione da SARS-CoV-2 e/o allo stato di vaccinazione.

Risultati Centodiciassette (32.3%) pazienti e 39 (23.6%) CS hanno contratto un'infezione da SARS-CoV-2. Quaranta (34.2%) pazienti hanno avuto un flare di AI entro un mese dall'infezione, di cui 3 (7.5%) hanno avuto necessità di cambiare terapia. La prevalenza dell'infezione da SARS-CoV-2, la gravità della malattia e il tasso di ospedalizzazione non erano significativamente diversi. Almeno una dose di vaccino anti-SARS-CoV-2 è stato somministrata in 331 (91.4%) pazienti e 147 (89.1%) CS. Nell'arco di 48 ore, 102 (30.8%) pazienti hanno sviluppato EA correlati al vaccino; 52 (15.7%) pazienti con almeno una dose di vaccino hanno sperimentato un flare di AI, di cui 12 (23.1%) hanno avuto necessità di cambiare terapia.

Conclusioni Un tasso significativamente più alto di flare di AI è stato osservato tra i pazienti che hanno contratto l'infezione da SARS-CoV-2 vs. quelli senza infezione. I pazienti con AI hanno sperimentato flare dopo la vaccinazione anti-SARS-CoV-2, anche se ciò non è risultato statisticamente significativo.

INTRODUCTION

1.1 Inflammatory arthritis

1.1.1 General aspects of seronegative spondyloarthritis

Definition

Seronegative spondyloarthritis (SpA) is a group of chronic inflammatory rheumatic disease that include six main different clinical phenotypes: ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), enteropathic spondyloarthritis (En-SpA associated with chronic inflammatory bowel disease such as ulcerative colitis and Chrons disease), undifferentiated spondyloarthritis (USpA), juvenile spondyloarthritis (JSpA). The adjective seronegative is related to the absence of rheumatoid factor (RF) in these patients. AS and PsA are the most frequent subtypes and those with a course more inauspicious. The severity of such pathologies is closely related to the degree of their activity and to the speed with which the anatomical damage is established, with consequent loss of mobility and physical function and impairment of quality of life.

SpA are characterized by common clinical, histo-pathological and radiographic aspects and most importantly by being seronegative [1], a concept introduced by Moll and colleagues [2] in 1974.

Clinically, in all subtypes of SpA, a common pattern of articular and peri-articular involvement can be found, which may be characterized by mono-oligo peripheral arthritis, spondylitis and/or sacroiliitis, tendinitis and/or tenosynovitis, enthesitis and dactylitis. The systemic involvement allows to frame the disease in a defined clinical entity: uveitis for AS, psoriasis for PsA, inflammatory bowel disease for En-SpA. However, patients who do not show these typical clinical patterns can be classified as suffering from USpA [3–8] a concept introduced from 1990s since the Moll's criteria did not include the undifferentiated forms.

In the early 1990s, two groups of SpA classification criteria were proposed, including the undifferentiated forms, in order to merge the different clinical entities in a single spectrum of disease: in 1990 were proposed the Amor criteria, subsequently modified in 1991 by the European Spondyloarthropathy Study Group (ESSG). This introduced a new concept of SpA as single clinical entity with two main phenotypes: a mainly axial SpA, characterized by lower back pain, and mainly peripheral SpA, characterized by peripheral inflammation [9].

Subsequently, with the advent of new imaging methods, especially magnetic resonance (MR), the Assessment of Spondyloarthritis International Society (ASAS) updated again the previous criteria introducing new classification criteria in 2009 for the axial forms and in 2011 for the peripheral forms. This was a very important innovation because typical findings of organic damage such as erosion, sclerosis and syndesmophytes (vertical bridges of bone between two contiguous vertebrae) are visible only belatedly on X-ray, causing a delay in the diagnosis of the disease [10].

Epidemiology

SpA mainly affect younger subjects, with a peak incidence around the third decade. The prevalence of these diseases is around 2% but shows wide geographical variability (higher in Nordic populations), due both to the type of population studied (some data refer to well-defined ethnic groups) and to the differences in the methodology used to estimate their prevalence (e.g. 1.9% in Germany, 2.5% in Alaska). In Italy, the prevalence of SpA is estimated at around 1%. The most common subtype is PsA (0.42% - 95% CI 0.31-0.61), followed by AS (0.37% - 95% CI 0.23-0.49). Moreover, the different prevalence of SpA reported in literature can be ascribed to the different classification criteria used. There is no significant difference in the prevalence between the two sexes. Lastly, the difference in geographical distribution could reflect a different prevalence of HLA-B27. Moreover, data suggest that HLA-B27 and sex could be associated with a more severe disease in terms of joint structural damage [11]. Recent studies indicate that patients with advanced axial SpA shows a prevalence of HLA-B27 near to 80% with a ratio of 3 to 1 between males and females, while in early SpA the prevalence of HLA-B27 is near to 50%, and the males/females ratio is about 1. A recent review of 36 studies showed a prevalence of axial radiographic SpA, using the New York criteria (Table I), of 3.19% in the United States, 2.38% in Europe, 1.67% in Asia, 1.02% in Latin America and 0.74% in Africa. Since the radiological signs appear late in the disease, the prevalence appears slightly higher

using the clinical criteria of the EESG, Amor criteria or the ASAS criteria.

Table I: New York criteria for inflammatory sacroiliitis [12]

	Radiological findings
0	Normal
1	Suspicious changes (some blurring of the joint margins)
2	Minimum abnormality (small, localized areas with erosion or sclerosis, with no alteration in the joint width)
3	Unequivocal abnormality (moderate or advanced sacroiliitis with erosion, evidence of sclerosis, widening, narrowing or partial ankylosis)
4	Severe abnormality (complete ankylosis)

Etiopathogenesis and risk factors

Predisposing genetic factors (e.g. HLA-B27) are relevant in the etiopathogenetic process of SpA. HLA-B27 is closely related in particular to the axial involvement, therefore mainly with AS, but also with the other forms (Table II) [13]. Prevalence of HLA-B27 in different subtypes of SpA was recently estimated: HLA-B27 is associated in particular with AS (70-90%), but also with the other subtypes such as ReA (30-60%), PsA (20-50%), En-SpA (10-40%) and USpA (25-70%).

Table II: Prevalence of HLA-B27 in seronegative spondyloarthritis [13]

Diseases	**HLA-B27 frequency**
Ankylosing spondylitis (AS)	75-90%
Non-radiographic spondyloarthritis (Nr-SpA)	75-90%
Reactive arthritis (ReA)	30-60%
Psoriatic arthritis (PsA)	20-50%
Enteropathic spondyloarthritis (En-SpA)	10-40%
Enthesitis related arthritis (ERA)	50-80%
Undifferentiated peripheral spondyloarthritis (USpA)	25-70%

However, the prevalence of HLA-B27 in general population is about 6% in Italy (about 8% in US), but only 0.5% have a diagnosis of SpA, so most subjects, despite the genetic predisposition, will not develop the disease. Therefore, environmental factors are also necessary for the development of the disease: although not all the mechanisms that lead to the disease and that affect its evolution are definitively clarified, in many cases the trigger is represented by intestinal (Salmonella, Shigella, Yersinia, etc.) or urogenitals (Chlamydia, Mycoplasma, etc.) infection, but also trauma, both physical and psychological. Furthermore, it should be stressed that smoking is a risk factor both for developing SpA and for more severe disease [1].

Moreover, different HLA appear to be associated with PsA such as HLA-B16/B17/B27/B38/B39 and HLA-Cw6 (mainly associated with cutaneous psoriasis).

The primary site of the SpA inflammatory process is the enthesis, that is the point of bone insertion of ligaments, tendons and other fibrous/cartilaginous components of the locomotor system. This involvement is responsible for most of the clinical manifestations typical of the SpA, both axial and peripheral, such as sacroiliitis, spondylitis, enthesitis and oligoarthritis.

In addition, extra-articular manifestations such as ocular (acute anterior uveitis, conjunctivitis), mucocutaneous (psoriasis, blenorragic keratoderma, balanitis circinata), cardiac (aortic insufficiency, atrioventricular conduction disorders) and intestinal (chronic colitis) involvement should be mentioned.

Classification and diagnosis

In 1990 Amor created the first classification criteria based on the patient clinical symptoms, the radiological findings, the genetic background and the response to anti-inflammatory treatment (Table III) [14]. These criteria were modified in 1991 by the ESSG (Table IV) [15]. The characteristics of inflammatory spinal pain are expanded in Table V [16].

Table III: Amor classification criteria for SpA (1990) [14]

A.	**Clinical symptoms**	**Score**
1.	Lumbar or dorsal pain at night or morning stiffness in the lumbar or dorsal region	1
2.	Asymmetric oligoarthritis	2
3.	Buttock pain, unspecified	1
	Alternating buttock pain	2
4.	Dactylitis	2
5.	Heel pain or other enthesopathy	2
6.	Iritis	1
7.	Non-gonococcal urethritis or cervicitis within one month before the onset of arthritis	1
8.	Acute diarrhea within one month before the onset of arthritis	1
9.	Past or current psoriasis and/or balanitis and/or chronic inflammatory bowel disease (ulcerative colitis or Crohn disease)	2
B.	**Radiographic findings**	
10.	Sacroiliitis (bilateral grade >2 or unilateral grade 3)	3
C.	**Genetic background**	
11.	Presence of HLA-B27 and/or family history of Ankylosing spondylitis, reactive arthritis, psoriasis, uveitis or chronic inflammatory bowel disease	2
D.	**Response to anti-inflammatory treatment**	
12.	Clear-cut improvement within 48 hours after NSAIDs intake or rapid relapse of the pain within 48 hours after NSAIDs discontinuation	2

Despite the new ESSG criteria, the Amor criteria still had the advantage of being able to classify as USpA even those forms which do not have at least one of the two major ESSG criteria (inflammatory spinal pain or peripheral arthritis). However, a common limitation of these criteria is that they are not reliable for early diagnosis, considering the wide variability of clinical presentation, especially at the initial stage of the disease when there are no even radiographic findings: from the onset of the disease (inflammatory lower back pain) to the radiological findings, a long time interval may elapse, over 5 years in 50-70% and over 10 years in 15-25% (Figure 1) [17].

Table IV: ESSG criteria for SpA (1991) [15]

Inflammatory spinal pain (hystory or present symptoms of spinal pain in back, dorsal or cervical region, with at least 4 of the following: (a) onset before the age of 45, (b) insidious onset, (c) improved by exercise, (d) associated with morning stiffness, (e) at least 3 months duration)

or

Synovitis (past or present asymmetric arthritis or arthritis predominantly in the lower limbs)

and

One or more of the following:
- Family history: first- or second-degree relative with AS, spondylitis, psoriasis, acute uveitis, reactive arthritis or inflammatory bowel disease
- Past or present psoriasis diagnosed by a physician
- Past or present ulcerative colitis or Crohn disease diagnosed by a physician and confirmed by radiography and/or endoscopy
- Past or present buttock pain alternating between the right and left gluteal regions
- Past or present spontaneous pain or tenderness at examination of the site of insertion of the Achilles tendon or plantar fascia (enthesitis)
- Bilateral grade 2-4 sacroiliitis or unilateral grade 3-4 sacroiliitis according to the New York criteria
- Episode of diarrhea occurring within 1 month before the onset of arthritis
- Nongonococcal urethritis or cervicitis within 1 month before the onset of arthritis

Table V: Inflammatory lower back pain (criteria that can be extended to the entire spine) [16]

Factors which differentiate the back pain produced by spondylitis from the back pain due to other causes:
- Onset of back pain before the age of 45
- Insidious onset
- Persistence for at least 3 months
- Associate with morning stiffness
- Improvement with exercise
- Good response to anti-inflammatory drugs
- Night pain

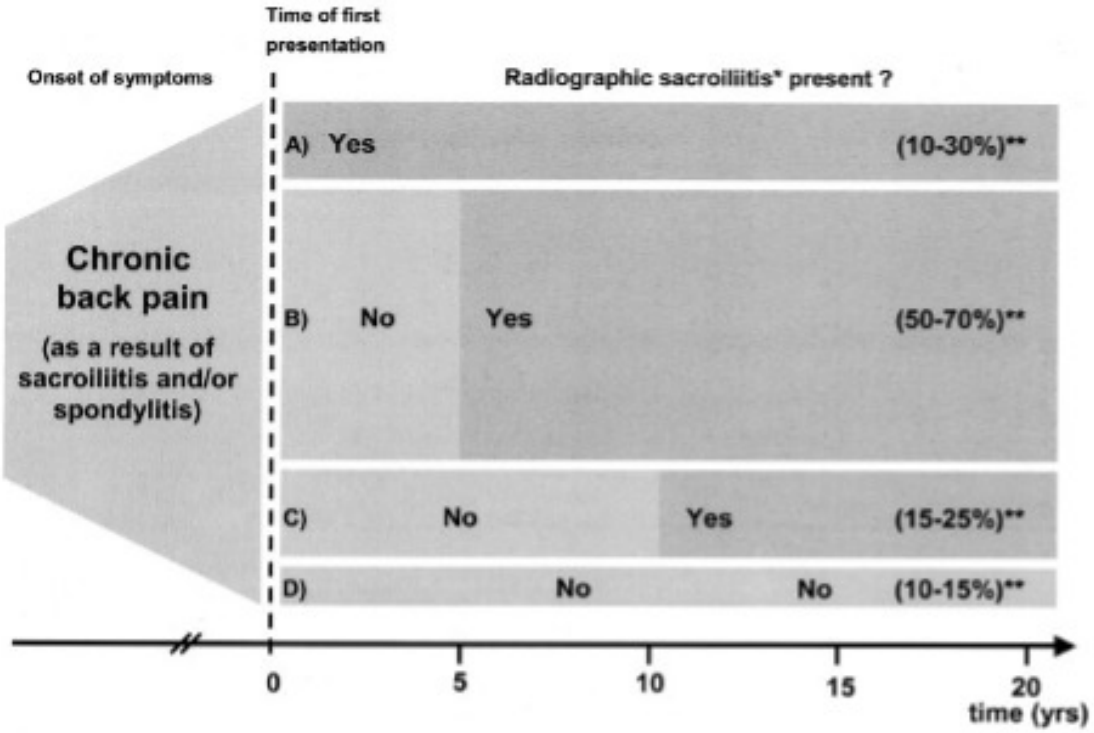

Figure 1: Axial SpA progression [17]

Therefore it has been proposed to include in the concept of axial SpA also an early-stage disease defined as non-radiographic spondyloarthritis (nr-SpA), where the sacroiliac involvement can only be detected with MR. Many patients may also develop radiological findings over the years, but as seen in clinical practice, not all subjects show an evolution from a non-radiographic to a radiographic disease [17, 18]. This window of time represents a real opportunity for early drug treatment, aimed at stopping the evolution of the disease and preventing the progression of anatomical damage.

The progression can be classified into three stages (Figure 2) [17, 18]:

1. Evidence of sacroiliitis only on MR (nr-SpA)
2. Radiographic sacroiliitis
3. Appearance of syndesmophytes

With the advent of new imaging methods, in particular MR, the ASAS group developed, in 2009, new criteria which allow the diagnosis also in non-radiographic stage, combining clinical, laboratory and imaging techniques, in particular MR which allows to highlight the inflammation of the sacroiliac joints at the onset of the disease [18] by detecting bone marrow edema, which is the manifestation of early bone inflammation [19].

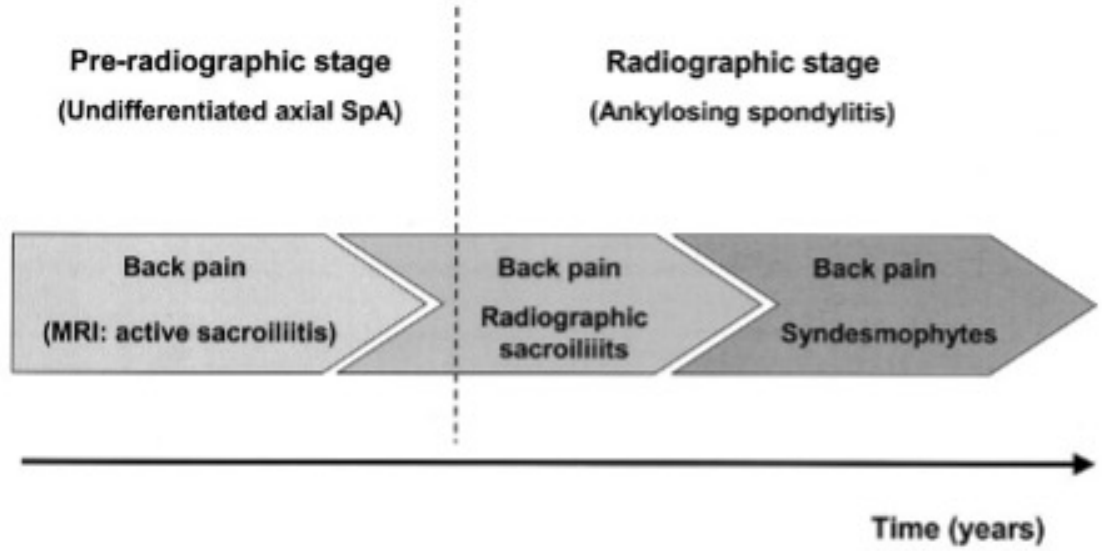

Figure 2: Stages of ax-SpA progression [17]

First, in 2009, was published the new diagnostic algorithm for the axial SpA contemplating the presence of sacroiliitis on the imaging methods (bone marrow edema on MR or radiographic sacroiliitis according to New York Criteria) associated with at least one of the clinical signs peculiar of this group of disease (inflammatory lower back pain, peripheral arthritis, enthesitis, uveitis, dactylitis, psoriasis, inflammatory bowel disease, response to treatment with NSAIDs, family history, HLA-B27 positive, increased inflammatory indices) or the presence of HLA-B27 positive with at least two of these manifestations (Figure 3) [20].

Similarly to the criteria for the axial SpA, an algorithm for peripheral SpA was also developed in 2011 (Figure 4) [21]. In this case, for the diagnosis, is necessary the presence of at least one clinical manifestation among psoriasis, inflammatory bowel disease, preceding infections, HLA-B27 positive, uveitis, presence of sacroiliitis on imaging (bone marrow edema on MR or radiographic sacroiliitis according to New York Criteria) or at least two among peripheral arthritis, enthesitis, dactylitis, inflammatory lower back pain, family history for SpA). It should be remembered, that peripheral SpA are usually asymmetric, mono or oligoarticular, predominantly of the lower limbs.

The ASAS criteria are more performing, compared to the ESSG criteria and Amor criteria, in classifying the different forms of SpA and represent a useful support to clinical studies and rheumatological practice.

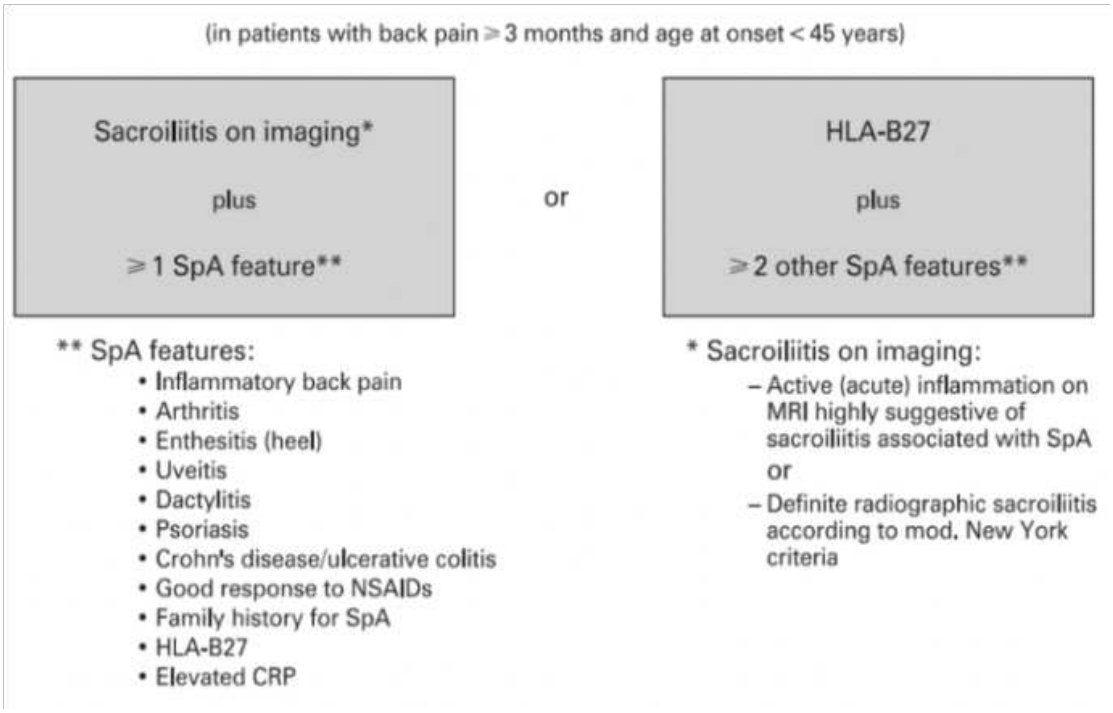

Figure 3: ASAS criteria for Axial SpA [20]

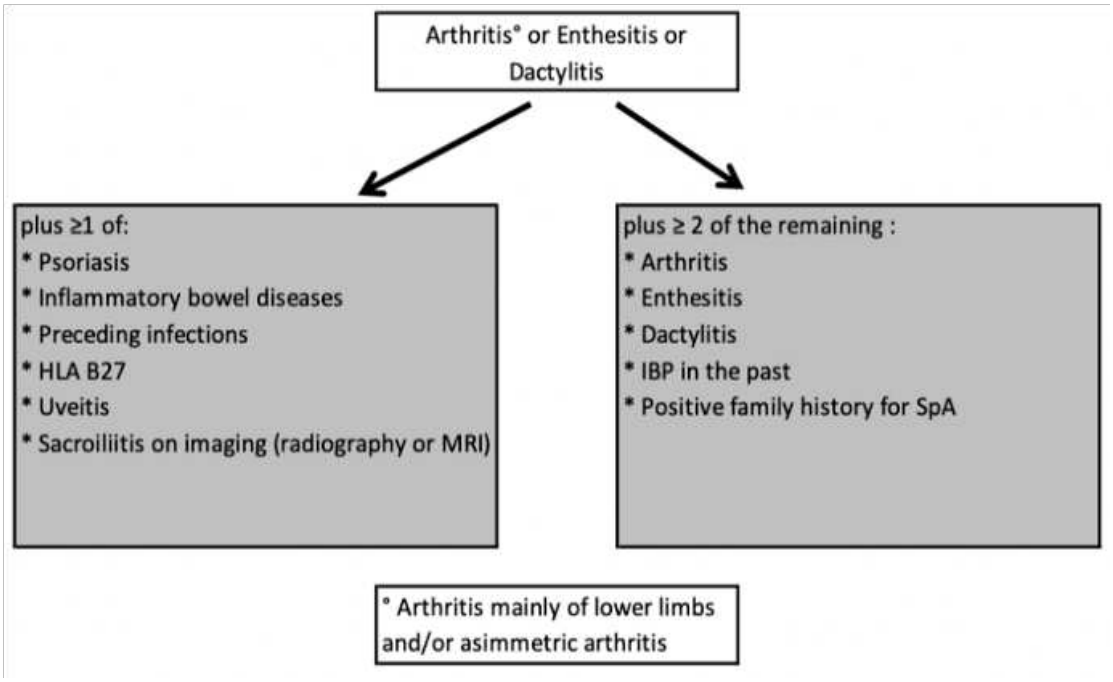

Figure 4: ASAS criteria for peripheral SpA [21]

1.1.2 Ankylosing spondylitis

Definition

Ankylosing spondylitis (AS) is a chronic inflammatory disease with predominant axial involvement, although sometimes it may also affect peripheral joints and have systemic extra-articular manifestations [1].

Epidemiology

The prevalence of AS is about 0.1-1.4% and the incidence is about 1 per 10 thousand Caucasians per year. The prevalence of the disease is closely related to the frequency of HLA-B27, described in the Caucasian population up to 6-9%, while in North America, in some Indian tribes, prevalence can even reach 50% of the population. Males/females ratio is approximately 2/1. The average age of onset is around 26 years, however, cases with juvenile onset are not uncommon, while cases after 45 years are rare. Survival is roughly the same of the general population, if not for cases complicated by extra-articular manifestations.

Etiopathogenesis

Although the pathogenic mechanism of the disease is still not fully clarified, AS is certainly a multifactorial disease that is caused by the interaction of genetics, environmental and immunological factors.

Genetic predisposition Regarding genetic predisposition, HLA-B27 is present in 95% of cases of AS, validating the hypothesis that genetic susceptibility is determinant for the onset of the disease. In addition, epidemiological studies have shown a frequency of AS and HLA-B27 three times higher in northern european countries than in the south populations. It has been observed that about 1-2% of the population with HLA-B27 develops the disease; this percentage is higher in patients who have family members affected by AS and with HLA-B27 positive, where prevalence reaches 10-20% [1]. The involvement of HLA-B27 was also observed in mouse models: Hammer and collaborators observed an axial-SpA-like disease in transgenic mice expressing human HLA-B27 associated with β2-microglobulin [22]. HLA (Human Leukocyte Antigen) is a class I protein of the Major Histocompatibility Complex (MHC-I), and is present in all nucleated cells. It has a function of controlling the immune response mediated by T cells. The genes encoding HLA are highly polymorphic so there are several types of HLA.

For HLA-B more than 1000 different alleles have been described. The HLA-B27 allele has 130 subtypes (B27:01 to B27:105), which differ only in some bases. AS is particularly associated with HLA subtypes B27:02, B27:04 and B27:05 [23]. The subtypes B27:06 and B27:09 seems not to be associated with AS. The role of this allele in the pathogenesis can be explained precisely by its role in presenting self-peptides to the CD8+ cytotoxic lymphocytes (CTL). These seem to be actived by a mechanism of molecular mimicry and this theory has recently become widely accepted: a bacterial agent activates CTL, and the analogy between bacterial epitopes and some articular self-peptides presented by HLA-B27 determines the cross-CTL reaction, which result in an inflammatory state with a consequent tissue damage. This hypothesis is supported by the identification of specific CTLs for HLA-B27 directed against self-epitopes in the synovial fluid of patients with AS. Further scientific evidence also suggests that HLA-B27 could be able to interact with CD4+ T lymphocytes and with molecules present on the surface of natural killer (NK) cells and monocytes, although the role of this interaction has not yet been clarified [24, 25]. Moreover, an altered post-translational modification of the HLA-B27 protein has an important role because it determines the accumulation of aberrant chains within the endoplasmic reticulum, the consequent activation of inflammatory pathways such as that of NF-κB, and the subsequent secretion of pro-inflammatory cytokines such as TNF-α [26].

As for the HLA-B27 negative forms, there are other alleles that can be found in these patients, including HLA-B7, -B22, -B40, - Bw42. These patients have a disease with particular characteristics compared to the HLA-B27 positive forms: less family predisposition, later age of onset, milder and more nuanced symptoms, slower progression and above all rare presence of uveitis, which is instead more frequent in the HLA-DR8 and DRB1-01 positive forms [1]. An association with HLA-C1 alleles has also been demonstrated [27].

Environmental factors The importance of environmental factors as a trigger of AS, in patients with a genetic and immunological predisposition, is underlined by the fact that about 60% of patients affected by AS experienced a bacterial infection before the onset. The pathogens mainly involved seems to be Yersinia, Campylobacter, Salmonella, Shigella and in particular Chlamydia. The latter has also been found during PCR studies in the synovial fluid of some AS patients [28]. Some other studies showed that ANKLE mice (the mice model of AS) develop the disease if they contract a bacterial infection, unlike when placed in aseptic environment.

Recent studies also proposed that the entrapment of pathogens resistant to phagocytosis by intestinal, tonsillar and periodontal macrophages, may be crucial in the molecular mimicry mechanism [29].

Finally, also traumatic factors have an important role through the activation of chronic joint inflammation, confirmed by both clinical experience and scientific literature [30].

Immunological factors Several studies show that AS patients have an aberrant cytokine production, in particular TNF-α, IL-12, IL-22, IL-23, IL-17 and IFN-γ, while IL-10, which have a strong anti-inflammatory role, was found to be significantly decreased in case of infection of endogenous bacteria of the Bacteroides group [31].

An important discovered in understanding the pathogenetic process of AS was the identification, at the level of the enthesis, of pre-T lymphocyte (CD3+/CD4-/CD8-) responsible for the production of pro-inflammatory cytokines. Furthermore, recent studies on mouse models have shown that increased IL-23 levels are sufficient to induce the chronic inflammation typical of the disease, while the inhibition of IL-23 through monoclonal antibodies significantly improves the signs of inflammation in joints and enthesis [32]. The knowledge of the immunological factors that mediate the activity of the disease are fundamental to understand the therapeutic rational.

Pathological anatomy

As the name "spondylo-entheso-arthritis" suggests, AS affects both the joints, in this case in particular the sacroiliac joints, and the enthesis (insertion of a tendon, ligament, capsule, or fascia into bone), in particular those of the vertebral bodies. Initially, at both of these sites, subcondral granulation tissue is formed, consisting of lymphocytes, plasma cells, mast cells and chondrocytes. This leads in time progress to the development of sclerosis and erosion in the margins of the joints which are gradually replaced by fibrocartilaginous tissue and subsequently by bone tissue, as an attempt of reparation, with evolution towards complete joint fusion and consequent ankylosis. This process ends in the formation of syndesmophytes (particularly associated with AS), by differentiation of mesenchymal cells into chondrocytes then into osteoblasts that finally leads to the formation of bone bridges between adjacent vertebral bodies, with a progression that results in a typical radiographic picture called "bamboo cane spine" [33–36].

Moreover, typical is the formation of peri-articular enthesophytes. Finally, in the AS the sacroiliac joints involvement is often bilateral, unlike a possible involvement in other SpA, such as PsA, in which it is often monolateral.

Clinical manifestations

As all seronegative SpA, AS has mainly an articular involvement but can also have an extra-articular involvement.

AS traditionally begins with an inflammatory lower back pain as described in Table V [16]. Usually the pain begins at lumbar region and has an ascending progression over time up to affect the cervical region. The pain is typically insidious and poorly defined, sometimes referred to the region of the iliac crest or the great trochanter. At first, the pain may be monolateral or alternating, but within a few months after the onset of the disease it becomes persistent and bilateral. This leads to a serious limitation especially in the extension of the lumbar spine, but also in the movements of the vertebral column in forward and lateral flexion. Furthermore, a possible complication is the reduction in thoracic expansion during deep inspiration. Lower back pain may radiate along the back side of the thigh to the popliteal fossa which occurs alternately on one side and then on the other, and is therefore defined as "tilting". Morevore, inflammation often involves the acromioclavicular, sternoclavicular, sternocostal and costovertebral joints which may cause chest pain exacerbated by coughing or sneezing.

The patient shows a marked alteration of the posture with a strong reduction of lumbar lordosis, accentuation of the dorsal kyphosis, contracture in flexion of the hips and of the knees and fixed posture in anterior flexion, with inability to lie prone. Especially in advanced stages of the disease, postural alterations become really severe with skeletal deformities and disabilities.

Peripheral joint involvement may affect 30% of patients, often in the female sex and often as an oligoarticular asymmetric arthritis. Extra-axial involvement frequently involves the hips and is manifested by an inguinal pain radiated even to the knee with reduced rotation and abduction. In addition, an Achilles or patellar tendinitis may appear, as well as dactylitis.

Axial involvement can lead to complication which often result in a destruction of the intervertebral disc or spondylodiscitis. Fractures are not uncommon and may occur especially in the cervical region as well as atlo-epistrofean subluxations with characteristic manifestations such as headache, rigidity, hyperreflexia of the limbs, tremors and myoclonus. Some patients can develop a destruction of the

femoral head due to a severe coxitis. Finally, the disease can lead to cauda equina syndrome with urinary and faecal incontinence, paresthesia and motor disorders to the lower limbs due to denervation at the level of the sacral roots [1, 16, 37].

As for extra-articular involvement, one of the most frequent manifestations, that can sometimes be the first manifestation of the disease, is anterior uveitis (or iridocyclitis). This condition affects 25-40% of AS patients and is often acute and unilateral but it can be alternating or with a tendency to contralateral recurrence. The patient may complain eye pain with redness, photophobia, hyperlacrimation and decreased vision. Signs include hyperemia of the paracorneal conjunctiva (ciliary flush or limbal injection). Sometimes uveitis can lead to complications, such as severe and irreversible loss of vision (especially if uveitis is not recognized, is treated poorly, or both), cataracts (secondary to the pathological process and/or treatment with corticosteroids), cystoid macular edema (the most frequent cause of reduced vision in patients with uveitis), glaucoma (secondary to the pathological process and/or treatment with corticosteroids), retinal detachment, banded keratopathy, neovascularization of the retina, optic nerve or iris, reduction of intraocular pressure [38].

Complications can also affect the cardiovascular system, in case of long-term disease, manifested mainly by the development of ascending aortitis, aortic insufficiency and conduction abnormalities, especially atrioventricular block [39]. Rarely patients can develop myocarditis, endocarditis or pericarditis. However, these manifestations can also be present as isolated events linked to the presence of the HLA-B27. It should not be forgotten that like all chronic inflammatory diseases, arteriosclerosis is accelerated, with possible development, in progress of time, of hypertension, ischemic heart disease and cerebrovascular events.

Another complication of AS is respiratory damage, since the thoracic stiffness, secondary to the damage of the costovertebral joint, disturbs the ventilation mechanism with the appearance of a restrictive syndrome on respiratory function tests. Pleuropulmonary lesions have also been described, notably fibrosis of the upper lobes of the lung, interstitial infiltration, and pleural thickening, although they represent a rare and late manifestation of the disease.

The development of nephritis is mainly linked to the use of NSAIDs while renal amyloidosis is also a complication present in a long-lasting disease.

The links between AS and inflammation of the gastrointestinal tract have been highlighted in various studies, in fact, in patients with AS, chronic inflammatory bowel disease appears during the course of the disease in 2 to 18% of cases, with a delay of up to 20 years. About 30% of AS patients undergo endoscopic

investigations for suspected intestinal lesions [1, 40].

Finally, these manifestations are often accompanied by general symptoms such as fatigue, fever, weight loss, anorexia and night sweats.

Diagnosis

Usually the time interval between the onset of symptoms and the diagnosis of AS is the longest among rheumatic diseases (usually 5-7 years). One of the main causes of this diagnostic delay is due to the fact that the main symptom of presentation, lower back pain, is a very common symptom even in the general population [41]. Another important reason is the fact that the appearance of X-ray signs of sacroiliitis is quite late compared to the onset of symptoms and at the same time is difficult to diagnose AS before the typical structural changes visible on imaging are present.

The diagnosis of AS is based essentially on clinical manifestations, however, imaging methods are crucial to confirm the diagnostic suspicion, define the stage of the disease, follow its evolution over time and evaluate the response to therapy.

Laboratory There are no diagnostic laboratory tests for AS. The term "seronegative SpA" comes from the fact that tests for RF are usually negative. The inflammatory state leads to an increase in ESR and CRP, both in the onset and in AS relapses, whose negativity does not allow to exclude the diagnosis. There is often also a normochromic-normocytic anemia due to the chronic inflammatory state.

HLA-B27 typing cannot be used as a diagnostic test in all patients with lower back pain: its presence or absence is not sufficient to confirm or exclude the diagnosis of AS since this test never has a 100% of sensitivity or a specificity.

In severe cases, an increase in IgA, hypoalbuminemia and an increase in alkaline phosphatase is possible.

In cases of acute arthritis, synovial fluid should be drawn and analyzed despite there being no specific features except for a marked inflammatory fluid [1].

Imaging As mentioned above, X-ray and MR are both fundamental not only to confirm the diagnosis but also to monitor the evolution of the disease over time.

The first level imaging in case of diagnostic suspicion is definitely X-ray. The alterations evidentiable by traditional radiology begin at the level of the sacroiliac joints and follow an evolution described by the New York criteria [42].

This classification divides the radiographic signs into 5 degrees (Table I). Such lesions begin as fine erosion, unilateral at least initially and especially on the iliac side, which over time are replaced by areas of subcondral sclerosis. These aspects become increasingly marked and bilateral as the disease progresses until the complete loss of the joint space with structural continuity between the two articular sides and the appearance of bone ankylosis. As for the involvement of the spine, it usually follows the appearance of the sacroiliitis, although the alterations may also be contemporary. Early signs are the erosion of the anterior corners of the vertebral bodies (Romanus sign) and the sclerosis with loss of normal vertebral concavity (vertebral squaring). A late radiological sign is the presence of "shiny corners", that is an accentuation of the radiopacity of the vertebral angles for marginal sclerosis, which represents the physiological evolution of early erosion. The most characteristic aspect, but also late, is the appearance of syndesmophytes, ossification of the peripheral side of the fibrous ring that, starting at the edges of the vertebral bodies, extend along the entire height of the disc by joining like a bridge the contiguous vertebrae involved. The syndesmophytes initially are found mainly at the dorso-lumbar level and then extend, in case of severe and long-lasting disease, to the whole spine that will assume the characteristic "bamboo cane" appearance. At such an advanced stage of the disease, X-ray can sometimes also show other characteristic signs: the "track sign" (diffuse ossification of the interapophyseal joints that determines two radiopaque vertical lateral bands similar to the train tracks) and the "dagger sign" (ossification of interspine ligaments that determines the appearance of a central vertical band). If both these signs are present we speak of "rack railway".

However, conventional radiology presents significant limitations in the identification of early alterations, often resulting in little sensitivity, especially in young patients or patients with a short history of inflammatory lower back pain, making the use of other imaging methods necessary. MR is the method that, through the identification of bone edema, allows an early diagnosis of sacroiliitis and/or spondylitis even after a few weeks since the onset of an inflammatory lower back pain and many years before the radiographic changes are detectable. Subcondral bone edema will be visible as a circumscribed area of hyperintensity in the STIR T2-weighted sequences or T1-weighted sequences after gadolinium-based contrast agent with hyperintensity impregnation of the subcondral bone and joint space [43]. If MR abnormalities are identified, a diagnosis of nr-SpA can be established.

Moreover, peripheral enthesis can also be studied using traditional radiology where entesophites can be visible, that is calcification-ossification phenomena of enthesis [44].

Computed tomography (CT), although it has a higher sensitivity in detecting fine erosion and initial sclerosis, is used as second-level imaging, only in case of doubts in the interpretation of X-ray and MRI.

The scintigraphy is an examination today little used since, although highly sensitive, it is little specific since the frequent hypercaptation of the sacroiliac joints also in healthy subjects.

Finally, ultrasonography (US) is used exclusively to identify enthesitis or peripheral arthritis: in this sense, it is also possible to use power doppler which will evaluate the presence of an altered vascularization [20].

Clinimetric evaluation

Numerous indices have been developed in order to evaluate objectively the severity, both in terms of disease activity and quality of life, progression and response to therapy of AS. Some of these indices are specific for AS, others are also used for the evaluation of other inflammatory arthritis.

Aspecific indices 1) Visual Analog Scale (VAS): pain assessment using the visual analog scale 0-100 mm, in which 0 corresponds to "no pain" and 100 to "worst pain ever felt" [45]. 2) Patient Global Assessment (PGA): assessment of patient's health status using visual analog scale 0-100 mm, in which 0 corresponds to "excellent" and 100 to "very bad" [46]. 3) Health Assessment Questionnaire (HAQ): functional disability assessment using a questionnaire consisting in 24 questions divided into 8 categories; for each question, the patient can choose among 4 options (0=without difficulty, 1=with some difficulty, 2=with much difficulty, 3=impossible). The final value (from 0 to 3) is the result of the sum of the highest score of each category divided by the number of categories (8) [47]. 4) Leeds Enthesitis Index (LEI): evaluation of the tenderness of the enthesis of the lateral epicondyle humerus, medial condyle femur, Achilles tendon; each tender enthesis has a value of 2 and the final score goes from 0 to 6.

Specific indices 1) Bath Ankylosing Spondylitis Functional Index (BASFI): self-assessment scale to evaluate the impact of AS in daily actions and movements and the residual functional capacities; it consists of 10 VAS of common actions each with a score from 0 (easy) to 100 (impossible) [48]. 2) Bath Ankylosing

Spondylitis Disease Activity Index (BASDAI): self-assessment scale to evaluate the disease activity; it consist of 6 VAS of the most relevant symptoms of AS each with a score from 0 (none) to 100 (very severe) [49]. 3) Bath Ankylosing Spondylitis Metrology Index (BASMI): an index to evaluate the mobility of the spine which considers tragus-wall distance, lumbar flexion at Schober's test, lateral flexion, cervical rotation and intermalleolar distance; each item has a score of 0 (normal mobility), 1 (partial mobility) and 2 (very impaired mobility) and the final score varies from 0 to 10 [50]. 4) Ankylosing Spondylitis Disease Activity Score (ASDAS): a score to evaluate disease activity based on 4 VAS (lower back pain, morning stiffness, global health and swelling-tenderness of peripheral joints) and levels of ESR or CRP; the final score is stratified as <1.3 (remission), 1.3-2.0 (low disease activity), 2.1-3.5 (high disease activity) and >3.5 (very high disease activity) [51]. 5) Maastricht Ankylosing Spondylitis Enthesitis Score (MASES): a score which evaluates 13 enthesal sites (I and VII cost-chondral joint, superior anterior iliac spine, posterosuperior iliac spine, iliac crest, spinous process of the 5th lumbar vertebra, insertion of the Achilles tendon on the heel); each site has a score of 1 if tender and the final score is represented by the the sum of each value [52].

Treatment

The aims of AS therapy are to reduce the intensity of pain and stiffness, to improve the patient quality of life and functional capabilities, to prevent progression of radiological damage and disability. The therapeutic approach is multidisciplinary and varies from simple educational interventions to surgery, sometimes necessary for the correction of deformities.

Pharmacological treatment First-line therapy for symptomatic patients are NSAIDs; several studies suggest that continued use of NSAIDs in AS prevents X-ray progression more than use on demand [53]. However, an individualised risk assessment should be considered, in consultation with the rheumatologist, before the long-term daily use of NSAIDs. Speaking of which, the recent introduction of COXIB drugs (selective inhibitors of cyclo-oxygenase type II), was very important, especially due to the better safety profile and lower incidence of side effects at gastrointestinal level; in particular, recent studies reported that celecoxib particularly prevents the radiological progression of the disease. In addition to their effect on axial involvement, NSAIDs are also effective in the control of peripheral enthesitis and peripheral arthritis. Nevertheless, since the lack of safety

in long-term use, both for NSAIDs and COXIBs, intermittent administration in periods of activity is preferred over continuous administration. It should be noted, however, that only a minority of AS patients are effectively treated with NSAIDs. Most do not have a satisfactory response and therefore need other therapies.

Systemic use of corticosteroids is not supported by scientific evidence, although oral steroid cycles can be used in patients refractory to NSAIDs and with very high ESR and CRP levels; in addition, local infiltrations of corticosteroids directly into the joint and periarticular area may be useful in cases of enthesitis or peripheral arthritis.

In contrast to RA, conventional synthetic disease modifying rheumatic drugs (csDMARDs) such as methotrexate (MTX) and sulfasalazine (SSZ) have little effect in axial disease, but may be useful in a patient with peripheral involvement or in case of unavailability of biological disease modifying rheumatic drugs (bDMARDs).

Biological drugs, in fact, have been one of the most important innovations from a therapeutic perspective for AS: in particular for patients refractory to treatment with at least two NSAIDs for at least a month, the guidelines recommend the use of anti-TNF-α, (infliximab, adalimumab, certolizumab pegol, golimumab and etanercept). The biological characteristics of anti-TNF-α drugs and the respective dosage regimen used in AS are reported in Table VI. There are several randomized clinical trials data in favor of these drugs that have proven very effective in reducing laboratory inflammation indices, joint pain and morning stiffness, improving quality of life and functional abilities. In addition, it should be remembered that recent guidelines do not indicate the superiority of one drug over another in the treatment of AS.

After anti-TNF-α, new discoveries in the pathogenetic mechanism of AS led to the development of new drugs with a different mechanism of action such as anti-IL-17, first of all secukinumab. Recently, ixekizumab, used especially in PsA, has been approved for both AS and nr-SpA. Characteristics are reported in Table VII. Although this drug has proven effective in reducing disease activity and slowing radiographic progression after 4 years of follow-up in over 80% of treated patients, guidelines still suggest the use of anti-TNF-α as a first-line therapy for the greater data available in terms of long-term safety [1, 54, 55].

Table VI: Anti-TNF monoclonal antibodies

Anti-TNF-α	**Biological features**	**Dosage**
Adalimumab	Human monoclonal antibody	Administered subcutaneously at a dose of 40 mg every 2 weeks.
Etanercept	Human p75 receptor fusion protein of tumor necrosis factor α (TNF-α) bound to the modified Fc portion of human immunoglobulin G1 (IgG1)	Administered subcutaneously at a dose of 25 mg twice a week, or 50 mg once a week.
Golimumab	Human monoclonal antibody	Administered subcutaneously at a dose of 50 mg every month.
Certolizumab-pegol	Fab fragment of humanized recombinant antibody directed against tumor necrosis factor α (TNF-α) and conjugated with polyethylene glycol (PEG)	Administered subcutaneously at a dose of 400 mg (given in 2 subcutaneous injections of 200 mg each) per weeks 0, 2 and 4. After the starting dose, the recommended maintenance dose is 200 mg every 2 weeks or 400 mg every 4 weeks. After at least 1 year of treatment, in patients with prolonged remission, a reduction in the maintenance dose of 200 mg every 4 weeks can be considered.
Infliximab	Human-murine monoclonal antibody chimeric	Administered with a first intravenous infusion of 5 mg/kg per week 0 followed by additional infusions of 5 mg/kg per week 2 and 6 from the first infusion, and then continue administration every 6-8 weeks. If a patient does not respond within 6 weeks (that is after 2 doses) he should not receive any further treatment with Infliximab.

Finally, recently, a JAK inhibitor (JAKi), upadacitinib, was also approved for both AS and nr-SpA. JAK inhibitors are part of the class of targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs). The JAK/STAT signaling pathway (JAnus Kinases/Signal Transducer and Activator of Transcription proteins) regulates the processes of cell proliferation, differentiation and apoptosis transducing cellular signals resulting from the interactions of cytokines or growth factors.

Table VII: Anti-IL-17A monoclonal antibodies

Anti-IL-17A	**Biological features**	**Dosage**
Secukinumab	Human monoclonal antibody	Administered subcutaneously at a loading dose of 150 mg every week for 5 weeks, followed by a maintenance dose of 150 mg every 4 weeks. If the patient has previously been treated with anti-TNF-α without success, the recommended dose is 300 mg with the same schedule.
Ixekizumab	Humanized monoclonal antibody	Administered subcutaneously at a loading dose of 160 mg (two 80 mg injections) per week 0, followed by a maintenance dose of 80 mg every 4 weeks. Discontinuation of treatment in patients who have not shown any response after 16-20 weeks of treatment should be considered. Some patients with a partial initial response may subsequently improve by continuing treatment beyond 20 weeks.

Four JAK proteins [JAK1, JAK2, JAK3 and TYK2 (TYosine-protein Kinase 2)] and seven STAT proteins (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6) have been identified so far. JAK1 plays a key role in mediating the signals of inflammatory cytokines, JAK2 in mediating erythropoiesis, while JAK3 plays an essential role in immune homeostasis and lymphopoiesis. In the signal transduction pathway, JAK phosphorylate STAT that modulate intracellular activity, including gene expression [56]. The main JAK inhibitors (Table VIII) approved for rheumatic diseases are:

- Upadacitinib: approved for AS, nr-SpA, PsA and RA.
- Tofacitinib: approved for PsA and RA.
- Baricitinib: approved for RA.
- Filgotinib: approved for RA.

A series of studies has shown that upadacitinib has allowed an improvement in inflammatory symptoms and signs of inflammation (CRP, ASDAS score and MR), physical function (BASFI) and quality of life. In addition, these studies also showed a slowdown in radiographic progression.

The endpoints were reached already after 4 weeks of treatment and the response was still positive after 64 weeks. In addition, the safety profile has remained as important as in studies for RA and PsA [57, 58].

Table VIII: JAK inhibitors

JAK inhibitor	**Biological features**	**Dosage**
Upadacitinib	Reversible JAK inhibitor with higher affinity for JAK1	Administered orally 15 mg once a day.
Tofacitinib	Reversible JAK inhibitor with higher affinity for JAK1/3	Administered orally 5 mg twice daily.
Baricitinib	Reversible JAK inhibitor with higher affinity for JAK1/2	Administered orally 4 mg once a day; a dose of 2 mg once a day may be appropriate in patients aged over 75 years, with history of chronic or recurrent infections or with persistent clinical benefit with a dose of 4 mg once a day.
Filgotinib	Reversible JAK inhibitor with higher affinity for JAK1	Administered orally 200 mg once a day.

bDMARDs and tsDMARDs are associated with higher rates of adverse events than csDMARDs, in particular, rates of severe infections have increased, although they tend to decrease over time [59]. For this reason, before starting therapy, it is necessary to exclude the presence of latent infections such as tuberculosis and hepatitis B and C; in these patients is therefore necessary to execute a chest X-ray, quantiferon test and serological investigation for hepatitis viruses. In addition, it is recommended to avoid biological agents (except rituximab) within 5 years after a cancer has been cured, although the data in the register do not suggest increased risk [60]. In case of remote history of lymphoma, rituximab (RTX) or even tocilizumab would be the drugs of choice. During pregnancy, the drug of choice is SSZ, while MTX and leflunomide are contraindicated due to their teratogenic effects; also bDMARDs are contraindicated in pregnancy, except certolizumab-pegol, which, due to its pharmacological characteristics, is not able to pass the blood-placental barrier and reach the fetus with the risk of immunodepression and infections [61].

Anti-TNF-α are absolutely contraindicated in patients with class NYHA III/IV heart failure due to the high mortality rate found in some studies [62]. JAKi have recently undergone a safety review as patients at risk of heart disease were more likely to have serious cardiovascular problems (such as heart attack, stroke or death from cardiovascular disease) [63] and had a higher risk of developing cancer and thromboembolic events than those treated with anti-TNF-α.

Non-pharmacological treatment Scientific literature unanimously agrees in recommending smoking cessation which worsens the quality of life and response to therapy and accelerates the progression of the disease. The management of obesity is also of fundamental importance in patients with AS, as recent studies indicate, again, a worsening of symptoms, quality of life and response to biological drugs. In addition, body weight control significantly reduces the risk of cardiovascular events to which patients with long-term AS may be more exposed. On the contrary, there are few studies about the usefulness of non-pharmacological therapies such as physiotherapy, balneotherapy, mud and thermal baths, with often heterogeneous results and improvements that seem to run out in the short term (6-15 months). However, ACR guidelines recommend regular aerobic light physical activity and physiotherapy to improve physical function, quality of life, cardiorespiratory function and chest expansion [64].

Prognosis

The prognosis is favorable if the disease is diagnosed at an early stage and if therapy is adequate: appropriate therapy should both treat the acute inflammatory episode and prevent any future flares. Negative prognostic factors are still subject to debate, but a negative effect on prognosis seems to have early hip involvement, the presence of peripheral joint involvement and/or dactylitis, a persistent inflammatory state (ESR and CRP consistently elevated despite therapy) and presence of bone marrow edema at MR. Mortality is higher in patients with long-term disease, particularly in cases of cardiovascular involvement [1].

1.1.3 Psoriatic arthritis

Definition

Psoriatic arthritis (PsA) is an inflammatory joint disease associated with cutaneous psoriasis. The first definition was given by Moll and Whright who described it as "an inflammatory arthritis in presence of psoriasis and in absence of rheumatoid factor" [2], but this definition did not include the possibility of diagnosing PsA even if only with the clinical or radiological confirmation of an enthesis involvement. In addition in 5-10% of cases, psoriasis appears after arthritis [1]. The American College of Rheumatolgy (ACR) identified PsA as a separate clinical entity in 1964, including it in seronegative SpA.

Epidemiology

The exact prevalence of PsA is not known since very different data have emerged between Western and Eastern countries: about 0.001% in Japan against 0.40% in Italy. This difference could be due to genetic and environmental factors but also to non-standard study methodologies [65]. PsA affects both sexes equally and usually in an age group between 40 and 50 years, rarely in children. Among patients with cutaneous psoriasis about 20-30% develop joint disease [66]. A prospective study showed that of 464 patients with cutaneous psoriasis, but without joint disease, 51 developed PsA during an 8-year follow-up and with an annual incidence of 2.7% [67].

Etiopathogenesis

The pathogenesis of PsA is multifactorial, due to the concomitant presence of a predisposing genetics, environmental and immunological factors.

Genetic predisposition Several studies have suggested that psoriasis is mainly linked to HLA-Cw6 allele, and lesser to HLA-DR7, while psoriatic arthritis is more strongly linked to HLA-B16, B38 and B39. In addition, about 30% of patients with PsA, especially in forms with greater axial involvement, may also exhibit positivity for HLA-B27. Mutations in single genes that result in psoriasis are rare and only affect a small subset of psoriasis patients. Several monogenic mutations have been associated with cutaneous psoriasis but not confirmed in PsA such as gain of function mutations in caspase recruitment domain-containing protein 14 (CARD14) located in the PSORS2 region on chromosome 17q2 [68, 69]

and polymorphisms in JAK2, SOCS1 and ETS1 genes [70]. However, a single-nucleotide polymorphism (SNP) in the collagen10A1 gene (rs3812111c.155A > T, COL10A1) [71] and mutations in IL-12B, IL-23A, TYK2, STAT3, TRAF3IP2 genes has been associated with PsA [72, 73]. Polymorphisms in IL-23R have been linked to genetic susceptibility in a number of human autoimmune diseases including PsA, psoriasis, and IBD [74, 75]. Nevertheless, the majority of genetic variants associated with psoriasis or PsA are not strong enough to explain the development of disease [76].

Environmental factors Environmental factors, such as stress, trauma, infection, diet and especially microbiota are possible triggers of chronic immune activation in genetically predisposed individuals [76, 77]. In particular, has been proven an association between upper respiratory airway streptococcal infection and PsA, since elevated levels of anti-deoxyribonuclease B antibodies against Streptococcus exotoxin were found, but them were absent in patients with psoriasis alone [78].

Immunological factors Both innate and acquired immunity are involved in the pathogenesis of PsA. Several studies have in fact shown the abundant presence of CD4+ and CD8+ cells both Th1 and Th17 in the synovial membrane resulting in abundant production of cytokines such as IL-1, IL-2, IL-12, IL-17, IL-23, IFN-γ, TNF-α [79]. This condition of uncontrolled inflammation lead to bone erosion and cartilage destruction but a fundamental difference with RA is that the bone architectural changes in PsA are characterized by the presence of both catabolic (bone erosion and osteolysis) and anabolic (enhanced bone formation, such as syndesmophytes and enthesophytes) bone changes [80]. Among these cytokines, established is the key role of TNF-α, to date the main therapeutic target, which has been shown not only to participate in the proliferation of keratinocytes in skin psoriasis, but also induce the destruction of cartilage by metalloproteases (MMPs), bone remodeling and the production of calcifications at the articular, periarticular and enthesitic level [81]. Recent studies have shown the proliferation of osteoclastic precursors in PsA patients and this seems to be induced by TNF-α: blocking TNF-α, in fact, will also block osteoclastic proliferation [82]. TNF-α plays a key role in the formation of erosion as it induces osteoclasts differentiation and suppress osteoblasts formation by triggering the expression of receptor activator nuclear factor-kB ligand (RANKL), stimulating the expression of Dickkopf-related protein 1 (Dkk-1) by synovial fibroblasts, further promoting the formation of erosion [83].

It also appears that TNF-α also stimulates the production of VEGF, the main stimulator of the angiogenesis process, and its receptors, playing a key role in the mechanism of bone remodeling and the maintenance of joint inflammation [81].

In addition, of considerable importance, especially from a therapeutic point of view, is the recent evidence regarding the role of the Th17 response in both skin psoriasis and PsA [84]. These findings support the use of anti-IL-12/23 therapies in patients with cutaneous psoriasis or PsA refractory to anti-TNF-α. Finally, other studies have shown the expression of IL-15 and IL-18 mRNA in endothelial cells below the synovial membrane [82].

Pathological anatomy

In patients with PsA, the development of proliferation of synoviocytes and of hypertrophy of the villi of the synovial membrane, as well as an inflammatory infiltrate of T lymphocytes and plasma cells secreting IgA and IgG predominantly perivascular with consequent thickening of the arteriolar wall involved, have been observed at the articular level. The joint inflammation causes the appearance of cartilaginous and bone erosion with subsequent fibrotic repair and the development, in time progress, of reactive bone with the presence of the classic syndesmophytes. Unlike RA, however, there is not the presence of synovial pannus. As for entesis both axial and peripheral insertions can be involved [85].

Clinical manifestations

As described by Moll and Whright in 1973, PsA can clinically manifest with five different types of joint involvement: classic PsA, asymmetric oligoarticular PsA, symmetric polyarticular PsA, ankylosing PsA and mutilating PsA [2].

Classic PsA This phenotype affects about 5-10% of patients with PsA and is characterized by an exclusive involvement of the distal interfalangeal joints (DIP), often associated with psoriatic onychopathy.

Asymmetric oligoarticular PsA This is the most frequent phenotype (about 60-70% of patients with PsA) and is characterized by an involvement of up to 4 joints asymmetrically, mainly those of the hands, but also knees and ankles. Particularly involved are the distal interphalangeal joints (DIP) and metacarpophalangee (MCP), while more rarely the proximal ones (PIP).

Moreover, dactylitis is also characteristic of this phenotype, which is the development of swelling of an entire finger due to the tenosynovial involvement of the soft tissue.

Symmetric polyarticular PsA This phenotype affects about 15-20% of PsA patients and is also known as RA-like although there are marked differences with RA such as predominantly DIP involvement, possible axial involvement and a lower tendency to evolution. Nevertheless, the differential diagnosis between these two conditions may be difficult, especially if the RF is positive.

Ankylosing PsA This phenotype is characterized by a clinical presentation with predominant axial involvement and a higher frequency of HLA-B27 positive. However, unlike AS, axial involvement is less extensive, sacroiliitis is unilateral and vertebral calcifications are coarser and known as pseudosyndesmophytes.

Mutilating PsA This phenotype of PsA is the rarest and is characterized by a marked bone erosion of the distal phalanges, which gives the appearance of "telescope-like fingers".

This classification is still widely used in clinical practice, however it is limiting as it does not fully describe the much wider heterogenicity of the clinical spectrum of PsA and does not consider the evolution that the disease may undergo during its course [86]. For this reason, in 2006, the CASPAR group published its classification criteria for PsA, which today represent the standard for the diagnosis of PsA: a diagnosis should only be made if the patient has evidence of an inflammatory joint disease established and at least 3 points from the characteristics described in Table IX [87].

Table IX: CASPAR criteria (classification criteria for psoriatic arthritis)

A patient must have inflammatory articular disease (joint, spine or entheseal) and ≥3 points from the following categories:	**Score**
1. Evidence of current psoriasis.	2
2. Evidence of personal or family history of psoriasis.	1
3. Typical psoriatic nail dystrophy including onycholysis, pitting, hyperkeratosis observed on current physical examination.	1
4. A negative test result for the presence of rheumatoid factor by any method except latex but preferably by enzyme-linked immunosorbent assay or nephelometry, according to the local laboratory reference range.	1
5. Either current dactylitis, defined as swelling of an entire digit, or a history of dactylitis recorded by a rheumatologist.	1
6. Radiographic evidence of juxta-articular new bone formation, appearing as ill-defined ossification near joint margins (but excluding osteophyte formation) on plain radiographs of the hand or foot.	1

Diagnosis

Laboratory Laboratory tests are not specific: ESR and CRP are used to monitor the course of inflammation, however, especially in oligoarticular forms, often the inflammatory markers are lower than expected by the clinical characteristics of the patient. Synovial liquid is markedly inflammatory but without any particular feature. In addition, genetic characteristics are less indicative than other forms of SpA [1, 88].

Imaging The radiographic picture of PsA is highly variable and strongly depends on the clinical phenotype of the disease. Radiological damage was observed in at least a quarter of early PsA patients and the presence of joint damage on radiography has been proved to be an independent predictor of a very aggressive disease [89, 90]. Two different patterns have been described for PsA: "row pattern" in which there is a mainly involvement of the DIP joints and sparing of the MCP joints; "ray pattern", in which all three joints of the finger are affected, and potentially also the wrist [91]. The "ray pattern" and the asymmetric joint involvement are useful to distinguish PsA from RA [92].

A typical but not specific finding in PsA, is "Pencil in Cup Deformity", a term referring to bone erosion in which narrowed end of metacarpal or phalanges (pencil) rests in the expanded end of the adjacent bone sharing the joint (cup). Other typical radiological changes include lysis of the terminal phalanges (acro-osteolysis), fluffy periostitis, as well as new bone formation at the site of enthesitis (especially in the plantar fascia and in the Achilles tendon), gross destruction of isolated joints and the occurrence of both joint lysis and ankylosis in the same patient [93].

In case of axial involvement, sacroiliitis is often unilateral and asymmetric, and pseudosyndesmophytes are usually para-marginal and do not extend from one vertebra to another as in AS [44, 94].

Although X-ray is one of the first-line diagnostic methods in most cases, MR is of paramount importance in young patients and those with early disease. It is highly sensitive in detecting all peripheral and axial joints involved in PsA, in order to assess in detail inflammation and structural damage and it is also fundamental in the evaluation of enthesitic involvement, which precede the bone involvement observable on radiography. Nevertheless signs of inflammation such as synovitis, tenosynovitis and bone marrow edema, are not specific for PsA.

In addition, US is also an excellent diagnostic tool for identifying the presence of synovial hypertrophy, joint capsule distension, bone erosion and enthesitis; power doppler may also be useful in the assessment of inflammation by finding increased vascularization. In peripheral PsA, US is more sensitive than X-ray, scintigraphy or MR, even in combination with clinical examination in PsA patients for the detection of joint involvement, both intraarticular (synovitis and erosion) and extra-articular, including bursitis, tenosynovitis and enthesitis [95–97].

Clinimetric evaluation

Aspecific indices Many non-specific indices, already treated for AS, are also used for PsA, such as VAS, PGA and LEI. In addition, other indices can be chosen on the basis of the considered phenotype of PsA: specific indices for AS, such as BASDAI, BASFI, BASMI, MASES and ASDAS-CRP in case of prevailing axial involvement, or specific indices for RA such as Disease Activity Score-28 for Rheumatoid Arthritis with CRP (DAS28-CRP), Clinical Disease Activity Index (CDAI) and Simple Disease Activity Index (SDAI), which will be described in detail during the discussion of the RA, in case of prevailing peripheral involvement, can be used. In addition, the assessment of the patients disability by HAQ is also useful in this case.

Specific indices However, there are also specific disease evaluation indices for PsA: 1) Psoriasis Area and Severity Index (PASI): assesses the severity of cutaneous involvement of psoriasis dividing the whole body into four parts (head, trunk, upper and lower limbs), and for each section the extent (calculated as percentage of skin affected by psoriasis) and severity (according to three specific parameters: erythema, infiltration and desquamation) of psoriasis is assessed; each of severity parameters can be defined as absent (0), mild (1), moderate (2), severe (3) and very serious (4), and the total score is the sum of the 3 scores for each zone [98]. 2) Disease Activity index for PSoriatic Arthritis (DAPSA): evaluates disease activity based on the severity of joint involvement (number of tender and swollen joints), CRP, patient VAS pain and PGA; numerical values for the five domains are summed to classify the disease activity as remission (0-4), low disease activity (5-14), moderate disease activity (15-28), high disease activity (>28) [99]. 3) Minimal Disease Activity (MDA): evaluates disease activity based on joint (number of tender and swollen joints), skin [body surface area (BSA) and PASI] and enthesitic involvement, HAQ, patient VAS pain and PGA; minimal disease activity is defined if the patient has 5 out of 7 of the following criteria: Tender Joints Count out of 68 (TJC-68) <1, Swollen Joints Count out of 66 (SJC-66) <1, PASI $\leq$1 or BSA $\leq$3, patient VAS pain $\leq$15, PGA $\leq$20, HAQ $\leq$0.5, and painful enthesitic points $\leq$1 [100].

Treatment

Pharmacological treatment As well in other SpA, also in PsA, drugs used are divided into NSAIDs, corticosteroids, csDMARDs and bDMARDs; what changes is the mode of their use.

NSAIDs are widely used in PsA and are sometimes sufficient on their own to control the disease at onset; anti-COX2 are generally preferred, since their low gastrointestinal toxicity. NSAIDs have proven to be effective in reducing inflammatory markers and patient VAS pain/PGA but are not effective on cutaneous psoriasis.

Both intraarticular and oral corticosteroids are useful in the most severe forms and in the treatment of exacerbations. Also they have shown to prevent the formation of bone erosion, but dosage should not exceed 10 mg/day of predinosolone or equivalent.

The most resistant forms, where it is necessary to use steroidal drugs continuously, require csDMARDs: the most suitable are SSZ, leflunomide and, above all, MTX. These drugs, which can also be used in combination, are also

able to act on cutaneous psoriasis. It should be noted that all these csDMARDs are effective on the phenotypes of PsA that affect peripheral joints, but are less useful in axial and enthesitic forms. The dosages of these drugs in PsA are the same as RA and are described in Table XIII

In patients refractory to traditional therapies, new biological drugs can be used: the most used are anti-TNF-α although, as already mentioned talking about the pathogenesis of the disease, also anti-IL-12/23 and anti-IL-17 have their rational use and has been proven to be very effective on both joint disease and cutaneous psoriasis, especially in patients not responsive to anti-TNF-α.

All anti-TNF-α drugs, (etanercept, adalimumab, infliximab, golimumab and certolizumab pegol) have been approved for PsA and have proven to be an effective therapy for all disease phenotypes of PsA and are recommended by the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) [101] and the EUuropean League Against Rheumatism (EULAR) [102], while only the first three have been approved also for cutaneous psoriasis. There is good evidence that anti-TNF-α can slow the radiographic progression in peripheral PsA both in naive patients and in patients refractory to csDMARDs [103] despite they have not proved useful in delaying the bone neoapposition. There is no evidence that the association of csDMARDs with anti-TNF-α can lead to additional benefits compared to monotherapy.

As seen in AS, also in PsA, anti-IL-17A biological drugs can be used, such as Secukinumab and Ixekizumab, whose characteristics have already been described, talking about the AS, in Table VII and remain valid in the same way for the PsA. With the latest findings about the pathogenesis of PsA, biological drugs directed against IL-12 and IL-23 have been developed, due to the greater importance of the Th17 response in the pathogenesis of this disease. Ustekinumab, an inhibitor of the p40 subunit shared by IL-12 and IL-23, was until recently the only monoclonal antibody of this group available in Italy. Recently, however, Guselkumab and Risankizumab, monoclonal antibodies targeting the p19 subunit of IL-23 but with no influence on IL-12, were also approved for both psoriatic arthritis and cutaneous psoriasis [104–106]. All these monoclonal antibodies, whose specific characteristics are extended in Table X and in Table XI, have been shown to be effective in the clinical improvement of the patient in the short term by acting both on the joint component and on enthesitis and dactylitis; in addition, they have been shown to be effective in slowing long-term radiographic progression. These goals have been achieved in naive patients but especially in patients not responsive to a previous therapy with anti-TNF-α [103].

Table X: Anti-IL-12/23 p40 monoclonal antibodies

Anti-IL-12/23 p40	**Biological features**	**Dosage**
Ustekinumab	Human monoclonal antibody	Administered subcutaneously at a loading dose of 45 mg per week 0 and week 4, followed by a maintenance dose of 45 mg every 12 weeks.

Table XI: Anti-IL-23 p19 monoclonal antibodies

Anti-IL-23 p19	**Biological features**	**Dosage**
Guselkumab	Human monoclonal antibody	Administered subcutaneously at a loading dose of 100 mg per week 0 and week 4, followed by a maintenance dose of 100 mg every 8 weeks.
Risankizumab	Humanized monoclonal antibody	Administered subcutaneously at a loading dose of 150 mg per week 0 and week 4, followed by a maintenance dose of 45 mg every 12 weeks.

However, a recent systematic review revealed that minimal disease activity is attained only in up to 17% of patients receiving csDMARDs and in up to only 57% of those receiving bDMARDs [107].

Apremilast is a drug that targets phosphodiesterase 4 (PDE4), recently approved in Italy for PsA and administered orally 30 mg twice daily. PDE4 is a PDE specific for cyclic adenosine monophosphate (cAMP) and is the main PDE in inflammatory cells. The inhibition of PDE4 increases intracellular cAMP levels, which in turn causes a downregulation of the inflammatory response by modulating the expression of TNF-α, IL-23, IL-17 and other inflammatory cytokines. Cyclic AMP also modulates levels of anti-inflammatory cytokines, such as IL-10. The efficacy of apremilast seems lower than monoclonal antibodies, particularly for higher levels of response, but its favourable safety profile can be an advantage in practice, especially in older patients and those with previous cancer [108].

Since the JAK-STAT signalling pathway can be activated by many important proinflammatory cytokines involved in the pathogenesis of PsA, such as IL-12/23 and IL-17, targeting the JAK-STAT pathway has a biological basis and has been

the subject of intense research in PsA [103]. Currently two JAK inhibitors have been approved for PsA: upadacinitb and tofacinitib. Both have proved useful in the clinical improvement of patients not responsive to previous therapeutic lines. The specific characteristics of JAK inhibitors have already been reported in Table VIII.

The choice of the first drug to begin treatment in patients with PsA, whether directly with a bDMARDs or a tsDMARDs or whether to reserve them for patients refractory to NSAIDs and csDMARDs, is still under discussion. Also, the same choice of the specific drug should be made on the basis of the patients medical history and comorbidities, disease activity and disease phenotype.

Non-pharmacological treatment The ACR guidelines recommend low intensity exercise, weight loss in overweight patients, massages, thermal baths and acupuncture: these are recommended despite the evidence of their effectiveness is limited specifically in PsA, with the exception of weight loss for which there are numerous data in scientific literature. Moreover, strong evidence of efficacy also exists for the cessation of the tobacco habit since, in addition to worsening the underlying inflammation of the disease and consequent clinical picture, it appears to be directly correlated with a lower efficacy of pharmacological treatment [109].

Prognosis

In most cases, PsA has a good prognosis, better than RA since it generally affects fewer joints. However, about 20% of patients with PsA have destructive or RA-like arthritis and in these cases the quality of life and disability can be particularly severe. For this reason the goal of therapy is to keep the disease in remission. Negative prognostic factors are: polyarticular involvement, high levels of CRP at the onset, positive anti-citrulline antibodies, presence of erosion in radiography [110]. In addition, these patients have a higher incidence of cardiovascular events, with a higher rate of mortality and morbidity than the general population, which therefore adversely affect the prognosis of the disease [111].

1.1.4 Rheumatoid arthritis

Definition

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune joint disease that affects the diarthrodial (or synovial) joints but also associated with systemic involvement quite relevant in the case of long-term disease. It can be defined as "persistent" instead of "chronic" as it can occur, although rarely, a lasting remission of the disease. The term "rheumatoid arthritis" was coined in 1859 by the British rheumatologist Alfred Baring Garrod [1].

Epidemiology

RA is the most common systemic inflammatory rheumatic disease since it affects an average of 0.5 to 1% of adults in the developed world, with an incidence between 5 and 50 individuals in 100,000 [112]. Some populations show higher prevalence such as some groups of native americans who have a prevalence rates about 5-6%, while other populations, such as those from the caribbean region, have lower than average prevalence rate. There is also an apparent decline of incidence from north to south and from urban to rural areas. More commonly, RA affects females aged between 40-50, while males tend to develop the disease more rarely and a little later [113]. However, there are also cases of juvenile RA onset in the form of juvenile idiopathic arthritis (JIA) and cases with senile onset, both with different clinical characteristics compared to the classical form.

Ethiopathogenesis

The pathogenesis of RA is multifactorial, due to the concomitant presence of a predisposing genetics, environmental and immunological factors. All these factors collaborate in determining the citrullination of arginine residues of peptides expressed in the synovial membrane, against which anti-Cyclic Citrullinated Peptide antibodies (aCCP) are produced. Such antibodies are very specific for RA although the positivity of RF, IgM directed against the Fc portion of the IgG and whose presence is a useful indicator of inflammation and autoimmune activity, is very sensitive.

Genetic predisposition Genetics is a very important aspect in the pathogenesis of RA: several studies have shown a concordance in monozygotic

twins of 15-40%, in heterozygous twins of 3.5-6%, in first-degree relatives of 1-2%, although there remains a 60% discordance in monozygotic twins [114].

The most important genetic associations are with several alleles belonging to HLA-DR1 and HLA-DR4 that share a highly preserved genetic region, for this reason known as "shared epitope" [115].

However, other predisposing non-HLA genes polymorphism have been identified, coding for proteins that regulate the immune system. First, PTPN22 gene polymorphism has proven to be associated with RA; it encodes a tyrosine phosphatase involved in antigen receptor signaling of B and T cells [116]. Another gene is CTLA4, encoding a peptide exposed on the surface of the T cells to inhibit co-stimulation and avoid an over-activation of lymphocytes during the immune response [117]. Another gene to mention is PADI4, encoding for the enzyme responsible for arginine citrullination [118]; this enzyme is expressed at the level of the joint synovial membrane and in neutrophils, eosinophils and macrophages while it is absent in lymphocytes and monocytes. Other loci and genes involved in inflammatory pathways that are involved in RA with a modest effect include STAT4, TRAF1-C5, CD40, IL-2/21, IL-2RA/IL-2RB, IL-6R, CCL21 and RNASET2 [119–125]. Importantly, besides disease susceptibility, some of the non-HLA genes have also been associated with severity and differences in seropositive and seronegative RA [126, 127].

The quantification of the genetic contribution has proved to be 50-65% [128]; interestingly, it has been recently shown to be higher in aCCP-positive RA (50%), compared to aCCP-negative disease (20%) [129].

Environmental factors In a genetically predisposed subject, several environmental factors act through epigenetic post-translational modifications by inducing the citrullination of some peptides. Among these, the main ones are: cigarette smoke, Porphyromonas gingivalis and Prevotella copri [130–132]. They act on the mucous membranes, respectively bronchial, oral and intestinal, at which level stimulate the citrullination of peptides. Other environmental risk factors associated with aCCP production appear to be intestinal dysbiosis, obesity and hormonal factors [130, 133, 134].

Immunological factors The citrullinated peptides formed under the probable pressure of environmental factors are transported by dendritic cells to the satellite lymph nodes, where the citrullinated antigens are exposed to T-cells which, by not recognizing them as self, initiate an autoimmune response against such peptides

forming aCCP, present on average already ten years before the clinical onset of the disease. The synovial membrane exposes such citrullinated peptides and the circulating antibodies can bind them at this level, form the immunocomplexes that activate the complement system and amplify the inflammatory process against the synovium mediated mainly by IL-1, IL-6 and TNF-α, which are therefore the main therapeutic targets in RA. At the level of the synovial membrane there may be the recruitment of lymphocytes T and B, the formation of lymphatic nodules with germinal centers, the local production of aCCP, RF and pro-inflammatory cytokines with a progressive amplification of the inflammatory process. The transition from this phase of "asymptomatic autoimmunity" to the clinical onset of the disease is still not quite clear. One of the main predisposing factors of this passage seems to be represented by the articular overload that induces a subclinical inflammation of the synovial membrane that, however, in the predisposed individual, can lead to apoptosis of the synoviocytes. The last step is the activation of osteoclasts, responsible for joint and bone damage.

In addition, the risk of RA is also increased by 1.53 fold in patients with other immune-mediated inflammatory diseases, such as systemic lupus erythematosus (SLE), Sjögren syndrome, AS or Hashimoto thyroiditis [135].

Pathological anatomy

The synovial membrane undergoes hyperplasia and hypertrophy, thickens (normally it consists of 2 or at most 3 layers of cells that in RA become 7 or more) and develops many villous folds. In this way, the "synovial pannus" is formed and begins to erode the bone not covered by cartilage ("bare bone"). At the same time the granulocytes move into the synovial fluid and T cells, B cells and plasma cells at the synovial membrane level form a nodal-like tissue. Synovial pannus cells take on a neoplastic-like appearance, meaning they are not affected by contact inhibition. Fibrin deposits, fibrosis and necrosis are also present. About 30% of patients with RA develop localized rheumatoid nodules predominantly of the skin and subcutaneous tissue, especially of the areas subjected to friction (elbows, sacrum, extensory surface of the limbs, occiput, Achilles tendon) but also of organs like lungs. These nodules are granulomas formed by a central fibrinoid necrosis area surrounded by palisade of macrophages, all wrapped by lymphocytes, plasma cells and fibroblasts. They can be single or multiple, of various sizes (from a few millimeters up to several centimeters in diameter), superficial and mobile or deep and adherent to periosteum, tendons and synovial bursae.

Clinical manifestations

The onset of RA is generally insidious. The disease progresses more rapidly during the first 6 years, particularly in the first year.

Articular involvement The joint involvement is usually polyarticular, symmetrical, centripetal (begins from small peripheral joints, such as those of the hands and feet and propagates to large joints, passing through wrists, ankles, elbows), with additional character (can always hit new joints, but those already hit remain affected). The affected joint sites are almost all of the diarthrodial (or synovial) joints: the most frequently affected are those of the hands (except the DIP that are spared not being diarthrodial joints) but can also be affected the temporo-mandibular joints and C1-C2. Lumbar spine involvement is not characteristic of RA, but inflammation of the cervical spine (C1-C2) may cause erosion of the tooth of the axis, resulting in subluxation and then compression of the spinal cord.

It is possible, although rare, that RA begins with unconventional clinical pictures such as, for example, palindromic onset characterized by periods of remission and exacerbation of symptomatology until the clinical picture is definitively established. Another example is the polymyalgia-like onset with involvement exclusively on the scapular and pelvic girdles. Finally, rarely there may be an onset with monoarthritis of large joint.

The symptoms that characterize RA are: 1) Inflammatory joint pain, which occurs at rest, often at night, with a peak of maximum intensity after prolonged inactivity, attenuates with moderate physical activity while worsens with overload. 2) Very prolonged post-inactivity stiffness ($>$ 60 minutes), usually manifested in the morning. 3) At the level of the hands and feet there is generally a positive squeeze test: a slight compression is exercised at the level of the metatarsal or of the metacarpal and if this causes pain the test is positive. 4) Systemic symptoms such as asthenia, fever, weight loss. The joints involved show signs of joint inflammation (rubor, tumor, dolor, calor and functio laesa).

The course, as already mentioned, is chronic; evolution, if the disease is not recognized and cured, leads to deformities and joint ankylosis. About 80% of patients develop some permanent joint abnormality within 10 years. Typical joint deformities of the hands that can be observed are: 1) Ulnar deviation of the fingers with sliding of the extensor tendons. 2) "Swan neck" deformity: characterized by extension of the PIP and flexion of the DIP. 3) "Boutonniere" deformity: characterized by flexion of the PIP and extension of the DIP. 4) Hitchhikers thumb

(or "Z-shaped deformity of the thumb"): flexion of the MCP and extension of the IP.

In addition, deformities can also be observed at the joints of the feet: 1) Valgism of the big toe. 2) Hammertoe: the toe is bent at the middle joint, so that it resembles a hammer. 3) Mallet toe: the toe is bent in the joint nearest the toenail. 4) "Curly toe": flexion and adduction of the IP joints. 5) Flatfoot: caused by the collapse of the arch.

In addition, the patient may also develop carpal tunnel syndrome, due to synovitis of the wrist with compression of the median nerve, and Bakers popliteal cyst, with swelling and tenderness of the calf.

Not to forget is also that the senile and juvenile forms of RA tend to have particular clinical and laboratory characteristics: the juvenile form (JIA) often presents with a mono-oligoarthritis with positivity for anti-nucleus antibodies (ANA); on the contrary, in the senile form (after the age of 60) there is more frequently a negative RF and a clinical pictures with palindromic onset [1].

Extra-articular involvement Especially in patients with severe and long-term disease there may be also an extra-articular involvement. The most frequent of these manifestations is the development of rheumatoid nodules which, as already mentioned, are granulomatous lesions localized mainly in the skin and subcutaneous tissues but also in the lungs, where their presence could be configured as Caplan syndrome consisting of multiple well-defined nodules predominantly at the lung periphery [136].

In addition, these patients may also develop rheumatoid vasculitis of small vessels that predominantly affects the skin with the appearance of purpuric lesions (ulcers, digital necrosis and gangrene may also appear in cases of involvement of small vessels but of slightly larger diameter), but may also involve the peripheral nervous system with development of multiple mononeuritis, lungs with pulmonary alveolitis or even the kidneys [137]. Rheumatoid vasculitis is always associated with a severe clinical picture of RA and in most cases with a high-titer positivity of RF and aCCP, HLA-DRB1 positive genetics and rheumatoid nodules [138].

Finally, these patients may also develop serositis, as a manifestation of rheumatoid immunoflogosis, particularly pleuritis or pericarditis. More rare extra-articular manifestations are obliterant bronchiolitis, interstitial pneumonia, myocarditis, lymphadenopathy, scleromalacia and episcleritis [139–141].

Complications In patients with severe or long-lasting disease there is also a higher risk of complications, which cannot be classified as "extra-articular involvement" as their pathogenesis is due to the persistence of the inflammatory state of the RA not adequately controlled: 1) Amyloidosis: due to the extracellular deposit of Serum amyloid A (SAA) in particular in the kidneys. 2) Osteoporosis: partly caused by disuse and partly by chronic inflammation. 3) Early atherosclerosis: uncontrolled systemic inflammation stimulates the progressive growth of plaque with increased risk of rupture and, therefore, development of a cardiovascular event. In this regard, a study by John Hopkins University compared patients with RA who died from myocardial infarction with patients not suffering from RA, showing that patients with RA developed more vulnerable plaques and therefore at greater risk of complications. In fact, cardiovascular disease is still the main cause of death in patients with RA. This phenomenon is not specific for RA, but also affects SLE and vasculitis [142, 143].

Among the complications, Felty syndrome must be mentioned, considered a particularly severe clinical variant of RA, characterized by severe joint involvement associated with splenomegaly and neutropenia. There is also a severe extra-articular involvement and other complications of RA already discussed [144].

Finally, not to be underestimated is the neoplastic risk to which these patients are exposed: the greatest risk is certainly that of lung cancer, since smoking is a common risk factor for both diseases. However, the risk is also increased for other types of neoplasms such as Hodgkins lymphomas and non-Hodgkins lymphomas due to the condition of systemic inflammation. However, some studies suggest that patients with RA have a lower risk than the general population of colorectal and breast cancer [145, 146].

Diagnosis

No diagnostic criteria exist for RA and, unfortunately, there is no pathognomonic findings. RA should be suspected in patients with a compatible clinical presentation as described above, in the presence of acute-phase reactants such as an increase in ESR and CRP, the biomarkers RF and aCCP positive, and a characteristic radiological picture. Other causes of arthritis need to be considered, such as reactive arthritis, osteoarthritis, septic arthritis, connective tissue diseases [147].

In 2010, the ACR and the EULAR published the latest version of the classification criteria, which, although designed to make the case studies uniform, can also be useful to direct the diagnosis (Table XII) [148].

Table XII: The 2010 ACR/EULAR classification criteria for RA [148]

	Score
Target population (Who should be tested?): patient who	
1) have at least 1 joint with definite clinical synovitis (swelling)	
2) with the synovitis no better explained by another disease	
Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of ≥6 is needed for classification of a patient as having definite RA)	
A) Joint involvement	
- 1 large joint	0
- 2-10 large joints	1
- 1-3 small joints (with or without involvement of large joints)	2
- 4-10 small joints (with or without involvement of large joints)	3
- >10 joints (at least 1 small joint)	5
B) Serology (at least 1 test result is needed for classification)	
- Negative RF and negative aCCP	0
- Low-positive RF or low-positive aCCP	2
- High-positive RF or high-positive aCCP	3
C) Acute-phase reactans (at least 1 test result is needed for classification)	
- Normal CRP and normal ESR	0
- Abnormal CRP or abnormal ESR	1
D) Duration of symptoms	
- <6 weeks	0
- ≥6 weeks	1

These criteria have been developed to solve shortcomings of the former criteria, established by the American Rheumatism Association in 1987 [149, 150]. The new criteria classified a patient as suffering from RA in presence of at least one clinically swollen joint (synovitis) in absence of other diseases that explain such clinical features. Subsequently, these criteria allow a classification of patients according to the degree of joint involvement, the duration of symptoms, the titre of RF and aCCP antibodies and the values of ESR and CRP. Applications of these criteria provides a score of 0-10 and a patients with a score ≥6 can be classified as suffering from RA. Since publication, the criteria have been validated in many contexts and offer a sensitivity of 21% higher than the criteria of 1987, at the cost of a specificity less than 16%.

To allow early diagnosis, some red flags were identified: 1) Swelling of at least three joints, with major involvement of MCP and metatarsophalangeal (MTP) joints. 2) Positive squeeze test. 3) Morning stiffness $\geq$30 minutes 4) Positive response to NSAIDs [151].

Laboratory Normochromic (or slightly hypochromic) normocytic anemia appears in 80% of patients. Acute-phase reactants (ESR, CRP) have gained greater interest as their values reflect disease activity. Mild polyclonal hypergammaglobulinemia often appears [152].

RF is positive in about 70% of patients with RA. However, RF is not very specific as it may be present, although with lower titers, even in other inflammatory contexts, including connective tissue diseases, granulomatous disease (e.g. tuberculosis), chronic infections (e.g. viral hepatitis, bacterial endocarditis) and cancer. A low RF titre may also be present in 3% of the general population and 20% of the elderly [153].

ACCPs have a high specificity (90%) and sensitivity (about 77-86%) for RA and, such as RF, correlate with a worse prognosis [154].

In addition, antibodies against anticarbamilate protein (anti-CarP) may be useful for diagnosis in patients with aCCP negative where, in addition, predict increased radiological progression [155, 156].

Examination of synovial fluid is necessary in any newly arising joint effusion, to exclude other pathologies and differentiate RA from other inflammatory arthritis (e.g. septic and crystal-related arthritis). In RA, during active joint inflammation, synovial fluid is cloudy, yellow, sterile, and generally has white blood cell count of 10.000 to 50.000 μL; typically polymorphonuclear leukocyte predominate, but >50% of cells may consist of lymphocytes and other mononucleate cells. The crystals are absent.

Imaging The arthritis, on radiography, has erosive character that is characterized by an interruption of the cortical surface of the bone often accompanied by a loss of substance of the trabecular bone below [157].

Radiography still plays the main diagnostic role although it is not useful in early diagnosis. In the initial phase of the disease the radiography shows the presence of very suggestive elements of RA such as iuxta-articular osteoporosis, erosion in the bare areas and reduction of the joint space; swelling of soft tissues can also be found. In the late phase, on the other hand, the X-ray shows and extended subcondral erosion with formation of pseudocysts and geodes, more

widespread iuxta-articular osteoporosis, disappearance of the joint space and joint subluxations and dislocations [158].

The MR, on the other hand, is a second-level examination in RA despite being much more sensitive in detecting bone erosion earlier than radiography [159].

The US can also be useful in visualizing bone erosion. However, the significance of erosion in the US is different from that in the radiography because US-demonstrated erosion can undergo a "restitutio ad integrum". The US, moreover, allows to detect, through the Power Doppler (PD), signs of inflammation at the level of the joint or tendons [160].

Clinimetric evaluation

Assessment of the disease activity is critical for monitoring the effectiveness of therapy in RA patients after diagnosis. Some of these indices are specific for RA, others are also used for the evaluation of other inflammatory arthritis.

Aspecific indices Some evaluation indices already treated for AS and PsA, although not specific for RA, can be used, such as VAS, PGA and even HAQ for the assessment of the impact of the disease on daily functions.

Specific indices More specific disease evaluation indices for RA are: 1) Disease Activity Score-28 for Rheumatoid Arthritis with CRP (DAS28-CRP): assesses disease activity by a score calculated by an algorithm that exploits the number of tender and/or swelling joints on a count to 28 joints (shoulders, elbows, wrists, knees, MCP, PIP), CRP and PGA; the final score allows to classify disease activity as disease remission (<2.6), low disease activity (≥ 2.6 and <3.2), moderate disease activity (≥ 3.2 and ≤ 5.1) and high activity disease (>5.1) [161–163]. 2) Clinical Disease Activity Index (CDAI): assesses disease activity using only clinical data that is the number of tender and/or swelling joints on a count to 28 joints, PGA and provider global assessment; the sum of scores allows to classify disease activity as remission (≤ 2.8), low disease activity (>2.8 and ≤ 10), moderate disease activity (>10 and ≤ 22) and high activity disease (>22) [164]. 3) Simple Disease Activity Index (SDAI): assesses disease activity using only clinical data that is the number of tender and/or swelling joints on a count to 28 joints, PGA, provider global assessment and CRP; the sum of scores allows to classify disease activity as remission (≤ 3.3), low disease activity (>3.3 and ≤ 11), moderate disease activity (>11 and ≤ 26) and high activity disease (>26) [165].

Such scores allow to evaluate the achievement of the state of remission or low disease activity (LDA) that have been established as treatment targets. The ACR and the EULAR have recently developed new remission criteria using SDAI and CDAI criteria [166, 167].

Treatment

Since joint damage, clinical manifestations of the disease and consequently also the effects on the patients quality of life are the direct consequence of inflammation, the main therapeutic goal is to reduce it. The treatment of RA therefore requires, like other inflammatory arthritis, to monitor constantly the activity of the disease and to modify the therapy accordingly. If pharmacological treatment does not lead to an improvement of clinimetric indices (CDAI and SDAI as described in the aforementioned EULAR and ACR guidelines) treatment should be amended. In addition to pharmacological treatment, non-pharmacological interventions also play a key role in improving the patients quality of life, functional capabilities, psychological and social status. These two aspects of therapy intersect in a wearable way in the proper management of the patient with RA [168].

Pharmacological treatment CsDMARDs reduce inflammation and, by definition, reduce the progression of structural damage. According to the EULAR recommendations for managing RA [169], treatment should be started with MTX for csDMARDs-naive patients with moderate-high disease activity; long-term glucocorticoids association (>3 months) in these patients is not recommended while a short-term association (<3 months) is conditionally recommended since, often, a course of corticosteroids may be necessary to alleviate symptoms prior to the onset of action of csDMARDs [170]. The association of MTX with glucocorticoids has been found to be equally effective but with less toxicity than the association of MTX and bDMARDs or MTX and other csDMARDs [171–175]. In addition, it has been found that the combination of low doses of glucocorticoids provides greater structural protection than MTX alone [176–178].

MTX is considered the anchor drug [179] that also optimizes efficacy of bDMARDs despite it has not yet been clearly demonstrated that is superior to other csDMARDs clinically or structurally, since comparisons with SSZ or leflunomide showed similar outcomes, but the MTX dosage was lower than the one usually used today. However in patients with contraindications to MTX, therapy can be started with leflunomide or SSZ always in association with a short course of glucocorticoids.

In csDMARDs-naive patients with low disease activity, HCQ is conditionally recommended over other csDMARDs due to minor side effects, SSZ is conditionally recommended over MTX since less immunosuppressive, MTX is conditionally recommended over leflunomide because of its greater dosing flexibility and the lower cost. These indications are conditional since MTX should always be the first choice if the patient has negative prognostic factors.

In patients who have been treated with csDMARDs but not with MTX and who despite the therapy still have moderate-high disease activity the indication is conditionally in favor of starting therapy anyway with MTX in monotherapy, although the guidelines specify how in some cases the association of MTX and a bDMARDs or tsDMARDs may be necessary to obtain a more rapid response.

Oral MTX is recommended over subcutaneous a the beginning, due to the patient increased compliance and similar bioavailability to subcutaneous administration at typical starting dose [180], at a dose of at least 15 mg weekly or otherwise reaching that dose within the first 4 to 6 weeks [181]. This recommendation is conditional since there are few studies comparing different dosage regimens and the wide variability of clinicians and patients preferences in finding the tradeoff between benefits and risks associated with higher starting doses. This recommendation refers only to the initial prescription and should no limit any further dose escalation to provide higher efficacy. If the patient does not tolerate oral weekly MTX, the recommendation is to split the dosage over 24 hours, to switch to a subcutaneous administration and/or to increase the dosage of folic/folinic acid instead of switching to another DMARDs; the recommendation is conditional since, in any case, patient preferences plays a key role in this decision. If, on the other hand, the patient does not reach the target of disease activity, the recommendation is to switch to subcutaneous administration instead of adding or switching to another csDMARDs; again patient preferences plays a key role and therefore it is a conditional recommendation. The pharmacological characteristics and the dosages of all csDMARDs used are shown in Table XIII.

If the patient has an improvement in disease activity at 3 months and reaches the target at 6 months, the therapy started is maintained by reducing the dosage if remission is maintained over time. If, however, the patient does not reach these targets, the first-line therapy is considered failed [182].

Table XIII: csDMARDs

csDMARD	Biological features	Dosage
Methotrexate	Antimetabolite and folic acid analogue that interferes with cell replication, synthesis and repair of DNA by several mechanisms of which the main one is the inhibition of dihydrofolate reductase.	Administered orally or subcutaneously at a minimum dose of 7.5 mg and up to a maximum of 20 mg per week. The day after taking MTX the patient should take folic acid to compensate the depletion caused by MTX and mitigate its side effects especially in the liver.
Sulfasalazine	Molecule obtained by the fusion of an antibiotic belonging to the category of sulfonamides (sulfapyridine) and a NSAID (salicylic acid): sulfapiridin inhibits the synthesis of folic acid, salicylic acid inhibits cycloxygenase responsible for the synthesis of inflammatory mediators such as prostaglandins, prostaciclines and thromboxanes.	Administered orally in 500 mg tablets with a schedule of: 1 tablet per day for the first week, 2 tablets spaced 12 hours apart for the second week, 3 tablets (2+1) spaced 12 hours apart for the third week, 4 tablets (2+2) spaced 12 hours apart for the fourth week and to continue.
Leflunomide	Inhibitor of the enzyme dihydroorotate dehydrogenase resulting in antiproliferative activity.	Administered orally with a loading dose of 100 mg once a day for 3 days, followed by a maintenance dose of 10 to 20 mg once a day depending on the severity of the disease.
Hydroxychloroquine	Antimalarial belonging to the 4-aminoquinoline family.	Administered orally in 200 mg tablets with a dose of 400 to 600 mg per day; when a good therapeutic response is obtained, usually between 4 to 12 weeks, the dose can be halved.

When the first treatment line fails, the EULAR recommends to stratify for predictors of severe disease such as: 1) Presence of high disease activity despite the therapy; 2) Autoantibodies ACPA or RF at high titres; 3) Early joint damage on radiography; 4) failure of ≥ 2 csDMARDs. If patients have these risk factors, they should receive a bDMARD or a tsDMARD, whereas those without should change to or add another csDMARD again in combination with glucocorticoids.

In patients treated with csDMARDs but no with bDMARDs or tsDMARDs and who are not at target, a treat-to-target (TTT) approach is strongly recommended. Anyway, this recommendation requires prior dose optimization of MTX and only then the addition of DMARDs. This recommendation, however, is conditional for patients who have had an inadequate response to bDMARDs or tsDMARDs because the remaining available treatment options, the impact of non-inflammatory causes of pain, comorbidities and the patient preferences may have a more significant influence on the decision to follow a TTT approach in this population compared to patients who are bDMARD- and tsDMARD-naive. In this patients, an initial target of low disease activity is preferred because remission by established criteria may not be reachable for some patients [183] and because the failure to reach a specified target may be stressful for patients. However, treatment goals should be constantly reviewed over time and individualized to each patient. The recommendation is conditional because remission remain, however, an acceptable initial goal for patients with early disease and minimal exposure to bDMARDs and tsDMARDs, and patient preferences play always a key role.

BDMARDs used in patients with RA include anti-TNF-α, anti-IL-6 receptor, Abatacept (T-cell costimulation inhibitor), Rituximab (anti-CD20), and, in a small proportion of patients, anti-IL-1.

All anti-TNF-α, described in Table VI are currently also approved for RA with the same dosages seen for AS and PsA. All anti-TNF-α can be used in combination with MTX, if the patient tolerates MTX but is not in target of disease activity, or in monotherapy if the patient is not tolerant to MTX and other csDMARDs.

The main antibody directed against IL-6 receptor currently used is tocilizumab; sarilumab, also an antibody directed against IL-6 receptor has recently completed phase 3 trials and is approved for RA. Both can be used in combination with MTX, if the patient tolerates MTX but is not a target of disease activity, or in monotherapy if the patient does not tolerate MTX and other csDMARDs. Tocilizumab and sarilumab as monotherapy are more efficacious than anti-TNF-α and JAKi monotherapy [184]. The pharmacological characteristics and the dosages used are shown in Table XIV.

Table XIV: Ant-Il-6 receptor monoclonal antibodies

Anti-IL-6 receptor	**Biological features**	**Dosage**
Tocilizumab	Humanized monoclonal antibody	Administered intravenously at a dose of 8 mg/Kg (up to a maximum of 800 mg) every 4 weeks, adjusting the dose according to the level of liver enzymes, neutrophil counts and platelet counts.
Sarilumab	Human monoclonal antibody	Administered subcutaneously at a dose of 200 mg every 2 weeks adjusting the dose according to the level of liver enzymes, neutrophil counts and platelet counts.

Abatacept is currently the only inhibitor of T-cell costimulation, mediated by the binding of the CD28 of T-cells and the CD80/86 of antigen-presenting cells (APC), the latter inhibited by Abatacept, but its effect may reflect not only the targeting of T-cells, but also the inhibition of myeloid cell activation and migration.

Rituximab is the only anti-CD20 monoclonal antibody approved for the treatment of RA; CD20 is a B-cells surface antigen (expressed in pre-B-cells and mature B-cells while not found on stem cells and lost before switching from B-cells to plasma cells). Several studies have shown the importance of B-cells in the pathogenesis of RA [185] and especially in the most aggressive forms [186]. In particular, rituximab has been shown to be effective in patients not responsive to anti-TNF-α therapy [187]. The pharmacological characteristics and the dosages used are shown in Table XV and Table XVI.

All bDMARDs have been shown to be effective in reducing disease activity and damage progression in patients with active disease despite MTX therapy, when added to it although, as mentioned above, they are not superior to it when used in monotherapy.

As regards tsDMARDs, however, all those already described in Table VIII are currently approved as second-line therapy in patients not responsive to treatment with csDMARDs with the same dosages already seen for AS and PsA. Also in this case, they can be prescribed in addition to csDMARDs with which the patient is already in therapy or can be used in monotherapy.

If the patient still remain not at target, switching to a bDMARD or tsDMARD of a different class is conditionally recommended over switching to a bDMARD or tsDMARD belonging to the same class but patient and physician preferences play always a key role in the decision.

Table XV: T-cell costimulation inhibitors

T-cell costimulation inhibitor	**Biological features**	**Dosage**
Abatacept	Fusion protein consisting of the extracellular domain of antigen 4 associated with human cytotoxic T lymphocyte (CTLA-4) bound to the modified Fc portion of human immunoglobulin G1 (IgG1).	Administered intravenously by intravenous infusion lasting 30 minutes per weeks 0-2-4 and then every 4 weeks. The dose is 500 mg for patients <60 Kg, 750 mg for patients ≥60 Kg and ≤100 Kg, 1000 mg for patients >100 Kg.

Table XVI: Anti-CD20 monoclonal antibodies

Anti-CD20	**Biological features**	**Dosage**
Rituximab	Chimeric monoclonal antibody IgG1	Administered intravenously with two 1000 mg infusions for weeks 0-2. Further infusions should be assessed 24 weeks after the previous cycle: if the patient has benefited from the infusion it can be repeated after 24 weeks otherwise, if the patient does not show a therapeutic benefit within this time period, should be carefully considered whether to continue therapy.

Once the goal of low disease activity or remission is achieved and maintained for a period of time of at least 6 months, the revaluation of therapy can be taken into account. As for bDMARDs, the risk of a flare of the disease, after halving the dose or lengthening the interval between doses, is low, while the risk is high if the drug is discontinued completely, regardless of the type of biological [188–190].

In these cases patients usually respond very well back to the same agent, however it can not be considered ethical to leave patients at risk of exacerbation and that some of them can not regain the original response [191].

Non-pharmacological treatment The positive effect of moderate physical activity in reducing the overall impact of the disease and improving the quality of life [192], especially in older patients with more active disease [193], has long been well documented [194]. Physical activity is important not only for musculoskeletal symptoms, but also for several comorbidities, such as cardiovascular diseases [195, 196], which are remembered to be the leading cause of death in patients with RA, and psychological comorbidities such as anxiety and depression [197]. In this regard, anxiety and depression seem to be about 2-3 times more frequent in patients with RA than in the healthy population [198, 199] with a bidirectional relationship between joint symptoms and psychological condition [200], therefore several psychological interventions have proven to be useful in these patients such as, for example, education techniques, stress management, basic psychotherapies (such as relaxation techniques, supportive therapy, mindfulness), specific psychotherapies (such as cognitive-behavioral therapy, and hypnotherapy) [201–205].

Balneotherapy has been widely studied and used in musculoskeletal diseases [206]. In addition, several studies on mud pack treatment have been published with positive results [207, 208].

Obesity is a contributing factor to the activity of RA and the beneficial effect of weight loss on disease activity and physical functioning is well known [209–211]. Several diet types and dietary supplements have been studied in the RA, especially the mediterranean diet when combined with exercise has shown a positive effect on quality of life [212, 213]. Vitamin D supplementation is useful in patients with RA, with positive effects both on the activity of the disease, and on comorbidities, for example, osteoporosis [214]. There are also some data on the potential benefit of fish oil supplements and probiotic supplementation [215, 216].

Several other non-pharmacological treatment options have been studied in patients with RA, but with little evidence of efficacy such as acupuncture, occupational therapy, orthosis and assistive devices.

Finally, smoking cessation is surely fundamental especially since its association with a lower response to drug therapy [217] and to prevent the risk of lung cancer. However, data about the effect of smoking cessation on the activity of the disease are poor.

Prognosis

Without adequate therapy, the prognosis is usually poor: within a year, 75% of cases already have the first bone erosion and this has a significant impact on disability. A fundamental aspect is that once the inflammation has managed to induce a damage and therefore a disability, it continues to progress even if the inflammation is extinguished.

RA reduces life expectancy by 3-7 years: the most important cause of death in patients with RA are cardiovascular comorbidities [218], although infections and gastrointestinal bleeding have also been shown to be significant causes of mortality; pharmacological treatments and the onset of cancer may also be responsible [219].

Finally, at least 10% of patients are severely disabled despite adequate treatment. Caucasians and women have a worse prognosis, as well as patients with extra-articular involvement, older age at the onset of the disease, inflammation in >20 joints, early erosion, smoking, high levels of RF, aCCP and ESR.

1.2 SARS-CoV-2

1.2.1 Definition

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a SARS-related virus strain, family Coronavirus, genus Betacoronavirus, subgenus Sarbecovirus, discovered in late 2019; it is the seventh coronavirus recognized to infect humans. Coronavirus Disease-2019 (COVID-19) refers to the virus syndrome. The first known cases involved mainly market workers in Wuhan, China, in December 2019, later, in the first weeks of January 2020, scientists detected strange pneumonia in these subjects caused by this new coronavirus. Coronaviruses were already known to be associated with disease ranging from the common cold to more serious disease like the two previous coronavirus related epidemics, Middle Eastern Respiratory Syndrome (MERS, epidemic in 2012 in Saudi Arabia) and Severe Acute Respiratory Syndrome (SARS, epidemic at the end of 2002 in Guangdong province in China) [220]. Only six coronaviruses (229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV) were previously known for the ability to infect humans [221]. SARS-CoV-2 is considered less lethal than SARS-CoV and MERS-CoV but more contagious [222].

1.2.2 COVID-19 pandemic

The World Health Organization (WHO) declared the outbreak a public health emergency of international concern from January 30, 2020, to May 5, 2023. The first confirmed case of COVID-19 dates back to 31 December 2019, but already on 8 December it seems that the first patients with symptomatic disease appeared. On 1 January 2020, the authorities ordered the closure of the market and the isolation of those presenting signs and symptoms of infection. The first confirmed death occurred on 9 January 2020. The epidemic was declared a public health emergency of international interest (PHEIC) by WHO on 30 January 2020.

Epidemiology

As of 7 June 2023, there are 767.364.883 confirmed cases in the world since the start of the pandemic with 6.938.353 deaths attributable to COVID-19. The doses of vaccine administered in the world are 13.356.281.548 (Figure 5) [223].

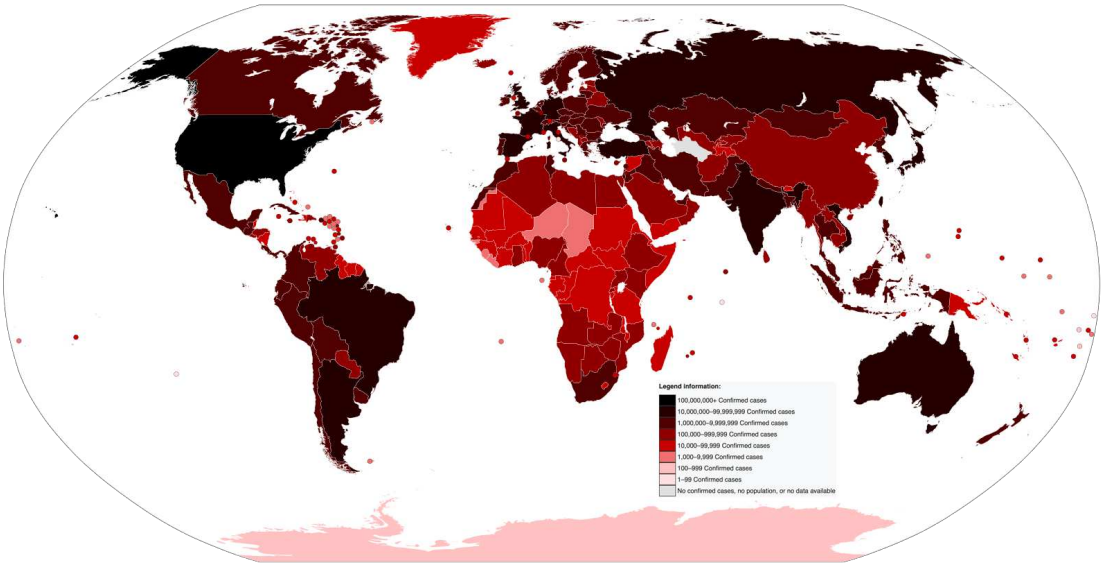

Figure 5: Cumulative confirmed COVID-19 cases on 30 April 2023 [224]

Individuals of all ages are at risk of SARS-CoV-2 infection and severe disease, however, the likelihood of severe COVID-19 is greater in people over the age of 65, residing in nursing homes or in a long-term care facility, those who are not vaccinated or have a poor response to anti-SARS-CoV-2 vaccines and those with chronic comorbidities. Regarding comorbidities, cardiovascular disease, chronic kidney disease, chronic obstructive lung disease, diabetes with complications, neurocognitive disorders, cystic fibrosis, cancer, congenital or acquired immunodepression, chronic liver disease, pregnancy and obesity significantly increase the risk of severe COVID-19. The risk increases even more in patients with multiple comorbidities [225].

1.2.3 Virology

Structure

Each SARS-CoV-2 virion has a diameter of about 50-200 nanometers, like other coronaviruses, consists of four structural proteins, known as: S protein (spike), E protein (envelope), M protein (membrane) and N protein (nucleocapsid) [226]. N protein contains the genome while S, E and M proteins create the viral capsid. S protein is the one that allows the virus to attach the membrane of a host cell by exploiting the angiotensin converting enzyme 2 (ACE2) [227–229]. S protein consists of a S1 subunit containing the receptor-binding domain (RBD) and is responsible for the initial attack of the virus on the host cell, and a S2 subunit responsible for fusion with the host cell membrane for the insertion of viral RNA.

SARS-CoV-2 RBD has different conformation than SARS-CoV RBD [230–232] and this explains both its greater affinity for ACE2, and the ability of SARS-CoV-2 to more easily evade the host immune system [233]. Due to the relevance of S protein in host cell attack and virus proliferation, a continuous selection process caused by immunization has led to the prevalence of viral variants with changes in the sequence of this protein. In contrast, the other proteins of the virus, which did not undergo selective pressure, did not develop significant changes over time and are also among the most preserved among the various pre-existing human coronaviruses. For example, one of the first mutations detected, known as D614G, was already detected in early 2020, becoming the most widespread in the world since March 2020 [234]; this mutation is derived from a missense mutation (substitution of an aspartate to a glycine in position 614) [235] and in most cases is accompanied by other minor mutations [234].

Genome

The genome of SARS-CoV-2 consists of a single RNA helix of about 30.000 bases, of which 89% are identical to those of SARS-like-CoVZXC21 (spread in bats) [236] and 82% are identical to those of SARS-CoV [237]. Recently, in addition, a work published in the journal Nature has identified species of bats living in the caves of northern Laos with a genomic sequence identity greater than 96% and high structural similarity [238].

Coronaviruses in general have high genetic plasticity, but the viral evolution of SARS-CoV-2 is slowed by the RNA proofreading capability of its replication machinery. For comparison, the in vivo viral mutation rate of SARS-CoV-2 was found to be lower than that of influenza [239].

Replication cycle

The virus spreads from infected individuals mainly from respiratory droplets and aeresols produced when speaking, breathing, coughing or sneezing, reaches the mucous epithelial cells in the upper airways and oral cavity [240] and penetrates into the cells by exploiting S protein to attach to the binding sites of the host cell ACE2 receptor. Indirect contact through contaminated surfaces is another possible cause of infection. Subsequently, proteases such as TMPRSS-2/furin cleaved the S protein to allow the virus to enter host cells by endocytosis [241, 242].

Single-stranded 30 kb RNA is released directly into the cytoplasm and uses the viral replication-transcription complex (RTC) to produce RNA and viral proteins. Virions are assembled with N protein encapsulated RNA and a "mantle" consisting of M, E and S proteins. Once released, viral particles can infect other cells in the lower airways (type II pneumocytes) and enterocytes in the gastrointestinal tract [243–245]. The degree of infectivity of the virus during the incubation period is not certain, but it has been seen that the virus reaches a peak of charge in the pharynx after about four days after infection and in the first week of symptoms, while this concentration subsequently decreases progressively [246] (Figure 6).

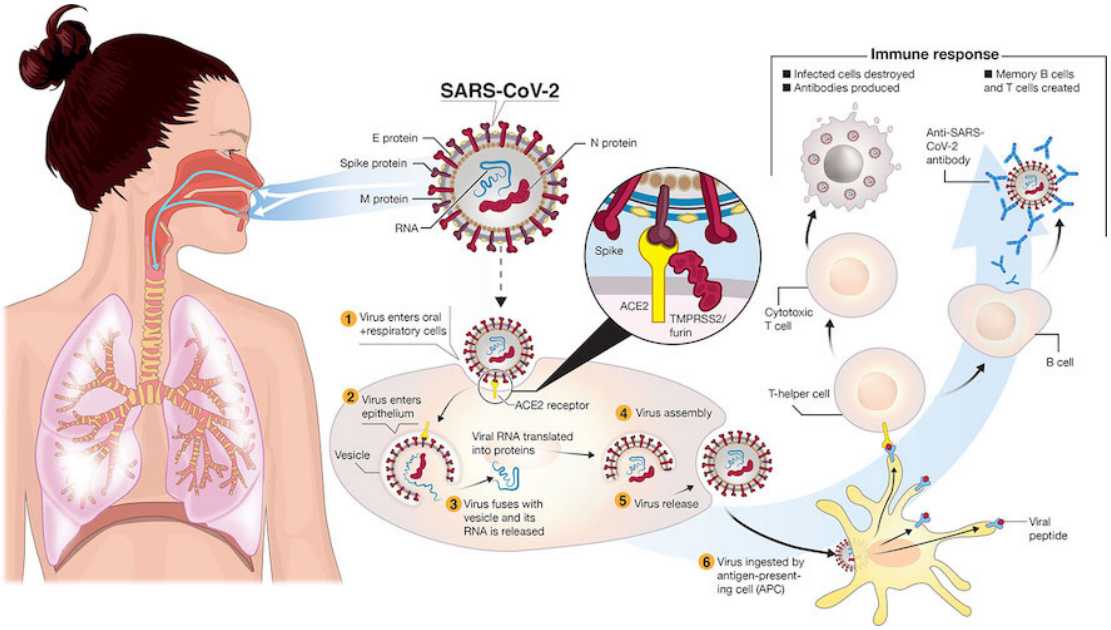

Figure 6: SARS-CoV-2 replication cycle [245]

Variants

Viral variants are generated by the continuous selective pressure to which the virus is subjected, both for natural active immunization in infected subjects and by artificial active immunization using vaccines introduced from December 2020. Mutations associated with new viral variants determine, in fact, not only increased virulence and transmissibility but also antigenic mutations that could interfere with the effectiveness of vaccines and of certain drugs and the sensitivity and specificity of diagnostic tests [247].

There are many thousands of variants of SARS-CoV-2, which can be grouped into much larger groups for which different nomenclatures have been proposed: Nextstrain divides the variants into five clades (19A, 19B, 20A, 20B and 20C)

while the Global Initiative on Sharing All Influenza Data (GISAID) divides them into seven (L, O, V, S, G, GH and GR). Since December 2020, the WHO has assigned greek letters to several identified variants.

Thus the initial classification of variants distinguished:

- Alpha (Lineage B.1.1.7): emerged in the United Kingdom in September 2020, characterized by the appearance of mutations N501Y and P681H that determine greater transmissibility and virulence [248, 249].
- Beta (Lineage B.1.351): emerged in South Africa in May 2020, characterized by the appearance of mutations K417N, E484K and N501Y that determine greater transmissibility and changes in antigenicity.
- Gamma (Lineage P.1): emerged in Brazil in November 2020, characterized by the appearance of mutations K417N, E484K and N501Y that determine greater transmissibility, virulence and changes in antigenicity.
- Delta (Lineage B.1.617.2): emerged in India in October 2020, characterized by increased transmissibility and changes in antigenicity.
- Omicron (Lineage B.1.1.529): emerged in Botswana in November 2021. It was designated as a VOC in November 2021 and quickly became the dominant variant worldwide. Sub-variants of Omicron BA.1, BA.1.1 and BA.2 emerged in early 2022. Sub-variants BA.4 and BA.5 and, more recently, other such as BQ.1, BQ.1.1, XBB and XBB.1.5 are more transmissible than previous variants and are not susceptible to any of the anti-SARS-CoV-2 antibodies developed for the treatment and prevention of COVID-19 [250, 251].

In March 2023, the WHO decided to completely rework the classification of variants, evaluating the Omicron sub-variants independently [252, 253]. It provides for:

- Variants of interest (VOI): variants that present genetic changes that determine or could determine greater transmissibility, virulence, immune response evasion, resistance to therapy and vaccines, that have a growth advantage over other variants resulting in increased cases over time and prevalence, and which have an epidemiological impact suggesting an emerging risk to global public health. This group includes the variant XBB.1.5 (Kraken) emerged between October 2022 and January 2023 and that becoming the prevailing variant in the WHO countries in March 2023;

this sub-variant appears to possess a higher transmissibility than XBB, but there is no evidence that may cause a more serious disease.

- Variants of concern (VOC): variants that meet the definition of VOI and that also meet at least one criteria, compared to the other variants, among the presence of changes that determine a greater clinical severity of the disease, the ability to bring about a change in the epidemiology of COVID-19, a substantial impact on the ability of health systems to provide care to patients with COVID-19 or other disease and the consequent need for major public health interventions, ability to bring about a significant reduction in the effectiveness of the vaccines available for protection against serious disease. The Alpha, Beta, Gamma, Delta and Omicron variants have been downgraded to "previously circulating VOCs". Moreover, with this new classification greek letters will be attributed only to VOC and no longer to VOI.

- Variants under monitoring (VUM): variants presenting genetic changes that are suspected to affect the characteristics of the virus and its epidemiology, but for which the evidence is still weak. Currently, several sub-variants of Omicron are included in this group, such as: BQ.1 (Cerberus) and its derivative BQ.1.1, BA.2.75 (Centaurus) and its derivative CH.1.1, XBB (Gryphon), XBF (Bythos).

  The Cerberus sub-variant (BQ.1 and BQ.1.1) became the predominant Omicron sub-variant in circulation in the WHO countries in January 2023. It derives from VOC Omicron 5 (BA.5) and has a greater ability to evade immune defenses and to resist to the treatment with monoclonal antibodies than BA.5. However, there is no evidence that Cerberus can cause a more severe disease than BA.5.

  Gryphon is a sub-variant generated by a recombination process between BA.2.10.1 and BA.2.75 and has additional mutations in the S protein. Some evidence suggests that this sub-variant is the one with the greatest ability to evade the immune response while there is no evidence about the ability to cause a more severe disease.

  Bythos is a sub-variant generated by a recombination process between BA.5.2.3 and CJ.1 (derived from BA.2.75.3). Its controlled because its believed to have greater transmissibility, but theres no evidence that it could induce a more serious disease.

The European Centre for Disease Prevention and Control (ECDC) has proposed its own classification similar to that of the WHO with greater specificity for the European situation. Also in this case there are VOC, VOI and VUM identified with the same criteria of the WHO, however there are differences in the classification of the single sub-variant [254]:

- VOI: BA.2.75 (including its derivatives such as XBF and XBK), BQ.1 (Cerberus), XBB (Gryphon), XBB.1.5 (Kraken).
- VOC: since 3 March 2023, BA.2, BA.4 and BA.5 sub-variants have also been downgraded from VOC, as these parental lineages no longer circulate.
- VUM: XBC (recombinant variant between Delta and Omicron 2 - BA.2), BN.1, CAP.1.1, XAY.

At the moment, symptoms of SARS-CoV-2 variants appear to be similar to those caused by the original virus. The exception seems to be the variant Omicron and its sub-variants whose symptoms seem to be milder and with a shorter duration, despite the risk of developing severe disease for sensitive individuals has not changed. Vaccines remain effective in preventing severe form of COVID-19 even when induced by variants, although some of them may partially decrease effectiveness. With regard to the drugs adopted so far in the treatment of COVID-19, the only case of reduced efficacy appears to exist for some monoclonal antibodies approved by studies carried out on the original virus. The diagnostic tests currently in use are also effective in detecting variants even if they are unable to determine which variant affect the patient [255]. In this regard in Europe, the ECDC recommends randomly sequencing at least 500 samples each week with priority for those derived from: vaccinated patients who have developed a reinfection despite the good immune response, patients admitted in structures that deal with immunocompromised patients positive to SARS-CoV-2 for long periods, patients arriving from countries with high incidence of SARS-CoV-2 variants. In addition, sequencing is important in the event of a sudden increase in cases and a change in the performance of diagnostic tools or therapies [256].

1.2.4 Infection and trasmission

Reservoirs and carriers

The origin of SARS-CoV-2 is confirmed to be animal, with bats as a natural reservoirs and subsequent passage to humans [228]. Differences between bat

coronavirus and SARS-CoV-2 suggest that humans may have been infected by an intermediate host, although the source of introduction into humans remains unknown [257, 258]. A study published in July 2020 suggested that pangolins are an intermediate host of SARS-CoV-2-like coronaviruses [259] but subsequent studies have not demonstrated their contribution to spillover since Pangolin virus are too different from SARS-CoV-2.

Survival in the environment

SARS-CoV-2 can survive in the environment for a time varying according to the considered surface. Respiratory droplets containing the virus can survive in the air, the main route of transmission, for several hours: 25% of viruses still maintain virulence after a little over an hour and 12.5% of the viruses persists after about 3 hours [260]. On stainless steel, the half-life of the viruses is about 5 hours, on plastic about 6 hours, on cardboard about 3 hours, on brass about 45 minutes [261]. The risk of survival in water, on the other hand, is low since sanitation systems for drinking water and swimming pools should remove or inactivate the virus.

Infection of the host

It has not been clarified how the virus could have moved from cold-blooded hosts to warm-blooded hosts. A homologous recombination event may have mixed a virus of subgenus A (Embecovirus, viruses similar to SARS Bat CoVZXC21) with the receptor binding protein of an unknown Beta-CoV [262].

Person-to-person transmission

Diffusion occurs through large respiratory droplets that can travel short distances or through aerosols of small respiratory particles that can be carried in the air for several hours and travel long distances before being inhaled. Factors such as the number of infected people in the room and the distance between them, the duration of time spent with infected people, the size and the ventilantion of airspace, the activity that generates aerosols can contribute to this risk [263].

Facilities at increased risk of infection include nursing homes, hospital facilities, schools, prisons as well as crowded and poorly ventilated environments such as religious services, gyms, bars, nightclubs, restaurants. Residents of nursing homes are also at high risk of serious illness due to the age and underlying comorbidities. Furthermore, it should also be considered that the social determinants of health

(living and working conditions, lifestyle, social network, education and health literacy, access to health services) affect a wide range of health risks and outcomes, such as exposure to SARS-CoV-2, COVID-19 severe infection and death, as well as access to testing, vaccinations and treatment [264, 265].

The virus appears to be transmitted mainly by symptomatic subjects as described in a meta-analysis that showed that asymptomatic individuals were 42% less likely to transmit the virus [266]. The WHO estimates that the basic breeding number (R0) representing the potential transmissibility of the virus from person to person is between 1.4 and 3.8. This value indicates the number of other people to whom a newly infected patient can transmit the disease, thus qualifying the new SARS-COV-2 as infectious as the SARS-CoV responsible for the 2002-2004 epidemic.

Studies conducted before the appearance of SARS-CoV-2 showed that ACE2 is also expressed in the lingual mucosa and that coronaviruses are also present in the feces of infected patients [267]. The oro-faecal transmission of SARS-CoV-2, however, is not yet confirmed. A study in hospitalized patients for COVID-19 showed that the virus was present in the feces of 53% of patients [268] for periods ranging from 1 to 12 days and in 17% of patients fecal tests remained positive even after the negative oropharyngeal tests [269], indicating the oro-faecal transimission can remain even after the elimination of the virus at the respiratory level. However, it is unlikely that the orofecal pathway is an important factor in the pandemic. In fact, the released SARS-CoV-2 virus is rapidly inactivated in the gastrointestinal tract and appears to be excreted mainly in a non-infectious state [270].

As in other respiratory viral infections, the possibility of reinfection has been observed for SARS-CoV-2. Data on prevalence, risk factors, timing and severity of reinfection are constantly updated and probably also depend on the variant considered. Reinfection can also affect vaccinated subjects although with less severe forms than those of unvaccinated subjects and compared to primary the infection.

Probably infection with SARS-CoV-2 confers a certain degree of immunity to reinfection and protection against severe forms of COVID-19; however, the duration and effectiveness of immunity after COVID-19 depend on multiple viral and host factors and are therefore complicated to estimate. Anti-SARS-CoV-2 antibody titers are highly variable after infection, and may not provide complete protection against new variants that have emerged in the meantime.

1.2.5 Pathogenesis

The virus accesses the host cells of the respiratory tract through the binding between the S protein and the ACE2 which is very represented in type II pneumocytes of the lungs. Therefore, the levels of expression of ACE2 in each tissue is related to the severity of the disease in that tissue. Some unconfirmed studies have suggested that reducing ACE2 may have protective effects [271, 272], while others that increasing ACE2 using angiotensin II receptor antagonist drugs may be protective [273]. The virus also affects the gastrointestinal tract since ACE2 is abundantly expressed in the glands of the gastric epithelium, as well as in the enterocytes of the small intestine and in rectum [268, 274]. The ACE2 is also expressed in the heart, in fact the virus can cause both acute and chronic myocardial damage [275].

The pathogenesis is described in detail in Figure 7 and consists of several direct and indirect damage mechanisms.

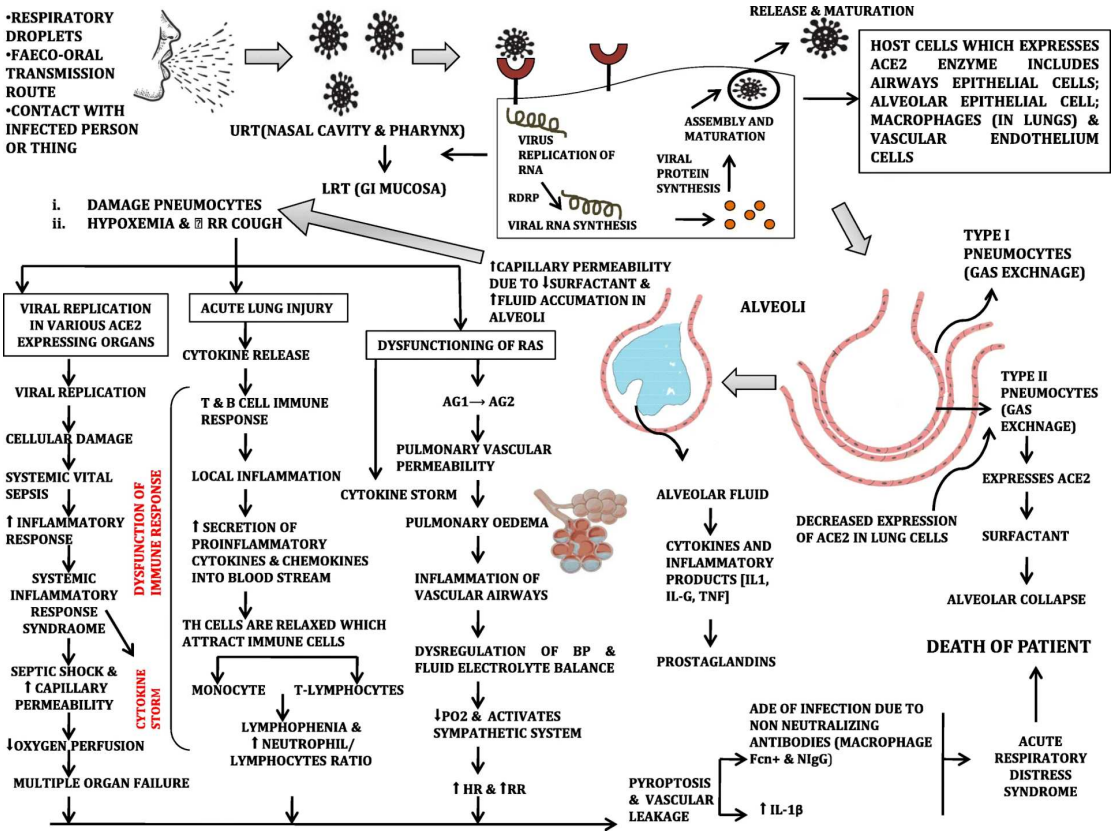

Figure 7: Pathogenesis of COVID-19 [276]

Pulmonary epithelial and endothelial damage and cytokine storm

The virus exploits pneumocytes to replicate and consequently causes their death by apoptosis. Since type II pneumocytes are fundamental in surfactant production in alveoli, they collapse, favoring the development of pneumonia and acute respiratory distress syndrome (ARDS) in patients with severe disease. In addition to this direct cell damage, rapid viral replication results in dysregulation of the immune system with intense cytokine and chemokine production, culminating in a cytokine release syndrome (CRS), lethal to host cells [277]. This mechanism provides that the macrophages, recruited in the alveolar space, secrete IFN that stimulates the further production of different inflammatory cytokines by the pulmonary epithelial cells themselves:

- IL-6, IL-1β and IL-8 promote the recruitment of cytotoxic T cells and neutrophils, which produce reactive oxygen species (ROS) and leukotrienes that contribute to acute lung damage. In addition, the neutrophil extracellular traps (NET), extracellular chromatin networks, microbicidal proteins and oxidizing enzymes that are released by neutrophils to contain infections seem to play a key role in the pathophysiology of COVID-19 as if not properly regulated, they have the potential to propagate inflammation and determine microvascular thrombosis.

- TNF-α is responsible for apoptosis of pulmonary epithelial and endothelial cells and the consequent deterioration of the alveolar capillary barrier, with alteration of the vascular wall and alveolar edema.

- Granulocyte-macrophage colonies stimulant factor (GM-CSF) mediates intercellular communication between Th1 cells and CD14+/CD16+ monocytes, responsible for the induction and amplification of tissue infiltration by macrophages.

- Interferon inducible protein 10 (IP-10) promotes the migration of T cells, monocytes and natural killer cells to the lungs.

- Transformed growth factor-β (TGF-β) promotes tissue remodeling and pulmonary fibrosis.

- IL-1β, IL-6, TNF-α, in addition, increase the expression of cell adhesion molecules (CAM) and vascular endothelial growth factor (VEGF) in pulmonary endothelium, thereby increasing endothelial permeability [278].

To prove this, COVID-19 patients with ARDS have classical serum biomarkers of CRS, including high CRP, lactate dehydrogenase (LDH), D-dimer, and ferritin [279].

The massive production of inflammatory cytokines and chemokines contributes to the damage of endothelial cells, already damaged by the direct cytopathic damage of the virus (the endothelial cells of the vessels and pulmonary capillaries express a high density of ACE2). This leads to an increase in vasal permeability and the formation of pulmonary edema, but also microvascular pulmonary thrombosis [280].

Since lymphocytes and neutrophils are involved in SARS-CoV-2-induced lung damage, in patients with COVID-19 disease and particularly in patients with severe forms, lymphopenia, both CD4+ and CD8+ and increased neutrophil levels were observed.

Dysregulation of the renin-angiotensin-aldosterone system (RAAS)

ACE2 catalyses the conversion of angiotensin I (vasoconstrictor) to angiotensin 1-9 (vasodilator), or angiotensin II to angiotensin 1-7. The latter through the MAS receptor, promotes the release of vasoactive peptides such as nitric oxide (NO), bradykinin and prostaglandin E2 (PGE2) with vasodilator and anti-inflammatory effect. The reduction of the expression of the molecules of ACE2, exploited by SARS-CoV-2 for the entrance in the host cell, determines an imbalance of the ACE/ACE2 activity with consequent accumulation of angiotensin II that, in turn, through the angiotensin type-1 receptor (ATR1), increases vascular permeability and promotes tissue damage [281]. In addition, this leads to the activation of NF-κB pathway, involved in immune regulation and currently considered one of the most important checkpoints involved in pro-inflammatory events related to COVID-19. Activation of NF-κB, mediated by IL-6 through the intracellular JAK/STAT3 pathway, leads to the production of additional pro-inflammatory cytokines including IL-6 itself, through a positive feedback mechanism [282].

Other characteristics of COVID-19 pathogenesis

In addition, the pathogenesis of COVID-19 would suggest a pattern of immune dysregulation regarding timing, localization, quality and quantity of immune response. A viral infection normally leads to a coordinated immune response, by pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) along with activation of numerous cytokines and

chemokines. In the case of SARS-CoV-2 infection seems that this model is being subverted as described in Figure 8. This condition, termed "immunological asynchrony" contributes to aberrant inflammatory response, cytokine storm, and lymphopenia [283]

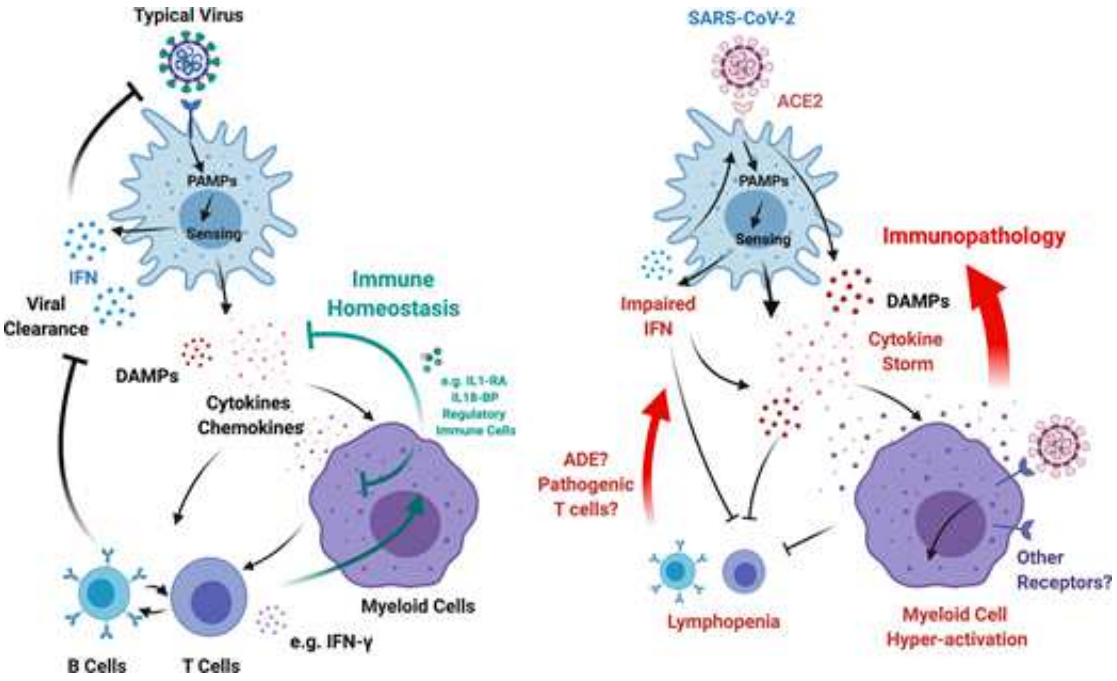

Figure 8: Immunological asynchrony in COVID-19 pathogenesis [283]

In addition, other interesting evidence about the pathogenesis of COVID-19 suggests that:

- Neuropylin-1 enhances the infectivity of SARS-CoV-2 suggesting the presence of alternative entry mechanisms to ACE2 [284, 285].
- The high glycosylation profile of SARS-CoV-2 would constitute a "glycanic mask" reducing viral immunogenicity [286].
- SARS-CoV-2 acts as antagonists for IFN and other innate immune elements [287].
- SARS-CoV-2 reduces adaptive immunity resulting in ineffective viral clearance along with failure to temper innate immune responses.
- Antibodies produced against SARS-CoV-2 have also been shown to be pathological by distorting macrophage responses, leading to fatal acute lung injury through severe hypercytokinemia [288].

Genetics

There are still no definite results regarding a personal genetic predisposition that would affect the risk of contracting SARS-CoV-2 infection or developing severe forms of COVID-19. However, some interesting results, although still to be confirmed, emerged from an Italian study that identified HLA-DRB1*15:01, -DQB1*06:02 and -B*27:07 HLA alleles that could represent markers of susceptibility [289].

Other studies have shown an increased risk of developing severe COVID-19 in patients with polymorphisms of IFN-related type I genes [290] or with IFN type I-neutralizing IgG antibodies [291].

Other studies suggested that ACE2 or TMPRSS2 DNA polymorphisms were likely associated with genetic susceptibility to COVID-19 [292].

1.2.6 Pathological anatomy

The most characteristic histological feature of COVID-19, although not pathological, is diffuse alveolar damage (DAD), characterized by squamous metaplasia, intra-alveolar hemorrhage, necrosis, formation of hyaline membranes and hyperplasia of type II pneumocytes [293]. The pulmonary picture resembled that found in ARDS and is characterized by the presence of DAD with fibromyxoid exudates and cytopathic viral changes in pneumocytes. Lung damage consists of a first exudative phase (variable duration, on average about 10 days, and characterized by a different degree of interstitial edema, acute pulmonary inflammation, hyperplasia of type II pneumocytes and hyaline membrane formation) and a second proliferative phase (characterized by remodeling of the alveolar wall with proliferation of fibroblasts and myofibroblasts, extracellular matrix deposition and accumulation of intra-alveolar fibrin). In pulmonary endothelial cells, however, structural damage is observed characterized by the interruption of intercellular junctions, cellular swelling, narrowing of the capillary lumen and loss of contact with the basal membrane [294]. If intra-alveolar fibrin deposition is the main histological feature, there is a histological picture known as acute fibrinous and organizational pneumonia (AFOP) [295].

In addition, other interesting histological findings include disseminated intravascular coagulation (DIC) [296], leucoeritroblastic reactions in the blood [297] and microvesicular steatosis in the liver [298].

1.2.7 Clinical manifestation

Patients infected with the virus, after an incubation period ranging from 2 to 14 days (with an average of 5.1 days) [299], usually have flu-like symptoms, such as fever (in over 90% of cases), dry cough (over 80% of cases), fatigue, shortness of breath (about 20% of cases) and dyspnea (about 15% of cases). Gastrointestinal symptoms such as diarrhea, conjunctivitis and cutaneous rashes or even hemoptysis are less common. Other more specific symptoms of SARS-CoV-2 infection are partial or total loss of sense of smell (dysosmia or anosmia) or taste (dysgeusia or ageusia) that may persist for quite a while even after healing.

In patients with paucisintomatic or mild disease, symptoms usually resolve within about a week, although some patients with mild symptoms may subsequently worsen, progressing to severe disease. In the more severe forms of COVID-19 the disease has, instead, a longer duration. These patients with long-term disease, however, although molecular tests may remain positive up to 3 months are generally not considered infectious, as the virus is rarely able to be cultivated from the upper respiratory tract of patients after 10 days of disease.

The National Institute of Health (NIH) has classified the possible clinical presentations associated with COVID-19 into [300]:

- Asymptomatic or presymptomatic infection: patients who test positive for SARS-CoV-2 but who have no symptoms consistent with COVID-19. Some of these patients, however, it was observed that might have radiographic signs of COVID-19 pneumonia [301].

- Mild illness: patients who have any of the various signs and symptoms of COVID-19 excluded shortness of breath, dyspnea or abnormal chest imaging. Most of these patients can be managed on an outpatient basis or at home through telemedicine. In addition, routine imaging or specific laboratory evaluations are not indicated. However, patients over the age of 50 and those with underlying comorbidities are at high risk of disease progression and are candidates for antiviral therapy.

- Moderate illness: patients who show evidence of lower respiratory disease during clinical assessment or imaging but who have an oxygen saturation measured by pulse oximetry (SpO_2) $\geq$94%. In this case lung disease can progress rapidly so patients with moderate disease should be closely monitored.

- Severe illness: patients who have SpO_2 <94%, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%. These patients may experience rapid clinical deterioration and must be hospitalized to receive oxygen supplementation.

- Critical illness: patients who have respiratory failure, septic shock or multiple organ dysfunction. These patients are managed in intensive care and their clinical management should include treatment with immunomodulators and, in some cases, the addition of remdesivir. These patients should also receive treatment for any comorbidities and nosocomial complications.

1.2.8 Complications

One of the most frequent complications in COVID-19 patients are superinfections that can complicate treatment and prognosis. Older patients or those with chronic comorbidities and/or immunodepression are at higher risk. The use of immunomodulators such as dexamethasone, IL-6 inhibitors (e.g. tocilizumab, sarilumab) or JAK inhibitors (e.g. baricitinib, tofacitinib) has been seen to increase the risk further. However, when these therapies are used appropriately, the benefits outweigh the risks.

Infectious complications in patients with COVID-19 have been classified by the NIH [300] into:

- Coinfections at presentation: although rarely, already at presentation the patient may present a concomitant infection of influenza viruses or other respiratory viruses. Cases of community-acquired pneumonia have also been reported although this is a rare occurrence with a prevalence ranging from 0% to 6%. Antibacterial therapy is generally not recommended unless there is additional evidence of bacterial pneumonia (e.g. leukocytosis or focal infiltrates on imaging) [302, 303].

- Reactivation of latent infections: limited data have shown a risk of reactivation for hepatitis B virus (HBV), mycobacterium tuberculosis (TB), herpes simplex virus (HSV) and varicella zoster virus (VZV) in COVID-19 patients receiving immunomodulators as treatment [304–306].

- Nosocomial infections: hospitalized patients with COVID-19 may acquire common nosocomial infections, such as hospital-acquired pneumonia

(including ventilator-associated pneumonia), catheter-related bloodstream bacteriemia or fungemia, urinary tract infection associated with catheter and clostridium difficle infections.

- Opportunistic fungal infections: for example aspergillosis and mucormycosis, have been reported in hospitalized patients with COVID-19. Although these infections are the rarest among those listed, they may be more common in patients undergoing mechanical ventilation and may be fatal [307–309].

Other complications that have been observed in COVID-19 patients with severe disease are: arrhythmias, cardiomyopathy, thromboembolism [310, 311], DIC, hemorrhages and Guillain-Barré syndrome.

Another rare complication of the infection in children is the multi-system inflammatory syndrome (MIS-C); it has characteristics similar to those of Kawasaki disease, in fact, children have fever, tachycardia, signs of inflammation, heart, gastrointestinal and renal involvement from 2 to 6 months after a generally mild or even asymptomatic SARS-CoV-2 infection [312]. Vaccination appears to be highly protective against the development of MIS-C. A similar multi-system inflammatory syndrome has also been reported in young and middle-aged adults and is called multi-system inflammatory syndrome in adults (MIS-A) [313].

1.2.9 Diagnosis

Diagnosis of the infection

There are two main types of COVID-19 diagnostic tests: real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen tests. In both cases, the samples for analysis include nasopharyngeal, oropharyngeal, nasal mediaturbinate and anterior nostril swabs, sputum and bronchial fluid [314]. In addition, viral nucleic acid was also found in samples of the gastrointestinal tract or blood, even when respiratory samples are negative [269].

RT-PCR is the gold standard test for COVID-19 due to its increased sensitivity and specificity. PCR tests, however, may remain positive for at least 3 months after initial diagnosis, regardless of symptoms.

Antigen tests are less sensitive, particularly in the early stages of infection when the viral load is lower, therefore, it may be necessary to confirm the result of the antigen test with an RT-PCR. On the other hand, antigen tests are less likely to remain positive after the resolution of the infection because they detect only higher viral loads.

Finally, serological tests should not be used to diagnose COVID-19 disease, as antibodies usually become detectable only 1-3 weeks after the onset of symptoms, but are indicated to evaluate a previous infection.

Laboratory

Laboratory tests should include a complete blood count with protein profile, metabolic profile, liver and kidney function tests. In addition, inflammatory markers such as CRP, D-dimer and ferritin should also be included in the assessment. In blood tests, infected subjects may have lymphopenia, increased liver transaminases, lactate dehydrogenase, D-dimer, ferritin, and inflammatory markers such as CRP.

Pulmonary viral damage observed in ARDS forms by COVID-19 may be indirectly confirmed by the increase of some biomarkers such as:

- Surfactant protein D: indicator of alveolar damage of type II pneumocytes, the value of which would seem to be inversely correlated with the PaO_2/FiO_2 ratio in those patients.
- Angiopoietin-2
- Soluble E-selectin
- Intercellular adhesion molecule 1 [294]

All these biohumoral markers are considered predictive of ARDS and admission to intensive care [278]. The presence of marked lymphopenia and neutrophilia would also appear to be a risk factor for ARDS [315].

Measurement of oxygen saturation is also recommended in all patients. Although pulse oximetry is useful for estimating blood oxygen levels, pulse oximeters may not accurately detect hypoxemia under certain circumstances. Several published reports compared the measurements of peripheral oxygen saturation measured by pulse oximetry (SpO_2) and arterial oxygen saturation measured by arterial blood gas analysis (SaO_2) are particularly discordant in patients with darker skin pigmentation and at the lower intervals of SpO_2 [316]. Additionally, occult hypoxemia appears to be associated with an increased risk of multi organ failure and hospital mortality [317]. Despite the limitations of pulse oximetry, a household pulse oximeter can be a simple means of evaluating a patients general clinical status [300].

Blood gas analysis

In these patients hypoxemia, hypocapnia and respiratory alkalosis are observed. In advanced stages, however, hypercapnia appears, indicating the loss of muscle compensation capacity. Always check the PaO_2/FiO_2 ratio, which in a patient with normal oxygen blood pressure (PaO_2 80-100%) and in ambient air (FiO_2 21%) is equal to 480. When the value of PaO_2/FiO_2 drops below 300 we can speak of respiratory failure (mild between 300 and 200, moderate between 200 and 100, severe below 100). So, blood gas analysis also helps to understand that the patient is getting worse, and it can give indication to begin invasive ventilation even in anticipation of an imminent rapid worsening of the picture [318].

Imaging

The initial assessment of patients with proven COVID-19 should include, except for patients with asymptomatic or paucisintomatic disease, chest imaging (X-ray, ultrasound or CT) and an electrocardiogram. Imaging can be decisive not only for the diagnosis of pneumonia, but also for monitoring and prognostic evaluation of the patient.

X-ray Interstitial pneumonia is the predominant clinical manifestation of COVID-19, that is, pneumonia characterized by edema and inflammatory cell infiltrates in the interstitial spaces (between alveolar walls), while only in the most advanced stages of disease, these begin to fill the hollow spaces, first subtotally (ground glass) and then completely (consolidation). However, interstitial pneumonia of COVID-19 does not have a specific manifestation but similar to that of other pneumonia and interstitial diseases [318].

CT The American College of Radiology states that CT should not be used either for screening or as a first line radiology, but only in hospitalized, symptomatic, or with specific clinical indications [319].

CT, particularly high-resolution CT (HRCT) has higher sensitivity in the early stages than X-ray. Even in this case, however, the findings are non-specific (similar pictures are also found in pneumonia from influenza virus, cytomegalovirus, other coronaviruses (SARS, MERS), streptococcus and atypical germs (chlamydia, mycoplasma).

CT frequently shows areas of ground-glass opacity, initially monolateral but with possible extension to the contralateal lung as disease progresses, associated with consolidation areas with patch distribution, mainly peripheral/subpleural, and with greater involvement of the posterior and lower lobes. The "crazy paving pattern" can also be present, characterized by the presence of "ground glass" areas superimposed on thickening of the interlobular and intralobular interstice [320].

In a more advanced picture of disease, moreover, ground glass areas increase in density, up to parenchymal consolidation. The evolution towards consolidation is spontaneous and characteristic of COVID-19, unlike other viral pneumonias that consolidate when a bacterial infection overlaps [321, 322].

Rarer, however, is the presence of only consolidation, cavitation, calcification, lymphadenopathy and pleural effusion.

CT therefore plays a fundamental role in the intermediate phase of the patients diagnostic process, to understand how it is necessary to be aggressive, fast and therapeutically impactful [318].

US Pulmonary ultrasound "bedside" has many advantages, starting with less contact with patients which reduces the risk of spreading the virus. The results on the pulmonary US in COVID-19 seem to correlate very well with the CT results and therefore also evolves with increasing clinical severity [318]:

- In the pre-symptomatic phase you can notice the presence of B lines alternating with areas with normal A lines and diaphragmatic hypomobility; this picture corresponds to the few ground glass areas visible at the CT mainly in the lower and rear fields.

- During the first symptomatic week, coalescent B lines appear that form "white patches" ("waterfall sign") and pleural line appears wrinkled; at the same time, on the CT, ground glass foci will be observed bilateral and more confluent. If the interstitial picture progressed further it would arrive at the so-called "white lung", that is, a completely white US picture with B lines that can no longer be distinguished from each other, typical finding of ARDS with a fully inflamed parenchyma and unable to ventilate. Even the X-ray would show large white fields.

- During the second symptomatic week, instead, the lines B appear dense and fixed compared to the pleural sliding ("dry lung pattern") while the CT will observe small bilateral subpleural peripheral consolidations.

- In the most severe form, progressively increasing the volume of the consolidated lung, we will observe hyperechogenic branched structures (containing air) and we talk about lung consolidation with open bronchi (the corresponding on the CT are aerial bronchograms) [323].

- Interruptions and thickening of the pleural line and small pleural effusions may also be present at any stage of the disease while larger pleural effusions are rare. Finally, US also allows to verify the possible presence of pneumothorax (absence of pleural sliding) and of new thickenings from bacterial over-infection.

The sensitivity of the US depends on several factors, in particular the severity of the disease, the operators experience and the quality of the scan. Specificity, on the other hand, is low as the presence of irregular B-lines or consolidation can be observed in any pneumonia or interstitial lung disease.

The execution of the thoracic ultrasound, if well planned, allows to avoid unnecessary CT, in fact, the CT should not be performed routinely to all patients, because in the first 48 hours can be negative, and late imaging feedback may not be useful to change the treatment approach.

1.2.10 Treatment

The choice of treatment depends on the severity of COVID and any complications, the characteristics of the patient and the availability of drugs and structures and may include the use of drugs (antivirals, corticosteroids and immunosuppressants), immunoglobulin anti-SARS-CoV-2 and mechanical ventilation. Very few drugs are known to effectively inhibit SARS-CoV-2.

Many other therapeutic possibilities initially used, are currently not recommended:

- Convalescent plasma: several randomized clinical trials failed to show significant benefit and suggest a potential association with increased need for mechanical ventilation [324]. However, it can still be used in non-hospitalized patients with mild to moderate disease and at high risk of progression to severe disease if no other treatment options are available.

- Nonspecific intravenous immunoglobulin and mesenchymal stem cell therapies are not recommended.

- Further immunomodulating therapies (e.g. interferon, kinase inhibitors and interleukin inhibitors), azithromycin, antiretroviral, chloroquine and

hydroxychloroquine, ivermectin: despite initially used in different classes of patients, to date are not recommended as there is not enough data in favor of use outside clinical trials.

Early treatment of mild COVID-19

For symptoms management in patients with mild COVID-19, paracetamol should be preferred over ibuprofen due to its increased safety. However, WHO and NIH do not oppose the use of NSAIDs for symptoms, and the FDA states that there is no evidence that NSAIDs worsen the symptoms of COVID-19.

Early treatment of mild or moderate COVID-19 at a high risk of progression to a serious disease

In Italy, two antivirals have so far been authorised for the treatment of COVID-19 in adult and adolescent patients (aged 12 years and over and weighing at least 40 kg) who do not require additional oxygen therapy and who have a high risk of developing a severe form of COVID-19:

- Veklury (remdesivir): is the first antiviral drug to have received authorization, initially only in patients with pneumonia requiring additional oxygen therapy. Since 30 December 2021, Veklury is also indicated for the treatment of COVID-19 in patients not hospitalized for COVID-19 and not in oxygen-therapy with onset of symptoms for no more than 7 days and in the presence of predisposing clinical conditions that represent risk factors for the development of COVID-19 severe. Remdesivir has been shown to reduce the percentage of people with hospitalization or deaths in the course of COVID-19 for any cause by 87% compared to placebo [325].

- Paxlovid (nirmatrelvir/ritonavir): the combination of nirmatrelvir and ritonavir should be started as soon as possible after diagnosis of COVID-19 and within 5 days from the onset of symptoms. Paxlovid has been shown to reduce the percentage of people with hospitalization or deaths in the course of COVID-19 for any cause by 88% compared to placebo [326]. Exacerbation of symptoms has been observed in some patients after nirmatrelvir/ritonavir treatment, diagnostic tests may return positive again, even in asymptomatic patients [327]. Paxlovid has a wide range of known and possible serious drug interactions, which must be evaluated before starting therapy [328]. In patients with chronic renal failure with eGFR 30-60 mL/min/1.73 m^2 the

dosage of nirmatrelvir is reduced to 1 tablet, while in patients with eGFR <30 mL/min/1.73 m^2 is contraindicated.

Another drugs used initially in this class of patients but subsequently withdrawn for poor evidence of effectiveness is Molnupiravir. It should be started as soon as possible after diagnosis of COVID-19 and within 5 days from the onset of symptoms. Molnupiravir is contraindicated for use in patients aged <18 years since it can alter bone and cartilaginous growth, in pregnancy as its teratogenic potential (women of childbearing age are advised to use a reliable contraceptive method during treatment with molnupiravir and for 4 days after the final dose, while men of childbearing age are advised to use a contraceptive method up to 3 months after the dose final). Molnupiravir has been shown to reduce the percentage of people with hospitalization or deaths in the course of COVID-19 for any cause by 3% [329]. Given its poor efficacy on March 10, 2023 has been withdrawn from use for lack of clinical benefits.

The pharmacological characteristics and dosages of such drugs are reported in detail in Table XVII.

Table XVII: Pharmacological treatment of mild or moderate COVID-19 at a high risk of progression to a serious disease

Antiviral drug	**Biological features**	**Dosage**
Nirmatrelvir/ritonavir	Major protease for viral replication inhibitor (nirmatrelvir) associated with a cytochrome 3A4 inhibitor (ritonavir) that acts as a booster increasing the half-life of the first drug	Administered orally with 2 tablets of nirmatrelvir 150 mg and 1 tablet of ritonavir 100 mg, twice daily and for 5 days.
Remdesivir	Nucleotide analogue	Administered intravenously with an infusion of 200 mg on the first day followed by two infusions of 100 mg on the second and third day (possible extension of the dose of 100 mg to the fifth day in case of partial response).
Molnupiravir	Nucleoside analogue	Administered orally with 4 tablets of 200 mg twice daily and for 5 days.

Bamlanivimab plus etesevimab, casirivimab plus imdevimab and sotrovimab, instead, belonging to the class of neutralizing SARS-CoV-2 monoclonal antibodies, initially were used according to availability in this class of patients. However, in April 2022, when the Omicron variant was the most widespread in the world, the FDA (Food and Drug Administration) recommended not using them because it is resistant [330]. Bebtelovimab, another SARS-CoV-2 neutralizing monoclonal antibody, which initially maintained activity against early variants of Omicron, was also withdrawn in November 2022 due to the lack of efficacy against the latest sub-variants of Omicron [331].

Treatment of severe COVID-19

The recommended drug therapy for severe infections include remdesivir, dexamethasone and immunomodulatory drugs such as baricitinib, tocilizumab, and sarilumab. Such drugs are used in different combinations depending on the stage of the disease in which the patient is: remdesivir is effective at the initial stage of the disease when viral replication is active, while anti-inflammatory and immunomodulatory drugs are more effective in later stages when the hosts inflammatory response and immune dysregulation carry the disease forward.

Patients requiring oxygen supplementation but no additional respiratory support In this class of patients the possible therapeutic choices are:

- Remdesivir: has been shown to accelerate clinical improvement in this class of patients compared to oxygen supplementation alone [332]. However, some open studies have not confirmed this benefit [333, 334]. It is given intravenously at a loading dose of 200 mg on the first day followed by a maintenance dose of 100 mg until the fifth days (the maintenance dose may be continued until the tenth day in patients requiring mechanical ventilation).

- Dexamethasone: showed a survival benefit only in patients requiring additional oxygen or mechanical ventilation as described in the RECOVERY study [335]. Other corticosteroids have also proven equally effective and can be used according to availability. Dexamethasone is given orally at a dose of 6 mg per day for ten days or until the patients discharge if it occurs before.

- Remdesivir plus dexamethasone: the combination of the two drugs is effective, in this class of patients, within the first 10 days of disease when both viral replication and host inflammation drive the clinical manifestation.

Patients requiring non-invasive ventilation (including high flow oxygen delivery systems) The treatment options include:

- Dexamethasone: recommended for all patients.
- Remdesivir: can be added in particular within 7-10 days from the onset of symptoms.
- Immunomodulatory drugs: are indicated in patients with rapid clinical deterioration or signs of systemic inflammation. Baricitinib (or tofacitinib upon availability) or tocilizumab (or sarilumab upon availability) may be used. Randomized (COV-BARRIER, ACTT-2) [336, 337] and open (REMAP-CAP, RECOVERY) [335, 338], clinical trials have shown survival benefits by adding immunomodulatory drugs in this class of patients. It must necessarily be considered the infectious risk in patients with concomitant severe bacterial or fungal infection, or at high risk of opportunistic infections.

Patients requiring mechanical ventilation or extracorporeal membrane oxygenation Dexamethasone is recommended for all patients of this class. The addition of tociluzumab should be considered for patients within 24 hours from the admission to the intensive care unit.

1.2.11 Prognosis

The prognosis of most patients is good, especially if young, without comorbidity and without severe COVID-19. Risk factors for a severe prognosis are: advanced age and male sex, the presence of comorbidities (mainly chronic lung conditions such as COPD, but also kidney injury, diabetes, hypertension, cardiovascular comorbidities, cancer, increased D-dimer, smoking and obesity [339]. The mortality rate varies from 0% for mild forms to 14.6% for severe forms of COVID-19 [340].

The term "Long-COVID" refers to the presence of long-term sequelae following an acute disease with symptoms that may persist for months (fatigue, weakness, pain, myalgia, dyspnea and cognitive dysfunction are commonly reported) [341].

1.2.12 Prevention

Vaccines

Vaccination is the most effective way to prevent serious disease and deaths from COVID-19. Since the beginning of the vaccination campaign in December 2021, the risk of death in unvaccinated subjects was 78 times higher than in vaccinated subjects [342].

Since the beginning of the vaccination campaign have been produced several types of anti-SARS-CoV-2 vaccines:

- mRNA vaccines: do not contain viral antigen, but are produced with a small synthetic fragment of messenger RNA (mRNA) coding for spike protein.
- Adenovirus viral vector vaccines: the adenoviral vector contains a fragment of DNA, or genetic material, that is used to produce the Spike protein of SARS-CoV-2, which then triggers the desired immune response.
- Protein subunit vaccines: contain a recombinant Spike protein of SARS-CoV-2 along with an adjuvant that triggers the desired immune response. There is a plausible causal relationship between the adenoviral vaccine and a rare and severe adverse event, vaccine-induced thrombosis with thrombocytopenia syndrome (VTT).
- Inactivated virus vaccines: contain the virus itself but chemically inactivated in culture.

All vaccines currently under study have been developed to induce a response that blocks the Spike protein and thus prevents cell infection.

Although anti-SARS-CoV-2 vaccination does not eliminate the risk of infection, it significantly reduces the risk of morbidity and mortality related to COVID-19, particularly in individuals who are at high risk of progressing to a serious disease [343].

mRNA vaccines The European Medicines Agency (EMA) and the Italian Medicines Agency (AIFA) have authorized two COVID-19 mRNA vaccines:

- mRNABNT162b2 (Comirnaty) of the pharmaceutical companies Pfizer-BioNTech: approved on 21 December 2020 and administered in two doses 21 days apart.

- COVID-19 Vaccine mRNA -1273 (Spikevax) of the pharmaceutical company Moderna: approved on 6 January 2021 and administered in two doses 28 days apart.

The two COVID-19 mRNA vaccines use molecules of mRNA, the transcript of the gene encoding the protein Spike. The vaccinated subjects cells produce the Spike protein that stimulates the immune system to produce specific antibodies against the Spike protein to counteract the entry of the virus into the cells. Vaccination also activates T cells that prepare the immune system to respond to additional exposure to SARS-CoV-2. The vaccine mRNA does not remain in the body, but degrades shortly after vaccination. Both are indicated for use from 6 months of age [344].

The booster dose (third dose) is a bivalent formulation, containing two mRNA of SARS-CoV-2 virus, one of the original SARS-CoV-2 strains and one of the other common BA.4 and BA.5 strains of the Omicron variant. A fourth booster dose is currently also indicated: a study conducted in Israel on 182.122 people who were 60 years old and who received the fourth vaccine dose, compared to subjects who received only the third dose of the vaccine Comirnaty, has proven an excellent effectiveness [345].

In almost all situations, mRNA vaccines and the protein subunit vaccines are preferred over the adenoviral viral vector vaccines due to the risk of serious adverse events.

Since Omicron 5 (BA.5) has become the most prevalent Omicron sub-variant worldwide, this has influenced research for effective vaccines against this variant:

- Comirnaty Original/Omicron BA.1: effective against the original SARS-CoV-2 and Omicron variant 1).

- Comirnaty Original/Omicron BA.4-5: effective against the original SARS-CoV-2 and the sub-variants Omicron BA.4 and BA.5.

- Spikevax Bivalent Original/Omicron BA.1: effective against the original SARS-CoV-2 and Omicron variant 1.

- Spikevax Bivalent Original/Omicron BA.4-5: effective against the original SARS-CoV-2 and against the sub-variants Omicron BA.4 and BA.5.

The most common side effect reported by those who received mRNA vaccines is mild-moderate pain at the injection site, which however resolves in a few days, while such subjects rarely report severe pain [346, 347].

There are also frequent reports of redness or swelling at the injection site. At the systemic level, the most common side effects were fatigue and headaches (more frequent after the second dose) and high fever (>38°C) [348].

As regards serious adverse effects, cases of lymphadenopathy have been reported, probably as a result of a robust immune response, which was resolved within 10 days. In addition, very rare cases of myocarditis and pericarditis have been reported, mainly in the two weeks following vaccination, most often after the second dose and in young men [349].

Adenovirus viral vector vaccines EMA and AIFA have so far authorised two COVID-19 viral vector vaccines:

- ChAdOx1-S (Vaxzevria) of the pharmaceutical company Astrazeneca and of the Oxford University: approved on 29 January 2021 and administered in two doses at least 28 days apart (up to 84 days apart).
- Ad26.COV2.S (Jcovden formerly COVID-19 Vaccine Janssen) of the pharmaceutical company Johnson & Johnson: approved on 11 March 2021 and administered in a single dose.

A viral vector vaccine uses a virus (usually an adenovirus rendered unable to replicate) to bring the gene encoding the Spike protein into the cell. The cells of the vaccinated subject produce the Spike protein from the introduced gene and the immune system activates against the protein and produces antibodies. Both are indicated for use from 18 years of age [344].

The most frequently reported adverse reactions for Vaxzevria vaccine are pain at the injection site, headache, myalgia and arthralgia, fatigue, fever (also >38°C) and nausea. Most adverse reactions are mild to moderate in severity and usually resolve within a few days from vaccination. If compared with the first dose, adverse reactions reported after the second dose were more mild and less frequent [350].

Following vaccination with Vaxzevria, the so-called "thrombosis with thrombocytopenia syndrome" also called "vaccine-induced immune thrombotic thrombocytopenia (VTT) has been observed very rarely. The component of the vaccine responsible for VTT is still unknown, but it is assumed that it is an adenoviral vector protein that leads to the formation of polyanions, negatively charged molecules that act, as heparin in heparin-induced thrombocytopenia (HIT). VTT includes severe cases that present as venous thrombosis, often in unusual locations such as cerebral venous sinus thrombosis, splanchnic venous thrombosis, as well as arterial thrombosis. Most cases occurred in the first three

weeks following vaccination, mainly in women under the age of 60 and some cases were fatal [351, 352].

In addition, another severe side effect of Vaxzevria vaccine of which very rare cases have been reported, is Capillary Leak Syndrome (CLS), a rare disorder characterized by acute episodes of edema that mainly affects the limbs, hypotension, hemoconcentration and hypoalbuminemia [353].

Finally, following vaccination with Vaxzevria, Guillain-Barré syndrome (GBS) has very rarely been reported [354].

The mild side effects of the Jcovden vaccine are the same, in intensity and frequency, of the Vaxzevria vaccine. In addition, cases of VTT, CLS and GBS were also observed for this vaccine, also in this case very rarely [352, 353, 355].

Protein subunit vaccines The EMA and AIFA have authorized two COVID-19 protein subunit vaccines:

- Nuvaxovid of the pharmaceutical company Novavax
- VidPrevtyn Beta of the pharmaceutical company Sanofi

Protein subunit vaccines are composed of protein fragments of the virus. In the production of this type of anti-COVID-19 vaccines, a portion of DNA coding for the Spike protein is inserted inside a baculovirus, exploited in vitro for the production and amplification of the Spike protein. Spike protein are then purified and compacted to obtain viral nanoparticles containing Spike protein. These particles, with the addition of an adjuvant molecule used to further stimulate the immune system, are injected into the human body to obtain the production of antibodies against the Spike protein of SARS-CoV-2.

Nuvaxovid is indicated for use in subjects at least 18 years of age, while VidPrevtyn Beta is only indicated for use as a booster dose in adults who have previously received a mRNA or adenoviral vector vaccine [344].

Inactivated virus vaccines The EMA and the AIFA have authorized only one inactivated virus vaccine: it is the Valneva of the pharmaceutical company of the same name.

Inactivated virus vaccines are produced by cultivating SARS-CoV-2 virus in cell cultures and subsequently by chemically inactivating it. When the vaccine is administered, the immune system identifies the inactivated virus as foreign and produces antibodies and T cells against it. Its use is currently indicated in subjects aged between 18 and 50 years [344].

Vaccination during pregnancy and lactation As for pregnant women, initially vaccination was indicated only for women at higher risk of exposure to the virus (e.g. health professionals) or at higher risk of developing a serious disease (e.g. women with chronic comorbidities), due to the lack of conclusive studies about its safety in this group of patients. The difficulty in obtaining data is due to the fact that pregnant women are not eligible for clinical trials.

To date, however, there is enough data on vaccination in pregnant and breastfeeding women, in particular for Pfizer-BioNtech and Moderna mRNA vaccines, therefore, both for the Centers for Disease Control and Prevention (CDC) and for the italian Istituto Superiore di Sanità (ISS) have expanded claims on vaccination against COVID-19 in pregnancy and lactation. In fact, over time several studies have proven that the vaccine is safe in pregnancy for both the fetus and the mother and that the benefit/risk ratio is better than that of SARS-CoV-2 infection for both [356, 357]. Vaccination with mRNA vaccines is, therefore, recommended to all pregnant women who wish to vaccinate in the second and third trimesters while there is still little evidence about safety in the first trimester [358]. In addition, if a vaccinated woman discovers that she is pregnant after receiving the vaccine, there is no reason to suggest abortion.

Lactating women can also be vaccinated without the need to stop breastfeeding, as it has been seen that vaccination does not expose the infant to risks, rather it allows him to take antibodies against SARS-CoV-2 through milk [359].

Social norms during the pandemic

In order to limit the transmission of SARS-CoV-2, the implementation of collective prevention measures was also important: maintaining the interpersonal distance of at least 1.5 meters, adhering to correct behaviors in terms of personal hygiene (periodically washing and disinfecting hands, sneezing or coughing in a handkerchief or in the elbow socket, wearing masks and gloves) and environmental hygiene (renew the air indoors by opening windows and keeping the rooms very clean).

Isolation and self-monitoring are important public health measures implemented during the pandemic to prevent the occurrence of additional secondary cases due to transmission of SARS-CoV-2 and to avoid overloading the hospital system. Persons tested positive for the diagnostic test (molecular or antigenic) for SARS-CoV-2 are subjected to home isolation, which consists in separating the subject positive to SARS-CoV-2 from healthy subjects in order to

prevent the spread of infection, during the period of transmission. The duration of the isolation was changed during the pandemic in accordance with new scientific findings and according to the rules in force in the country concerned. The quarantine, instead, concerns clinically healthy subjects who have been identified as close contacts of subjects diagnosed positive. Those who have had close contact with confirmed subjects positive to SARS-CoV-2 have been applied, in the last phase of the pandemic, the regime of self-monitoring, consisting in the obligation to wear respiratory protection devices on the go.

From the social point of view, however, as the SARS-CoV-2 pandemic was declared a world health emergency, the different countries have implemented preventive measures which have been modified according to the course of the pandemic in terms of infection and saturation of hospital facilities. These measures ranged from the obligation to wear the mask, closure of activities not considered viable, curfew, blocking social events, tracking of travel by self-certification.

From a health point of view, one of the most important innovations implemented in order to ensure the continuity of safe care has been the rediscovery of telemedicine [360].

1.3 The impact of SARS-CoV-2 pandemic on inflammatory arthritis

Since the beginning of the SARS-CoV-2 pandemic in late 2019, there has been growing concern among physicians and patients dealing with inflammatory arthritis (IA), regarding the risk of joint disease flare-up or developing more severe clinical manifestations of COVID-19 than the general population. Most of these patients are undergoing immunosuppressant therapy, mainly anti-cytokine therapy. Although this may result in a greater risk of developing severe infections, many cytokines such as IL-1, IL-6 and TNF-α are involved in the cytokine storm that determines the severity of COVID-19 [361].

Several studies have indicated a similar or slightly increased severity of COVID-19 and risk of hospitalization in patients with inflammatory joint disease, and more widely in rheumatic diseases, vs. the general population [362–368]. There was no association between SARS-CoV-2 infection and joint disease exacerbation [369, 370]. The serological response in patients with IA was surprisingly higher than expected based on reported symptoms [371], despite immunosuppressive therapy. However, some data suggests that patients with RA appear to have a higher risk of contracting SARS-CoV-2 infection [372], developing more severe COVID-19 [373] and joint disease exacerbation following infection [374], and need for therapeutic switch [375], vs. patients with SpA.

The launch of the COVID-19 vaccination campaign in late 2020 raised some concerns among patients and physicians about the risk of developing adverse events following immunization (AEFI) and joint disease exacerbation following the vaccination. Several studies have found that patients with IA, or rheumatic diseases in general, do not carry a higher risk [376–379] than the general population. Although joint disease flare-ups following vaccination appear to be very rare [380–382] or completely absent in some cohorts [383, 384], some studies conducted on larger populations found a slightly higher incidence of exacerbation than previously reported, estimated at <20% [385, 386]. These flare-ups are generally mild and easily manageable with therapy [387]. Predisposing factors include the use of corticosteroids, a history of other autoimmune disease and the presence of a previous exacerbation over the past 12 months [388].

The latest EULAR recommendations [389] and the ACR recommendations [390] concur on the importance of the COVID-19 vaccine in patients with rheumatic disease, stressing that there is a theoretical risk of a joint disease flare-up albeit much lower than the benefit conferred by immunization during a pandemic.

AIM OF THE STUDY

This study was designed to evaluate the impact of SARS-CoV-2 infection and/or vaccination in a cohort of patients with IA in Northeast Italy. The objectives of the study were:

- The primary endpoint was to evaluate the presence of joint disease flare-ups following SARS-CoV-2 infection or vaccination, by comparing disease activity indices before and after infection and/or vaccination.
- The secondary endpoints were:
  - The identification of possible predictive factors of flare-ups such as age, gender, comorbidity, baseline disease activity grade or class of anti-rheumatic drugs.
  - The risk of flare-ups between the two different clinical entities considered in the study (seronegative spondyloarthritis and rheumatoid arthritis) after infection and/or vaccination.
  - The incidence of SARS-CoV-2 infection, the severity of COVID-19 and any adverse events following immunization (AEFI) in cases and controls.

MATERIALS AND METHODS

3.1 Design, setting and study population

We conducted a retrospective cohort study and enrolled all consecutive consecutive patients with IA who attended the Spondyloarthritis Clinic at Padova University Hospital and Arthritis Clinic-San Bortolo Hospital (Vicenza), as well as healthy controls attending the Occupational Medicine Clinic for routine health surveillance activities between May 2020 and May 2022. Patients with IA and a confirmed diagnosis of AS according to the modified New York criteria [42], PsA according to the CASPAR criteria [87] and RA according to the ACR criteria [148] were included.

Exclusion criteria were:

- Patients under the age of 18.
- Patients unable or who refused to provide written informed consent.

No patients fulfilled the exclusion criteria.

All enrolled patients provided written informed consent, in accordance with the principles of the Declaration of Helsinki. Each participating Centre received the approval of the local Ethics Committee [approval no. CESC code: 4930/AO/2. URC: AOP2073], as well as the written informed consent for the anonymous use of personal data from every patient, in compliance with Italian Legislative Decree 196/2003.

3.2 Outcome measures

All the patients were evaluated through a telemedicine or face-to-face visit at one of the scheduled assessment visits and all data were collected through an interview and/or by reviewing the patients medical records.

3.2.1 Demographics and clinical data

Demographics and clinical data were collected as follows:

- Patient identification code
- Age
- Sex
- Type of rheumatic disease (AS, PsA or RA)
- Comorbidities (yes/no):
  - Metabolic disease:
    - Obesity (BMI >30)
    - Diabetes
  - Vascular disease:
    - Hypertension
    - Coronary heart disease
    - Cerebrovascular disease
  - Pulmonary disease:
    - Asthma
    - Chronic Obstructive Pulmonary Disease (COPD)
    - Pulmonary fibrosis
  - Neoplastic disease
- Ongoing medications (yes/no):
  - bDMARDs:
    - Anti-TNF-α
    - Anti-IL-17A
    - Anti-IL-23
    - Anti-IL-6
    - CTLA-4 Ig (abatacept)
    - Anti-CD20 (rituximab)
  - csDMARDs:
    - Methotrexate

    - Leflunomide
    - Sulfasalazine
    - Hydroxychloroquine
  - tsDMARDs:
    - Phosphodiesterase 4 inhibitors (apremilast)
    - JAK inhibitors
  - Corticosteroids

3.2.2 Disease activity

Disease activity was assessed by ASDAS-CRP [51] for AS and DAS28-CRP [161–163] for PsA and RA. Based on this score the disease activity was classified as follows:

- Remission: ASDAS-CRP < 1.3 or DAS28-CRP <2.6.
- Low disease activity: ASDAS-CRP ≥ 1.3 and < 2.1 or DAS28-CRP ≥ 2.6 and <3.2
- High disease activity: ASDAS-CRP ≥ 2.1 or DAS28-CRP ≥ 3.2.

3.2.3 SARS-CoV-2 infection and vaccination

Data on SARS-CoV-2 infection and vaccination were also collected as follows:

- SARS-CoV-2 infection (yes/no)
  - Date of the positive swab
  - Date of the negative swab
  - Symptoms of COVID-19
  - Severity of COVID-19
  - Hospitalization (yes/no)
  - Joint disease flare-up following infection (yes/no):
    - NSAIDs need to control flare-up (yes/no)
    - Therapeutic switch following the flare up (yes/no)
- Anti-SARS-CoV-2 vaccination (yes/no):
  - If not, reason for missed vaccination

- If yes:
  - Number of vaccine shots received
  - Booster shot (yes/no)
  - Date of the last vaccine shot
  - Type of vaccine received
  - Therapy stopped to allow vaccination (yes/no)
  - Adverse effects within 48 hours of vaccination (yes/no):
    - After what shot
    - Kind of side effect
  - Joint disease flare-up within 1 month (yes/no)
    - NSAIDs need to control the flare-up (yes/no)
    - Therapeutic switch following the flare-up (yes/no)

SARS-CoV-2 infection was considered only if documented in accordance with current laws in Italy, first only via nasopharyngeal swab for molecular tests, and later via nasopharyngeal swab for rapid antigen tests.

The severity of COVID-19 was assessed as indicated by the National Institute of Health (NIH): "asymptomatic or presymptomatic infection [no symptoms that are consistent with COVID-19]; mild illness [any of the various signs and symptoms of COVID-19, e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell but not shortness of breath, dyspnea, or abnormal chest imaging]; moderate illness [evidence of lower respiratory disease during clinical assessment or imaging and oxygen saturation measured by pulse oximetry (SpO2) $\geq$94% on room air at sea level]; severe illness [SpO2 <94% on room air at sea level, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, respiratory rate >30 breaths/min, or lung infiltrates >50%], critical illness [respiratory failure, septic shock, and/or multiple organ dysfunction]" [300].

Data regarding SARS-CoV-2 infection or vaccination status were compared with controls.

Side effects were assessed in accordance with the WHO guidelines on AEFI [391, 392].

All disease flares were documented in medical reports, laboratory evaluations, describing symptoms, disease activity score, and the patients clinical history. All patients were evaluated during telemedicine visits and those who needed to switch therapy were evaluated in face-to-face visits.

3.3 Statistical analysis

Data distribution (normal or not normal) was verified through graphical representation and then verified using the Shapiro-Wilk normality test. The data were expressed as mean (standard deviation) in case of normal distribution, and median (interquartile range IQR) in case of non-normal distribution, for continuous variables. Categorical variables were expressed as numbers (percentages). Baseline characteristics across the 3 groups (AS, PsA, and RA) were compared through the Mann-Whitney test for independent samples in the case of continuous variables and Chi-square ($\chi 2$) for categorical variables. Comparison between 2 groups (patients and controls) were performed using Wilcoxon rank sum/signed rank tests (as most data were not normally distributed) for continuous variables, and $\chi 2$ test for categorical variables, as appropriate. A logistic regression analysis was carried out to identify predictors of disease flare-ups. The following covariates were examined: age, sex, comorbidity, baseline disease activity grade, class of anti-rheumatic drugs and SARS-CoV-2 infection.

All statistical analyses were carried out using GraphPAD, PRISM9program and SPSS 27.0 statistical software; p values <0.05 were considered as significant.

RESULTS

The demographic and baseline characteristics of the study cohort and healthy control group are highlighted in Table XVIII.

Between May 2020 and May 2022, we enrolled a total of 362 patients, 182 (50.3%) females and 180 (49.7%) males with a median age of 57 years, IQR 47-66, and 165 HC, 22 (13.3%) females and 143 (86.7%) males with a median age of 45 years, IQR 34-52. Sex distribution between the two groups and age at enrollment were statistically significant ($p < 0.0001$ for both).

Among 362 patients with IA, 94 (26.0%) patients were affected by RA, 158 (43.6%) PsA, and 110 (30.4%) AS. Most patients, 223 (61.6%), were in clinical remission (ASDAS-CRP <1.3 or DAS28-CRP <2.6), 58 (16.0%) had low disease activity (ASDAS-CRP 1.3-2.0, DAS28-CRP 2.6-3.1), 81 (22.4%) had an active disease (ASDAS-CRP ≥2.1, DAS28-CRP ≥3.2).

Forty-one (11.3%) patients received steroid therapy with an equivalent dose of prednisone 5-15 mg daily. Methotrexate was the most frequently prescribed csDMARD in 91 (25.1%) patients, compared to leflunomide prescribed in 22 (6.1%), sulfasalazine prescribed in 16 (4.4%) and hydroxychloroquine prescribed in 8 (2.2%). Anti-TNF-α were the most frequently prescribed bDMARDs in 197 (54.4%) patients compared to anti-CD20 prescribed in 7 (1.9%), abatacept prescribed in 16 (4.4%), anti-IL-6 prescribed in 14 (3.9%), anti-IL-17 prescribed in 65 (18.0%), anti-IL-23 prescribed in 8 (2.2%). JAKi were prescribed in 13 (3.6%) patients and apremilast was prescribed in 8 (2.2%).

As regards comorbidities, 115 (33.5%) patients had cardiovascular comorbidities (hypertension, coronary heart disease, cerebrovascular disease), 28 (12.7%) had diabetes, 43 (18.9%) had obesity (BMI>30), 17 (4.9%) had pulmonary comorbidities (asthma, COPD, pulmonary fibrosis) and 17 (4.9%) had cancer. Cases had a statistically significant prevalence of cardiovascular diseases as well as obesity and diabetes ($p<0.0001$ for all), whereas there was no difference in pulmonary diseases and cancer vs. healthy controls.

Table XVIII: Demographic and baseline features of the study cohort and healthy control group

	Study cohort (n=362)	**Healthy control group (n=165)**	**p-value**
Females, n (%)	182 (50.3)	22 (13.3)	<0.0001
Median age, years (IQR)	57 (47-66)	45 (34-52)	<0.0001
Disease			
Rheumatoid Arthritis, n (%)	94 (26.0)		
Psoriatic Arthritis, n (%)	158 (43.6)		
Ankylosing Spondylitis, n (%)	110 (30.4)		
Therapy			
Steroids, dose prednisone 5-15 mg/day, n (%)	41 (11.3)		
csDMARDs			
- Methotrexate, n (%)	91 (25.1)		
- Leflunomide, n (%)	22 (6.1)		
- Sulfasalazine, n (%)	16 (4.4)		
- Hydroxychloroquine, n (%)	8 (2.2)		
bDMARDs			
- Anti-TNF-α, n (%)	197 (54.4)		
- Anti-CD20, n (%)	7 (1.9)		
- Abatacept, n (%)	16 (4.4)		
- Anti-IL-6, n (%)	14 (3.9)		
- Anti-IL-17, n (%)	65 (18.0)		
- Anti-IL-23, n (%)	8 (2.2)		
JAKi, n (%)	13 (3.6)		
Apremilast, n (%)	8 (2.2)		
Disease Activity ^			
Active Disease, n (%)	81 (22.4)		
Low Disease Activity, n (%)	58 (16.0)		
Remission, n (%)	223 (61.6)		
Comorbidities			
Cardiovascular disease, n (%)	115 (33.5)	15 (9.1)	<0,0001
Diabetes, n (%)	28 (12.7)	1 (0.6)	<0,0001
Obesity, n (%) *	43 (18.9)	0 (0)	<0,0001
Pulmonary disease, n (%)	17 (4.9)	6 (3.6)	ns
Cancer, n (%)	17 (4.9)	4 (2.4)	ns

IQR, interquartile range; csDMARDs, conventional disease modifying antirheumatic drugs; bDMARDs, biological disease modifying antirheumatic drugs; TNF-α, tumor necrosis factor α; CD20, cluster of differentiation 20 (B-lymphocyte antigen); IL, interleukin; JAKi, Janus kinase inhibitors.
^Disease activity evaluated by ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score-C Reactive protein) and DAS28-CRP (Disease Activity Score-C Reactive Protein), defined as remission (ASDAS-CRP <1.3, DAS28-CRP <2.6), low disease activity (ASDAS-CRP 1.3-2.0, DAS28-CRP 2.6-3.1), active disease (ASDAS-CRP ≥2.1, DAS28-CRP ≥3.2).
* Obesity evaluated by BMI (Body Mass Index), defined as BMI >30.0.

4.1 Impact of SARS-CoV-2 infection on inflammatory arthritis

Clinical characteristics relating to SARS-CoV-2 infection in our study population are reported in Table XIX.

Table XIX: Prevalence and severity of SARS-CoV-2 infection in inflammatory arthritis

	Inflammatory arthritis	**Healthy controls**	**p-value**	**OD (95CI)**
SARS-CoV-2 rate, n (%) ∘	117 (32.3)	39 (23.6)	0.05	1.54 (1.01-2.34)
SARS-CoV-2 severity *				
Asymptomatic, n (%)	9 (7.7)	1 (2.6)		
Mild, n (%)	95 (81.2)	35 (92.1)		
Moderate, n (%)	6 (5.1)	0 (0)	ns	
Severe, n (%)	2 (1.7)	1 (2.6)		
Critical, n (%)	5 (4.3)	1 (2.6)		
Hospitalization rate, n (%)	11 (9.4)	2 (5.1)	ns	
Death, n (%)	1 (0.9)	0 (0)	ns	

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
∘ SARS-CoV-2 infection was considered only if documented in accordance with current laws in Italy, first only via nasopharyngeal swab for molecular tests, and later also via nasopharyngeal swab for rapid antigen tests.
* SARS-CoV-2 severity in healthy controls calculated from 38 subjects.

One hundred-seventeen (32.3%) patients and 39 (23.6%) controls contracted a SARS-CoV-2 infection during the study period. However, the infection rate was not statistically significant (p=0.05 OR 1.5, 95% CI: 1.0 to 2.3).

Mild infection was the most frequent clinical presentation of SARS-CoV-2 infection: 95 (81.2%) patients vs. 35 (92.1%) of controls. Among the remaining, 9 (7.7%) patients and 1 (2.6) control had an asymptomatic infection, 6 (5.1%) patients and no control had a moderate COVID-19, 2 (1.7%) patients and 1 (2.6%) control had a severe COVID-19, 5 (4.3%) patients and 1 (2.6%) control had a critical COVID-19.

Hospitalization rate was higher among cases than healthy controls: 11 (9.4%) patients vs. 2 (5.1%) of controls, though not statistically significant.

One (0.9%) death due to COVID-19 was recorded in our study cohort.

The clinical features of inflammatory arthritis according to SARS-CoV-2 infection status are reported in Table XX.

Among the 117 patients who contracted SARS-CoV-2 infection, 62 (53.0%) were females and 55 (47.0%) were males. The median age of patients with infection was 55 (IQR 44-62) compared to 58 (IQR 48-68) of patients without infection and this difference was statistically significant (p=0.02).

Twenty-one (18.0%) patients with RA, 52 (44.4%) with PSA and 44 (37.6%) with AS had SARS-CoV-2 infection. The prevalence of SARS-CoV-2 infection was significantly different between the IA subgroups (p=0.03); in particular patients with AS had significantly higher infection rates vs. RA (p=0.01), as shown in Figure 9.

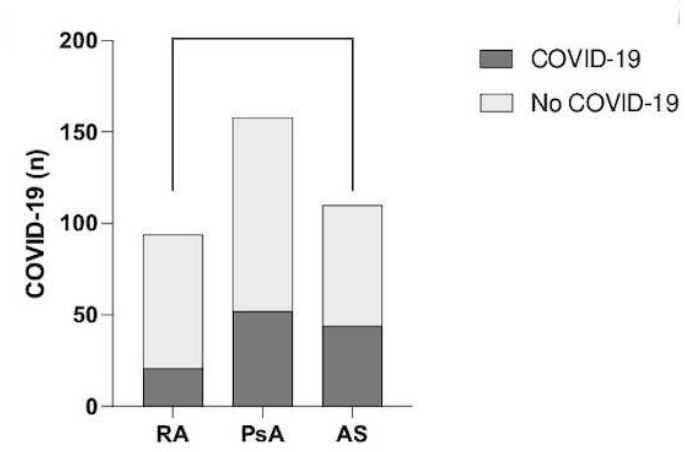

Figure 9: SARS-CoV-2 infection rate in the different inflammatory arthritis subgroups (AS vs. RA p=0.01)

Table XX: Clinical features of inflammatory arthritis according to SARS-CoV-2 infection status

	Total population (n=362)	**SARS-CoV-2 infection (n=117)**	**No SARS-CoV-2 infection (n=245)**	**p-value**
Females, n (%)	182 (50.3)	62 (53.0)	120 (49.0)	ns
Median age, years (IQR)	57 (47-66)	55 (44-62)	58 (48-68)	0.02
Disease				
Rheumatoid Arthritis, n (%)	94 (26.0)	21 (18.0)	73 (29.8)	
Psoriatic Arthritis, n (%)	158 (43.6)	52 (44.4)	106 (43.3)	0.03
Ankylosing Spondylitis, n (%)	110 (30.4)	44 (37.6)	66 (26.9)	
Therapy				
Steroids, dose prednisone 5-15 mg/day, n (%)	41 (11.3)	10 (8.5)	31 (12.7)	ns
csDMARDs				
- Methotrexate, n (%)	91 (25.1)	25 (21.4)	66 (26.9)	
- Leflunomide, n (%)	22 (6.1)	5 (4.3)	17 (6.9)	ns
- Sulfasalazine, n (%)	16 (4.4)	7 (6.0)	9 (3.7)	
- Hydroxychloroquine, n (%)	8 (2.2)	1 (0.9)	7 (2.9)	
bDMARDs				
- Anti-TNF-α, n (%)	210 (54.4)	67 (57.3)	143 (58.4)	
- Anti-CD20, n (%)	7 (1.9)	2 (1.7)	5 (2.0)	
- Abatacept, n (%)	16 (4.4)	4 (3.4)	12 (4.9)	
- Anti-IL-6, n (%)	14 (3.9)	1 (0.9)	13 (5.3)	ns
- Anti-IL-17, n (%)	65 (18.0)	25 (21.4)	40 (16.3)	
- Anti-IL-23, n (%)	8 (2.2)	5 (4.3)	8 (3.3)	
JAKi, n (%)	13 (3.6)	4 (3.4)	9 (3.7)	
Apremilast, n (%)	8 (2.2)	0 (0)	8 (3.3)	
Disease Activity ^				
LDA/Active Disease, n (%)	139 (38.4)	54 (46.2)	85 (34.7)	0.04
Remission, n (%)	223 (61.6)	63 (53.8)	160 (65.3)	
Comorbidities				
Cardiovascular disease, n (%)	115 (31.8)	35 (29.9)	80 (32.6)	
Diabetes, n (%)	28 (7.7)	8 (6.8)	20 (8.2)	
Obesity *, n (%)	43 (11.9)	15 (12.8)	28 (11.4)	ns
Pulmonary disease, n (%)	17 (4.7)	10 (8.5)	7 (2.8)	
Cancer, n (%)	17 (4.7)	9 (7.7)	8 (3.3)	

IQR, interquartile range; csDMARDs, conventional disease modifying antirheumatic drugs; bDMARDs, biological disease modifying antirheumatic drugs; TNF-α, tumor necrosis factor α; CD20, cluster of differentiation 20 (B-lymphocyte antigen); IL, interleukin; JAKi, Janus kinase inhibitors; LDA, low disease activity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^Disease activity evaluated by ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score-C Reactive Protein) and DAS28-CRP (Disease Activity Score-C Reactive Protein), defined as remission (ASDAS-CRP <1.3, DAS28-CRP <2.6), low disease activity (ASDAS-CRP 1.3-2.0, DAS28-CRP 2.6-3.1), active disease (ASDAS-CRP $\geq$2.1, DAS28-CRP $\geq$3.2)

* Obesity evaluated by BMI (Body Mass Index), defined as BMI >30.0.

Among patients who contracted a SARS-CoV-2 infection, 10 (24.4%) received steroid therapy. Methotrexate was the most frequently prescribed csDMARD in 25 (21.4%) patients with SARS-CoV-2 infection, compared to leflunomide prescribed in 5 (4.3%), sulfasalazine prescribed in 7 (6.0%) and hydroxychloroquine prescribed in 1 (0.9%). Anti-TNF-α were the most frequently prescribed bDMARDs in 67 (57.3%) patients with infection, compared to anti-CD20 prescribed in 2 (1.7%), abatacept prescribed in 4 (3.4%), anti-IL-6 prescribed in 1 (0.9%), anti-IL-17 prescribed in 25 (21.4%) and anti-IL-23 prescribed in 5 (4.3%). JAKi were prescribed in 4 (3.4%) patients with infection while no one received apremilast.

Sixty-three (53.8%) patients with IA in remission contracted a SARS-CoV-2 infection vs. 54 (46.2%) patients with active/low disease activity. The prevalence of COVID-19 was significantly higher in patients in remission vs. those with high/low disease activity (p=0.04 OR 0.62, 95% CI: 0.39 to 0.97) as shown in Figure 10.

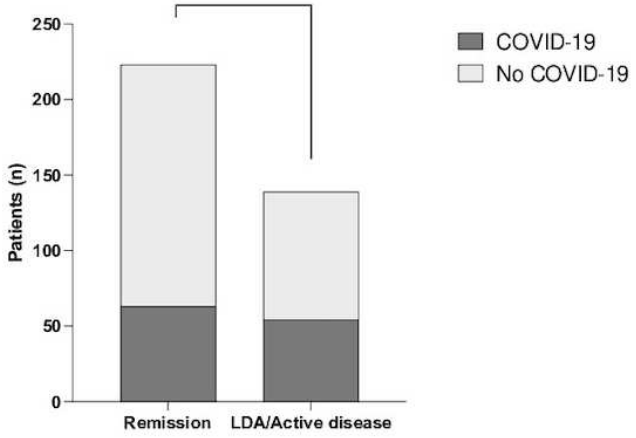

Figure 10: COVID-19 rate according to disease activity of inflammatory arthritis (p=0.04)

Thirty-five (29.9%) patients with SARS-CoV-2 infection had cardiovascular comorbidities, 8 (6.8%) had diabetes, 15 (12.8%) had obesity, 10 (8.5%) had pulmonary comorbidities and 9 (7.7%) had cancer.

We found no significant differences in terms of SARS-CoV-2 infection rate and severity according to the different therapies or comorbidities.

Forty (34.2%) patients experienced a flare within one month of COVID-19. A significantly higher rate of flare-ups was observed among patients who contracted a SARS-CoV-2 infection (p=0.01 OR 1.86, 95% CI: 1.16 to 3.05) vs. those without infection, as shown in Figure 11.

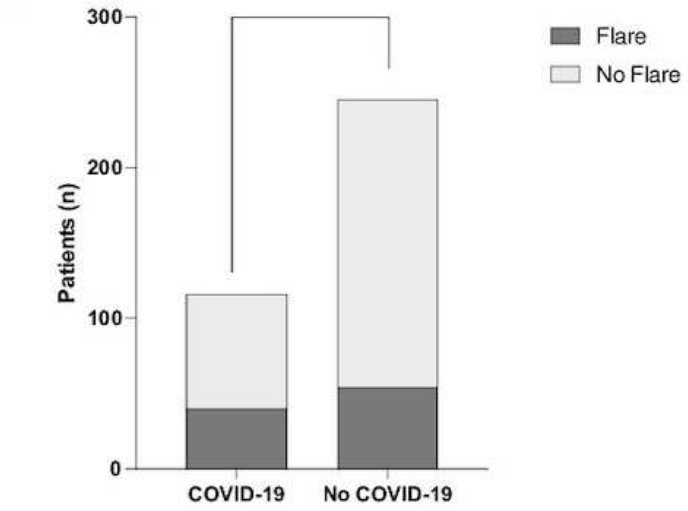

Figure 11: The prevalence of inflammatory disease flares in relation to SARS-CoV-2 infection

The median (IQR) ASDAS-PCR and DAS28-PCR during flares were 2.8 (2.4-3.7) and 3.5 (3.0-4.1), respectively significantly higher vs. before flares (p<0.001 for both). The need to switch to another therapy or initiate NSAIDs occurred in 3/40 (7.5%) and 13 (32.5%) patients, respectively.

4.2 Impact of SARS-CoV-2 vaccination on inflammatory arthritis

The clinical characteristics of patients with IA and vaccination status are reported in Table XXI.

Three-hundred-thirty-one (91.4%) patients received at least one dose of a SARS-CoV-2 vaccine, 166 (50.2%) females and 165 (49.8%) males, with a median age of 57 years, IQR 47-66. Eighty-two (24.8%) of these patients were affected by RA, 144 (43.5%) by PsA and 105 (31.7%) by AS. Among vaccinated patients, 39 (11.7%) received steroid therapy. Methotrexate was the most frequently prescribed csDMARD in 83 (25.1%) patients with vaccination, compared to leflunomide prescribed in 22 (6.6%), sulfasalazine prescribed in 16

(4.8%) and hydroxychloroquine prescribed in 7 (2.1%). Anti-TNF-α were the most frequently prescribed bDMARDs in 190 (57.4%) patients with vaccination, compared to anti-CD20 prescribed in 7 (2.1%), abatacept prescribed in 12 (3.6%), anti-IL-6 prescribed in 12 (3.6%), anti-IL-17 prescribed in 62 (18.7%) and anti-IL-23 prescribed in 8 (2.4%). JAKi were prescribed in 12 (3.6%) patients with vaccination while 8 (2.4%) patients received apremilast. Two-hundred-two (61.0%) patients with IA in remission received vaccination vs. 129 (39.0%) patients with active/low disease activity, although this difference was not statistically significant. One-hundred-six (32.0%) patients with vaccination had cardiovascular comorbidities, 26 (7.9%) had diabetes, 41 (12.4%) had obesity, 16 (4.8%) had pulmonary comorbidities and 17 (5.1%) had cancer. There was no difference in the SARS-CoV-2 vaccination rate between cases and healthy controls, as well as between the different subsets of IA. No difference was observed regarding the different therapeutic regimens and the comorbidities between vaccinated patients vs. unvaccinated. Most patients, 273 (83.2%), received Comirnaty (BioNTech/Pfizer) vaccine, compared to 28 (8.6%) who received Spikevax (Moderna) vaccine and 27 (8.2%) who received Vaxzevria (Oxford/Astrazeneca) vaccine. In our cohort 65.5%, 32.3%, and 2.1% of the patients received three, two, and one dose of the vaccine, respectively. Among healthy controls, 91.8% and 8.2% received two and one dose, respectively.

Thirty-one (8.6%) patients was unvaccinated, 16 (51.6%) females and 15 (48.4%) males, with a median age of 60 years, IQR 48-63. Twelve (38.7%) of these patients were affected by RA, 14 (45.2%) by PsA and 5 (16.1%) by AS. Among unvaccinated patients, 2 (6.5%) received steroid therapy. Methotrexate was the most frequently prescribed csDMARD in 8 (25.8%) patients without vaccination, compared to hydroxychloroquine prescribed in 1 (3.2%). No unvaccinated patients received leflunomide or sulfasalazine. Anti-TNF-α were the most frequently prescribed bDMARDs in 20 (64.5%) patients without vaccination, compared to abatacept prescribed in 4 (12.9%), anti-IL-6 prescribed in 2 (6.5%) and anti-IL-17 prescribed in 3 (9.7%). No unvaccinated patients received anti-CD20 or anti-IL-23. JAKi were prescribed in 1 (3.2%) patient without vaccination while no unvaccinated patients received apremilast. Twenty-one (67.7%) patients with IA in remission was unvaccinated vs. 10 (32.3%) patients with active/low disease activity. Nine (29.0%) patients without vaccination had cardiovascular comorbidities, 2 (6.5%) had diabetes, 2 (6.5%) had obesity, 1 (3.2%) had pulmonary comorbidities and no one had cancer. Fear was the most frequently recorded reason for not getting vaccinated in 18 (58.1%) unvaccinated patients,

compared to medical contraindication in 7 (22.6%) and other reasons in 6 (19.3%). The reasons for non-vaccination were similar between males and females: fear in 9 (60.0%) males and 9 (56.3%) females, medical contraindication in 3 (20.0%) males and 4 (25.0%) females, other reasons in 3 (20.0%) males and 3 (18.7%) females.

AEFI in patients with IA are highlighted in Table XXII. One-hundred-two (30.8%) vaccinated patients had AEFI within 48 hours, and among them 51 (50.0%) had fever, 43 (42.2%) arthralgia and 65 (63.7%) asthenia. Forty-three (42.2%) patients with active AI experienced AEFI within 48 hours vs. 59 (57.8%) with AI in remission, 15 (38.5%) patients who received steroid therapy experienced AEFI within 48 hours vs. 87 (29.8%) without steroid therapy, 36 (28.6%) patients who received csDMARDs experienced AEFI within 48 hours vs. 66 (32.3%) without csDMARDs, 95 (30.5%) patients who received bDMARDs experienced AEFI within 48 hours vs. 7 (36.8%) without bDMARDs, 64 (38.6%) females experienced AEFI within 48 hours vs 38 (23.0%) males. Eighteen (41.9%) patients with active AI had fever vs. 33 (55.9%) with AI in remission, 8 (53.3%) patients who received steroid therapy had fever vs. 43 (49.4%) without steroid therapy, 19 (52.8%) patients who received csDMARDs had fever vs. 32 (48.5%) without csDMARDs, 49 (51.6%) patients who received bDMARDs had fever vs. 2 (28.6%) without bDMARDs, 34 (53.1%) females had fever vs 17 (44.7%) males. Nineteen (44.2%) patients with active AI had arthralgia vs. 24 (40.7%) with AI in remission, 5 (33.3%) patients who received steroid therapy had arthralgia vs. 38 (43.7%) without steroid therapy, 17 (47.2%) who patients received csDMARDs had arthralgia vs. 26 (39.4%) without csDMARDs, 37 (38.9%) patients who received bDMARDs had arthralgia vs. 6 (46.2%) without bDMARDs, 27 (42.2%) females had arthralgia vs 16 (42.1%) males. Twenty-six (66.1%) patients with active AI had asthenia vs. 39 (60.5%) with AI in remission, 11 (73.3%) patients who received steroid therapy had asthenia vs. 54 (62.1%) without steroid therapy, 24 (66.7%) patients who received csDMARDs had asthenia vs. 41 (62.1%) without csDMARDs, 63 (66.3%) patients who received bDMARDs had asthenia vs. 2 (28.6%) without bDMARDs, 41 (64.1%) females had asthenia vs 24 (63.2%) males.

The prevalence of vaccine side effects was significantly higher in the control group vs. patients (44.9% vs. 30.8%, p=0.005 OR 0.55, 95% CI 0.37-0.82).

Fifty-two (15.7%) patients experienced a joint disease flare within one month of vaccination vs. 9 (29%) unvaccinated patients. However, the flare rate was not significantly different between vaccinated vs. unvaccinated patients. Twenty-three (14.4%) patients with active AI experienced a joint disease flare within one month of vaccination vs. 29 (17.8%) with AI in remission, 6 (15.4%) patients who received

steroid therapy experienced a joint disease flare within one month of vaccination vs. 46 (15.7%) without steroid therapy, 18 (14.3%) patients who received csDMARDs experienced a joint disease flare within one month of vaccination vs. 34 (16.6%) without csDMARDs, 48 (15.4%) patients who received bDMARDs experienced a joint disease flare within one month of vaccination vs. 4 (21.1%) without bDMARDs, 30 (18.1%) females experienced a joint disease flare within one month of vaccination vs 22 (13.3%) males.

There was a higher rate of active disease in the last 12 months among patients who experienced flares vs. those who did not (30.8 % vs. 20.1%), though the difference was not statistically significant. The median (IQR) ASDAS PCR and DAS28 PCR during flares were 3.2 (2.6-3.6) and 3.7 (3.1-4.6), respectively significantly higher vs. before flares (p=0.003 and p=0.04, respectively). Twelve (23.1%) and 29 (55.8%) patients switched to another therapy or initiated NSAIDs, respectively. In the multivariate analysis, we did not find any independent predictors of IA flares.

Thirty-five (10.5%) vaccinated patients had other AEs within 30 days of vaccination. Fifteen (9.9%) patients with active AI had other AEs within 30 days of vaccination vs. 20 (11.6%) with AI in remission, 7 (17.9%) patients who received steroid therapy had other AEs within 30 days of vaccination vs. 28 (9.6%) without steroid therapy, 12 (9.5%) patients who received csDMARDs had other AEs within 30 days of vaccination vs. 23 (11.2%) without csDMARDs, 33 (10.6%) patients who received bDMARDs had other AEs within 30 days of vaccination vs. 2 (10.5%) without bDMARDs, 26 (15.7%) females had other AEs within 30 days of vaccination vs 9 (5.5%) males.

Females reported a significantly higher rate of AEs both within 48 hours and within 30 days from the vaccination (p=0.003 and p=0.004). However, there were no statistically significant differences as regards sex-related adverse events in our cohort. Moreover, we did not find any significant difference regarding adverse events according to disease activity status and various therapeutic regimens.

Table XXI: Clinical features of inflammatory arthritis patients according to the vaccination status

	Total population (n=362)	**Vaccinated (n=331)**	**Unvaccinated (n=31)**	**p-value**
Females, n (%)	182 (50.3)	166 (50.2)	16 (51.6)	ns
Median age, years (IQR)	57 (47-66)	57 (47-66)	60 (48-63)	ns
Disease				
Rheumatoid Arthritis, n (%)	94 (26.0)	82 (24.8)	12 (38.7)	
Psoriatic Arthritis, n (%)	158 (43.6)	144 (43.5)	14 (45.2)	ns
Ankylosing Spondylitis, n (%)	110 (30.4)	105 (31.7)	5 (16.1)	
Therapy				
Steroids, dose prednisone 5-15 mg/day, n (%)	41 (11.3)	39 (11.7)	2 (6.5)	ns
csDMARDs				
- Methotrexate, n (%)	91 (25.1)	83 (25.1)	8 (25.8)	
- Leflunomide, n (%)	22 (6.1)	22 (6.6)	0 (0)	ns
- Sulfasalazine, n (%)	16 (4.4)	16 (4.8)	0 (0)	
- Hydroxychloroquine, n (%)	8 (2.2)	7 (2.1)	1 (3.2)	
bDMARDs				
- Anti-TNF-α, n (%)	210 (54.4)	190 (57.4)	20 (64.5)	
- Anti-CD20, n (%)	7 (1.9)	7 (2.1)	0 (0)	
- Abatacept, n (%)	16 (4.4)	12 (3.6)	4 (12.9)	
- Anti-IL-6, n (%)	14 (3.9)	12 (3.6)	2 (6.5)	ns
- Anti-IL-17, n (%)	65 (18.0)	62 (18.7)	3 (9.7)	
- Anti-IL-23, n (%)	8 (2.2)	8 (2.4)	0 (0)	
JAKi, n (%)	13 (3.6)	12 (3.6)	1 (3.2)	
Apremilast, n (%)	8 (2.2)	8 (2.4)	0 (0)	
Disease activity ^				
LDA/Active Disease, n (%)	139 (38.4)	129 (39.0)	10 (32.3)	ns
Remission, n (%)	223 (61.6)	202 (61.0)	21 (67.7)	
Comorbidities				
Cardiovascular disease, n (%)	115 (31.8)	106 (32.0)	9 (29.0)	
Diabetes, n (%)	28 (7.7)	26 (7.9)	2 (6.5)	
Obesity *, n (%)	43 (11.9)	41 (12.4)	2 (6.5)	ns
Pulmonary disease, n (%)	17 (4.7)	16 (4.8)	1 (3.2)	
Cancer, n (%)	17 (4.7)	17 (5.1)	0 (0)	
Vaccine type ∘				
Comirnaty (BioNTech/Pfizer), n (%)		273 (83.2)	-	
Spikevax (Moderna), n (%)		28 (8.6)	-	
Vaxzevria (Oxford/Astrazeneca), n (%)		27 (8.2)	-	
Vaccine shots ∘				
3 shots, n (%)		215 (65.6)	-	
2 shots, n (%)		106 (32.3)	-	
1 shot, n (%)		7 (2.1)	-	
Reason for missed vaccination, n (%)				
Total 31 (8.6)				
- Fear, n (%)		-	18 (58.1)	
- Medical contraindication, n (%)		-	7 (22.6)	
- Other, n (%)		-	6 (19.3)	
Males		-		
- Fear, n (%)		-	9 (60.0)	
- Medical contraindication, n (%)		-	3 (20.0)	
- Other, n (%)		-	3 (20.0)	
Females		-		
- Fear, n (%)		-	9 (56.3)	
- Medical contraindication, n (%)		-	4 (25.0)	
- Other, n (%)		-	3 (18.7)	

IQR, interquartile range; csDMARDs, conventional disease-modifying antirheumatic drugs; bDMARDs, biological disease modifying antirheumatic drugs; TNF-α, tumor necrosis factor α; CD20, cluster of differentiation 20 (B-lymphocyte antigen); IL, interleukin; JAKi, Janus kinase inhibitors; LDA, low disease activity.
^Disease activity evaluated by ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score-C Reactive protein) and DAS28-CRP (Disease Activity Score-C Reactive Protein), defined as remission (ASDAS-CRP <1.3, DAS28-CRP <2.6), low disease activity (ASDAS-CRP 1.3-2.0, DAS28-CRP 2.6-3.1), active disease (ASDAS-CRP ≥2.1, DAS28-CRP ≥3.2).
* Obesity is evaluated by BMI (Body Mass Index), defined as BMI >30.0.
∘ Vaccine shots calculated from 328 patients.

Table XXII: Adverse events in inflammatory arthritis patients after anti-SARS-CoV-2 vaccination

Total	AEs within 48 h (n=102)	p-value	Fever within 48 h (n=51)	p-value	Arthralgia within 48 h (n=43)	p-value
Active vs Inactive disease ^	43 (42.2) vs 59 (57.8)	0.46	18 (41.9) vs 33 (55.9)	0.23	19 (44.2) vs 24 (40.7)	0.84
Steroids treatment + vs - ∼	15 (38.5) vs 87 (29.8)	0.27	8 (53.3) vs 43 (49.4)	0.99	5 (33.3) vs 38 (43.7)	0.58
csDMARDs + vs - *	36 (28.6) vs 66 (32.2)	0.54	19 (52.8) vs 32 (48.5)	0.84	17 (47.2) vs 26 (39.4)	0.53
bDMARDs + vs - ∘	95 (30.5) vs 7 (36.8)	0.61	49 (51.6) vs 2 (28.6)	0.44	37 (38.9) vs 6 (46.2)	0.76
Females vs Males	64 (38.6) vs 38 (23.0)	0.003	34 (53.1) vs 17 (44.7)	0.54	27 (42.2) vs 16 (42.1)	0.99

Total	Asthenia within 48 h (n=65)	p-value	Disease flares within 30 days (n=52)	p-value	Other AEs within 30 days (n=35)	p-value
Active vs Inactive disease ^	26 (66.1) vs 39 (60.5)	0.68	23 (14.4) vs 29 (17.8)	0.44	15 (9.9) vs 20 (11.6)	0.71
Steroids treatment + vs - ∼	11 (73.3) vs 54 (62.1)	0.56	6 (15.4) vs 46 (15.7)	0.99	7 (17.9) vs 28 (9.6)	0.16
csDMARDs + vs - *	24 (66.7) vs 41 (62.1)	0.67	18 (14.3) vs 34 (16.6)	0.64	12 (9.5) vs 23 (11.2)	0.71
bDMARDs + vs - ∘	63 (66.3) vs 2 (28.6)	0.09	48 (15.4) vs 4 (21.1)	0.52	33 (10.6) vs 2 (10.5)	0.99
Females vs Males	41 (64.1) vs 24 (63.2)	0.99	30 (18.1) vs 22 (13.3)	0.29	26 (15.7) vs 9 (5.5)	0.004

AE, adverse events; csDMARDs, conventional disease-modifying antirheumatic drugs; bDMARDs, biological disease-modifying antirheumatic drugs.

^ "Active disease" includes both LDA (low disease activity) and active disease, "inactive disease" includes only remission; disease activity evaluated by ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score-C Reactive Protein) and DAS28-CRP (Disease Activity Score-C Reactive Protein), defined as remission (ASDAS-CRP < 1.3, DAS28-CRP $<$ 2.6), low disease activity (ASDAS-CRP 1.3-2.0, DAS28-CRP 2.6-3.1), active disease (ASDAS-CRP ≥ 2.1, DAS28-CRP $\geq$3.2).

∼ Prednisone 5-15 mg/day.

* csDMARDs include methotrexate, leflunomide, sulfasalazine, hydroxychloroquine.

∘ bDMARDs include anti-TNF-α, anti-CD20, abatacept, anti-IL-6, anti-IL-17, anti-IL-23, JAKi, apremilast.

DISCUSSION

5.1 The impact of SARS-CoV-2 infection

Overall, our study found a significantly higher risk of joint disease flare-ups within one month of SARS-CoV-2 infection. However, there was no difference in the rate of flare within the different IA subsets. At multivariate analysis, the only predictive risk factor for a flare-up was SARS-CoV-2 infection, thus confirming previous findings in the literature [369, 370]. Only 7.5% of patients who experienced flares needed a therapy switch.

The prevalence of SARS-CoV-2 infection was higher among cases than in the control group, though not statistically significant. Furthermore, there was no significant difference as regards the risk of hospitalization and the more severe course of COVID-19 between cases and healthy controls. Only one death occurred during the study, a patient with several comorbidities and long-term RA. Some recent data has shown that RA patients have a higher risk of contracting SARS-CoV-2 infection [372] and developing more severe COVID-19 [373] than patients with seronegative spondyloarthritis. Surprisingly, we found a significantly higher prevalence of SARS-CoV-2 infection in AS than in RA patients, in younger ones, and in those in remission. The cytokine profile involved in the pathogenesis of lung damage in COVID-19 is similar to that observed in the pathogenesis of joint damage in RA, with IL-1, IL-6 and TNF-α as key players [263, 393]. Instead, a central role in the pathogenesis of AS is attributed to IFN-γ, IL-12, IL-17, IL-22 and IL-23 [32]. Hence, the expectedly higher rate of infections in RA vs. AS group. Thus, we believe that the difference in the rate of infection between AS and RA group is due to the demographic differences between the two subgroups: patients with AS tend to be young adults, therefore much more exposed to social contacts that pose a risk of infection, as opposed to patients with RA who tend to be older and are therefore more likely to have limited their social contacts and followed the prevention rules more assiduously during the pandemic. The

difference cannot be attributed either to different therapies administered to the two subgroups: patients with AS were treated mainly with anti-IL-12, anti-IL-17, anti-IL-23, and anti-TNF-α whereas patients with RA were treated mostly with MTX, corticosteroids, anti-TNF-α, anti-IL-6, JAKi, abatacept, and rituximab. Finally, we did not found any differences in incidence of flares between the various subgroups despite the pathogenesis described possibly suggesting a greater risk for patients with RA.

The different prevalence of RA and seronegative SpA between our patients and the general population may be attributable to the fact that the Rheumatology clinic of Padova University Hospital is mainly dedicated to SpA. However, we did not find any difference regarding the risk of developing more severe COVID-19 and having a higher rate of joint disease flares between the IA subsets, and those with high disease activity.

Furthermore, we fail to demonstrate any impact of the different anti-rheumatic drugs on SARS-CoV-2 infection and/or COVID-19 course, in particular as it pertains to three recently reported aspects: the potential protective role of anti-TNF-α [394, 395] and the negative effect of corticosteroids and rituximab, the more severe forms of COVID-19 in patients with immune-mediated rheumatic diseases [365, 394, 395], and sulfasalazine. The effect of anti-TNF-α may stem from the fact that most of our patients suffering from seronegative SpA which presents some pathophysiological differences vs. RA, such as T17/T1 pathway balance [370]. We did not find a negative effect of rituximab, sulfasalazine or steroids, as previously reported [365, 394, 395], likely due to the low frequency of this treatment in our cohort.

5.2 The impact of SARS-CoV-2 vaccination

Although our findings showed no association between the SARS-CoV-2 vaccine and the occurrence of joint disease flare-ups, the latter were easily manageable with NSAIDs, as widely described in literature [380–382, 387]. It bears noting that 23% of the flares required a therapy switch in those patients with active disease in the last 12 months. Nonetheless, there was no difference in risk of flare within different IA subsets and therapy options. Unlike previous studies, we found no increased risk of flare in patients treated with steroids or immunosuppressive drugs, or in those who suffered a previous exacerbation in the past 12 months [388].

Moreover, we observed no other predisposing risk factors such as age, sex, or comorbidities despite a higher rate of active disease in the last 12 months in

patients who experienced flares vs. those who did not.

Finally, as widely described in the literature [378–381, 384], we observed no increased rate of AEFI than in the healthy controls. Female sex emerged as the only predisposing risk factor for AEFI both within 48 hours and 30 days despite no significant differences relating to disease activity status and different therapeutic regimens.

5.3 Limitations of the study

We would be remiss to not mention some of the limitations of our study. Firstly, the retrospective design may have resulted in recall bias.

Secondly, our cases and controls were not matched for age and sex, though there were no significant differences in the rate of infection and AEFI between the two groups.

CONCLUSIONS

This study allowed us to address many of the concerns expressed by our patients with inflammatory arthritis since the beginning of the pandemic: firstly, the most frequent one was about a possible increased risk of contracting SARS-CoV-2 infection and developing severe forms of COVID-19, due to immunosuppressive therapy; subsequently, patients also often asked about AEFIs and flares following COVID-19 vaccination. Our findings helped us reassure patients that they should have no concern about a higher risk of infection or a higher severity of COVID-19, and no reservations about the vaccine or continuing the therapy regularly. Generally speaking, our findings also serve as a further confirmation that vaccines are safe in patients with inflammatory arthritis and there is no increased risk of contracting SARS-CoV-2 infection and disease severity compared to the healthy population. The prevalence and severity of COVID-19, as well as the hospitalization rate were not significantly different in our cohort of patients with inflammatory arthritis compared with healthy controls. Moreover, COVID-19 vaccines were well tolerated and did not correlate with an increased risk of flares. Thus, vaccination is advisable in this subset of patients, especially considering their overall frailty. In addition, to confirm the excellent risk/benefit ratio of vaccination, we observed a significantly higher rate of flares among patients with inflammatory arthritis who contracted SARS-CoV-2 infection compared to those who did not. Even if did not reach the statistical significance the patients with flare were for most with higher disease activity before SARS-CoV-2 infection. Surely, the fact that we presented data retrieved directly from our center played a key role, since patients appeared to find it more reliable as compared to the early days of the pandemic when data was scarce and came from centers around the world.

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