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273rd ENMC International workshop: Clinico-Sero-morphological classification of the Antisynthetase syndrome. Amsterdam, The Netherlands, 27-29 October 2023

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ABSTRACT

Among the idiopathic inflammatory myopathies, patients harbouring an Antisynthetase syndrome exhibit a unique clinical picture, with characteristic signs such as myositis, interstitial lung disease, arthritis, rash, and/or fever. Characteristic morphological features on skeletal muscle biopsies differentiate Antisynthetase syndrome from other forms of myositis. Autoantibodies typically recognizing one of the members of the aminoacyl-tRNA synthetase family of proteins can be detected in the serum of such patients, with anti-Jo1 being most frequent. Until now, an international consensus definition of the Antisynthetase syndrome is lacking, hence this workshop has undertaken the task to inform about the clinical, morphological and autoantibody profiles of Antisynthetase syndrome. The authors also expand their aims by giving management and therapeutic strategies, and finally provide precise classification criteria for Antisynthetase syndrome.

1. Introduction

Some patients with an idiopathic inflammatory myopathy (IIM) have autoantibodies recognizing one of the aminoacyl-tRNA synthetase (aaRSs; anti-ARS). Accumulating evidence has shown that patients with autoantibodies against anti-ARS have a unique constellation of clinical features and muscle biopsy characteristics compared to IIM patients with dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM), inclusion body myositis (IBM), or other types of IIM. Specifically, these patients often have one or more of the following clinical features: myositis, interstitial lung disease (ILD), arthritis, rash, and/or fever [1]. Consequently, patients with antisynthetase autoantibodies are now considered by many to have a unique type of IIM, usually called the antisynthetase syndrome (ASyS). Nonetheless, some IIM classification criteria define patients with anti-ARSs and a rash as having DM while those without a rash are defined as having polymyositis (PM).

There remains some confusion about whether the ASyS is a distinct entity and, if so, how it should be defined. The organizers of this 273th European Neuro-Muscular Centre (ENMC) workshop welcomed 21 participants from nine countries (Belgium, Thailand, France, Germany, Denmark, The Netherlands, Sweden, United

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Kingdom and United States of America), comprising clinicians from different disciplines, laboratory specialists, researchers, and patient representatives to define the following:

- the clinical spectrum of ASyS, including both muscle and non-muscle related symptoms.
- the precise tools required for diagnostic evaluation of ASyS patients.
- the morphologic spectrum of the different types of ASyS-associated myositis.
- a minimal set of histologic features to make a diagnosis of ASyS.
- management strategies for patients with ASyS.
- classification criteria for ASyS.

To achieve these goals, participants presented about their area of expertise and the shared information was used in the discussions to achieve consensus.

2. The spectrum of organ involvement in ASyS

2.1. Definition of the knowledge lacunas in basic and clinical research, diagnostics and care, implications on trial readiness

Olivier Benveniste opened the workshop by explaining that the presence of one of the anti-ARS, anti-histidyl-tRNA synthetase, gave the name to the syndrome. However, our cells possess not one, but a set of 23 known aminoacyl-tRNA synthetases [2] which specifically charge their cognate tRNAs. Hence, up to 23 anti-ARS may exist. The most frequently observed anti-ARS are anti-histidyl-tRNA synthetase (Jo1), anti-alanyl-tRNA synthetase (PL12), and anti-threonyl-tRNA synthetase (PL7) present in up to 80–90% of ASyS patients [3]. Other rarer ones have been identified: anti-glycyl-tRNA synthetase (EJ), anti-isoleucyl-tRNA synthetase (OJ), anti-asparaginyl-tRNA synthetase (Ks), anti-lysyl-tRNA synthetase (*Sc*), anti-phenylalanyl-tRNA synthetase (ZO), anti-tyrosyl-tRNA synthetase (Ha), and anti-cysteinyl-tRNA synthetase (Ly) [4]. Furthermore, anti-glutaminyl-tRNA synthetase, anti-tryptophananyl-tRNA synthetase, anti-seryl-tRNA synthetase, anti-arginyl-tRNA synthetase, anti-methionyl-tRNA synthetase and anti-valyl-tRNA synthetase autoantibodies have been identified [2].

The first described clinical cases and subsequent series of cases suggest that the clinical presentation of ASyS remains fairly homogeneous regardless of which anti-ARS a patient has [2,4,5]. However, the clinical and radiological signs associated with the ASyS are not specific as patients positive for myositis-associated autoantibodies (MAA) such as anti-U_1RNP, Ku, PM/ScL may have very similar clinical presentations [6]. Therefore, the first gap in our knowledge is how to reliably test for anti-ARS and exclude patients with other myositis-specific-autoantibodies (MSA) and MAA who do not have the ASyS. A second challenge is that the ASyS is not recognized as a distinct type of IIM according to the widely used 2017 ACR/EULAR criteria of myositis [7]. Moreover, the ACR/EULAR criteria do not allow for patients without myositis to be classified as having the ASyS. Here, we will propose classification criteria for ASyS, including details on how autoantibody testing should be performed to confirm the diagnosis of ASyS and exclude patients with other forms of IIM.

Finally, the pathophysiology of ASyS is now better understood and continues to be studied intensely. Animal models of ASyS exist [8] and have been reproduced by multiple laboratories (see below). A positive correlation between levels of anti-Jo-1 Abs and ASyS disease activity has also been observed [9]. Taken together, these findings suggest that ASyS is a distinct type of IIM, and that this disease will require specific treatments, and that ASyS-specific efficacy outcome measures will be needed for future clinical trials.

2.2. Order of clinical manifestations and outcome of ASyS: differences and similarities related to the different autoantibodies

Yves Allenbach gave an overview of the clinical spectrum of ASyS. The ASyS represents a subset of multiorgan disease often including myositis characterized by inflammatory lesions not confined solely to the skeletal muscle. The clinical manifestations encompass a broad spectrum, which can vary depending on the specific antibodies present. Similarly, the timeline of onset of organ involvement varies among patients, as does the number of different organs affected.

The syndrome is associated with lesions affecting foremostly the: (i) skeletal muscles, (ii) joints, (iii) lungs, (iv) heart, and (v) skin. Musculoskeletal pain and respiratory signs are the primary reasons for consultations leading to diagnosis of this entity [10].

Approximately 70–80% of ASyS patients exhibit thoracic involvement in the form of interstitial lung disease (ILD). Likewise, 50–80% of patients present with myositis, and 50–60% manifest joint involvement (arthralgia and/or synovitis) [3,11,12].

Skin involvement is also prevalent, characterized by Raynaud's phenomenon observed in 40–50% of cases and/or mechanic's hands in 25–30% of cases [3,11,12]. Dermatomyositis-like skin lesions have been reported more rarely in ASyS patients [13].

Patients also often present with general signs of being unwell, predominantly fever in 30% of cases associated with an inflammatory syndrome [3,11,12].

Less frequently, patients may exhibit symptomatic myocarditis and/or pericarditis (3.5%) [14]. However, this involvement may be underestimated, as systematic screening has revealed cardiac abnormalities in at least 30% of patients at the time of diagnosis [15].

These observations have led to the definition of a syndrome characterized by a triad of myositis, arthralgia/arthritis, and interstitial lung disease. However, a majority of patients (50–70%) initially exhibit only one component of this triad, and only a minority (20–50%) display the complete triad after a median follow-up of 72 months [3]. Furthermore, 12–40% of patients retain a single affected domain throughout the follow-up, while 40% exhibit two out of the three triad elements [3].

Additionally, the clinical spectrum at diagnosis or during follow-up apparently depends on the type of antibody involved. Anti-Jo1 antibodies are the most prevalent (72%), whereas anti-PL7 and anti-PL12 antibodies are less frequent [3]. Together, these three antibodies account for 80–95% of ASyS cases in Europe and the USA [16]. Anti-EJ, anti-OJ, and anti-ZO antibodies are even more rarely reported.

The diagnostic delay for anti-Jo1 ASyS patients seems shorter than for other ASyS patients. Indeed, anti-Jo1 ASyS clinical presentation is often more pronounced, dominated by more frequent and/or inaugural musculoskeletal signs (particularly arthritis). Conversely, interstitial lung disease is most commonly observed in patients with anti-PL7, anti-PL12, and anti-EJ. ILD is the most frequent revealing feature in non-Jo1 ASyS patients, where it can either remain isolated or be associated with the onset of myositis, which is often less severe than in anti-Jo-1 ASyS patients. However, it is worth noting that lung-involvement remains highly prevalent in anti-Jo1 ASyS patients (50–80%).

This phenotypic difference between anti-Jo1 and non-anti-Jo1 ASyS patients also translates into a difference in outcome. The 5-year survival rate for non-Jo-1 ASyS patients is lower (70% vs. 90% for anti-Jo-1 patients), with mortality primarily driven by pulmonary causes (fibrosis and pulmonary hypertension) [17]. It is noteworthy that unlike in dermatomyositis (especially adult-onset

DM associated with TIF1γ and NXP2), there is no elevated cancer risk identified in the context of the ASyS [18].

While the pneumo-musculoskeletal syndrome in ASyS patients is well characterized, with outcomes linked to the severity of ILD, it is important to note that a substantial proportion of patients display limited organ involvement both at the time of diagnosis and throughout their follow-up. Anti-Jo-1 ASyS patients tend to exhibit a musculoskeletal-dominant phenotype, whereas those without Jo-1 antibodies often present with predominant lung involvement.

3. Anti-ARS detection

3.1. Anti-Jo1, anti-PL7 and anti-PL12 immunodiagnostics: antinuclear antibodies and specific tests (reliability)

Jan Damoiseaux initiated his presentation by explaining that among the expanding family of antisynthetase autoantibodies, the antibodies directed to Jo1, PL7 and PL12 are the most prevalent. Anti-Jo1 has a special position because this is the only autoantibody that is currently part of the EULAR/ACR classification criteria [7] and detection of this autoantibody is most often part of the routine diagnostic algorithm for systemic autoimmune rheumatic diseases (SARD) [19]. However, antibodies to Jo1 may also be detected in multiplex immuno-assays for myositis autoantibodies and, therefore, discrepant results for anti-Jo1 may be reported to the clinician due to the use of distinct testing procedures within the clinical laboratory.

Testing algorithms for SARD often start with screening for antinuclear antibodies (ANA) by HEp-2 indirect immunofluorescence assays (IFA). It was already concluded in the 256th ENMC workshop on myositis autoantibodies that HEp-2 IFA should not be used as screening assay in case of clinical suspicion for idiopathic inflammatory myopathy (IIM) [16]. This is even more apparent for the anti-ARS antibodies because the sensitivity of HEp-2 IFA for these antibodies, revealing a (dense) fine speckled cytoplasmic HEp-2 IFA pattern (AC-19 and AC-20), is rather low (~50%).

While immunoprecipitation is considered the gold-standard for detection of myositis autoantibodies, this technique requires extensive expertise and is only available in research settings. In routine clinical laboratories, multiplex immuno-assays are used. Comparison of a widely used line-immuno-assay (LIA) with immunoprecipitation has been summarized recently [20]. Kappa values (95% CI) for anti-Jo1 (n = 176), anti-PL7 (n = 324) and anti-PL12 (n = 324) are 0.52 (0.33–0.70), 0.82 (0.56–0.89) and 0.89 (0.56–0.89), respectively. Similarly, a comparison was made between three distinct multiplex immuno-assays [21]. In a cohort of 144 IIM patients, 24 out of 31 sera (sensitivity: 16.6 – 21.4%) were positive for anti-Jo-1 antibodies with concordance for 22 sera in all three assays. In a cohort of 240 control patients, only 1 out of 7 sera were positive (specificity: 99.6 – 97.1%). Positive results for anti-PL7 (n = 1 – 3) and anti-PL12 (n = 3) were very rare in this IIM cohort, preventing reliable conclusions from being reached about these. From these data, it is evident that future studies are needed with much larger patient cohorts, ideally including novel methods for the detection of myositis autoantibodies in comparison to immunoprecipitation.

3.2. Anti-EJ, anti -OJ and other anti-ARS immunodiagnostics: antinuclear antibodies and specific tests (reliability)

Sarah Tansley opened her presentation by explaining that autoantibodies directed against EJ, OJ, and other non-Jo1, non-PL7 and non-PL12 anti-ARS are collectively rare, making up just 6–8% of ASyS cohorts and 1.5–3% of IIM cohorts [22–24]. Available data are consequently very limited. The largest case series of anti-Zo consists of just 9 patients [5], there are only 3 confirmed reports of anti-Ha in the published literature [25] and only one case of the recently described anti-Ly [4]. The most common of these rare anti-ARS are anti-OJ and anti-EJ [22–24] but studies of commercial assays may include just one or two relevant patient samples. Anti-ARS have all been characterised using immunoprecipitation-based techniques and can reliably be detected by protein or RNA-immunoprecipitation. These assays are complex, time consuming and require expertise meaning they are unlikely to be practical in a clinical setting. Where utilised they should be carried out in reference laboratories with suitable experience in the technique.

There are now several different commercial assays available, which are used to detect different combinations of anti-ARS. An ELISA to detect several anti-ARS simultaneously (including anti-KS and anti-EJ), similarly to other ELISAs, appears to have high levels of agreement with immunoprecipitation. It is noted that this does not allow detection of individual anti-ARS without additional testing [26,27].

For blotting based assays, the sensitivity for different anti-ARS autoantibodies varies with manufacturer and autoantibody, but data are exceptionally limited with some studies containing just one or two relevant patient samples [21,26,28,29]. Individual rarity of these autoantibodies makes such assays very challenging to validate with patient sera and there is insufficient data in the published literature to draw firm conclusions on sensitivity and specificity. The rarity of these autoantibodies also creates practical problems. Studies of real-world data suggest that less than 25% of samples screened for MSA in a diagnostic setting are diagnosed with myositis spectrum disease [30,31]. For rare autoantibodies such as these, very low prevalence in the tested population means that even highly specific assays are likely to produce more false-positive than true-positive results.

Anti-OJ is somewhat unique amongst the anti-ARS discussed here, as its target autoantigen, isoleucyl tRNA synthetase, is part of a multi-tRNA synthetase complex (which also contains the tRNA synthetases QARS, KARS, DARS, RARS, MARS, LARS and EPRS). Reaction with additional tRNA synthetases within this complex has been seen with some anti-OJ sera, but all target isoleucyl-tRNA synthetase [32]. Anti-OJ autoantibodies target conformational and quaternary epitope structures of this complex and consequently blotting based assays, which utilise denatured antigen, are unlikely to be reliable. This has been highlighted as a concern across commercial line assays regardless of manufacturer and sensitivity for anti-OJ has been reported to be 0% in one study in comparison to protein-immunoprecipitation [27].

As with the more common anti-ARS, it is evident that future studies are needed with much larger patient cohorts to understand the reliability of commercial assays to detect these rarer anti-ARS in comparison to immunoprecipitation.

4. Lung involvement

4.1. Interstitial lung disease in ASyS: differences and similarities related to the different auto-antibodies

Yurdagul Uzunhan began her talk by emphasizing that pulmonary involvement is often the major component of systemic involvement in idiopathic inflammatory myositis (IIM) and particularly in antisynthetase syndrome (ASyS), where the prevalence of interstitial lung disease (ILD) is very high. Furthermore, the quality of life and mortality in ASyS patient are greatly affected by pulmonary involvement.

Respiratory manifestations in ASyS may also include respiratory muscle disease, leading to a restrictive pattern, as well as

dysphagia and swallowing disorders leading to inhalation pneumonitis and a biased assessment of ILD.

The ACR-EULAR classification criteria for adult and juvenile IIM do not take into account pulmonary involvement and many MSAs, making it difficult to classify patients as having IIM-related ILD. Some patients may therefore be misclassified, especially those who are hypo- or amyopathic. As a result, some might classify patients with ILD, MSA and a hypo- or amyopathic disease as having interstitial pneumonia with autoimmune features (IPAF) [33]. Obviously, IPAF should not be considered as a diagnosis in these cases of ASyS. The classification criteria for IPAF remain controversial and need to be better defined. A multicentre retrospective study was performed to evaluate the clinical features and outcomes of patients fulfilling IPAF criteria stratified by the presence of MSAs and MAAs. The results showed that patients fulfilling IPAF criteria with circulating MSA but not MAA have similar clinical features and outcomes to those with IIM-ILD, making these two groups largely indistinguishable. This supports a common diagnostic classification and management approach of MSA-associated ILD [34].

Three-quarters of patients with ASyS have ILD, whereas the proportion for the other overlap diseases in IIM patients is close to one third. Among ASyS patients, non-Jo1 antibody carriers have a higher prevalence of ILD [12]. ILD was more frequent (80% and 88% vs 67%, $p = 0.014$) whereas myositis was less frequent (44% and 47% vs 74%, $p < 0.001$) in patients with anti-PL7 and anti-PL12 compared to anti-Jo1 [11]. Different ASyS phenotypes are defined according to anti-ARS specificity: anti-Jo1 is most often associated with myositis, whereas anti-PL7 and anti-PL12 tend to be more restricted to the lungs [3,11].

Acute onset is a hallmark of ASyS, observed in almost 40% of patients with Jo1, PL7, PL12 and OJ-positive antibodies, rising to 74% in EJ-positive patients in a recent large multicentric cohort study [3].

CT scan is the main tool of evaluation, revealing different types of lesions and helping to classify ILD into different patterns, as defined by the ATS/ERS consensus for idiopathic interstitial pneumonias [35]. For instance, bilateral basal ground-glass opacities, linear reticulations and proximal bronchiectasis are associated with non-specific interstitial pneumonia (NSIP) pattern. NSIP is the most common pattern, as seen in other connective tissue diseases such as systemic sclerosis. Bilateral alveolar consolidations also occur, particularly in the context of acute onset, and define organising pneumonia (OP) pattern. Both patterns may be mixed (NSIP-OP). Usual interstitial pneumonia (UIP) with subpleural honeycombing and distal bronchiectasis is less common and dramatically less frequent in IIM- ILD than in rheumatoid arthritis-related ILD. Some features may be more frequent in CTD-UIP, including exuberant honeycombing, involvement of anterior upper lobes and the “straight-edge” sign [36]. In the worst cases of rapidly progressive ILD (RP-ILD), often leading to acute lung injury, the CT scan may show features of acute interstitial pneumonia with consolidations and extensive ground-glass opacities. The CT scan is important to assess for (i) the presence of fibrosing lesions, including traction bronchiectasis and reticulations, which are present in a high proportion at initial assessment, and for (ii) the extent of the lesions - usually bilateral and starting in the posterior and basal regions within the entire lung parenchyma. Some studies have attempted to describe CT patterns associated with different ASyS antibodies [37–39]. Organising pneumonia was found with a higher proportion in EJ-positive, whereas NSIP was found to be the most common pattern in PL7- and PL12-positive patients. Some authors considered that middle lobe traction bronchiectasis in ASyS-ILD could be a useful predictor of poor long-term disease outcome, but it cannot distinguish between antibody specificity. Finally, a cluster of reticulations, cysts and consolations seems to be associated with ASyS-ILD compared to other DM-ILD in one study, but the high number of Jo1-positive patients could not allow a conclusion regarding other ASyS-ILD [39].

Histological findings have been scarcely reported in ASyS-ILD and especially in non-Jo1 ASyS. Many selection biases may explain the heterogeneity of findings depending on the indication for lung histology, the timing of histology in the disease course and also the size of the lung specimens. Overlapping patterns are found with a higher prevalence of NSIP followed by histological UIP pattern mainly in PL7- and PL12-positive patients as shown in a recent systematic review [40].

The outcome of ILD in Jo1-positive shows no significant decline in forced vital capacity (FVC) over time in a Spanish study [41]. However, patients who died due to respiratory failure showed a statistically significant, steady, and progressive decrease in estimated mean FVC values over time compared to survivors and those who died from other causes [41]. Comparison of patients with and without improvement showed that age, UIP pattern and pneumomediastinum were associated with worse outcome, but these findings were not confirmed on multivariate analysis [42]. PL7- and PL12-positive patients had significantly more severe pulmonary function tests (PFT) at diagnosis, worse overall ILD outcome and shorter survival [11,17,43,44].

RP-ILD, a common life-threatening complication of IIM-ILD, is usually defined as rapidly progressive dyspnoea and hypoxaemia with worsening radiological ILD within three months of the onset of respiratory symptoms. A key feature of RP-ILD is the tendency to be resistant to high-dose glucocorticoids and immunosuppressants, which needs to be incorporated into a consensus definition, which is lacking. ASyS antibodies, especially anti-Jo1 antibodies, were detected less frequently in patients with RP-ILD compared to patients with chronic ILD in a large Chinese study, which showed that MDA5-positive patients have the worst risk of RP-ILD [45]. In the intensive care unit setting, MDA5 dermato-pulmonary syndromes had a significantly higher mortality than ASyS, with almost all of these patients dying in the (intensive care unit) ICU of refractory acute respiratory distress syndrome (ARDS) despite a high rate of extracorporal membrane oxygenisation (ECMO) usage (32%) [46].

The prevalence of progressive pulmonary fibrosis, as recently defined [33], is difficult to determine in patients with ASyS-ILD; its proportion has been reported to range from 18% to 44% [47–49]. No factors, including serotype, have been associated with this phenotype.

4.2. ILD and ASyS: how to assess disease activity

Next, Sonye Danoff described how to screen and measure ILD severity. While IIM is often considered a disease of muscles and skin, ASyS is more accurately defined as a disease of the lung, specifically ILD, with variable muscle and skin involvement [50]. While the presentation of the ASyS is variable, ILD is the common consistent feature. Thus, assessing lung involvement in ASyS is critical to the appropriate care of patients and the monitoring of therapeutic effect.

ILD can present in patients with previously recognized IIM or may be the first or only manifestation of ASyS [50]. In the former situation, the identification of ILD is typically triggered by patient symptoms (cough, dyspnea on exertion, increased fatigue). On physical exam, tachypnea or desaturation with ambulation may be noted on vital signs. Crackles may be present on chest auscultation; however, small lung fields to percussion and auscultation may be the only findings. Given the paucity of findings on exam, having a high degree of suspicion for ILD in any patient with ASyS is critical. The method for evaluation and

monitoring will be detailed below but should include complete pulmonary function testing (spirogram, lung volumes and diffusion capacity). A high-resolution chest CT scan is appropriate for evaluation in the case of abnormal pulmonary function testing or with a high pre-test probability of ILD.

Regarding patients presenting with ILD as the first manifestation of ASyS, the burden of evaluation typically falls on the pulmonologist evaluating the patient. The 2018 ATS/ERS/JRS Guidelines on evaluation of idiopathic pulmonary fibrosis recommend that patients with new ILD be evaluated with autoimmune serologies even in the absence of extrapulmonary manifestations [51]. Patient features which suggest a possible underlying autoimmune disease including ASyS include female, Black, age<60, presence of other features of autoimmunity on history such as Raynaud syndrome, arthralgias, myalgias, skin rash. However, as noted above, ASyS-ILD can present in the absence of other characteristic features. The working group recommends to send an anti-nuclear antibody, rheumatoid factor, full MSA panel, and anti-Ro52, anti-Scl-70, anti-U_1RNP antibodies and ESR, CRP, creatine kinase and aldolase on all new ILD patients; however, local practice may vary.

The presence of ILD in the setting of autoimmune diseases is suggested by restriction on pulmonary function testing as well as decreased diffusion capacity. Restriction alone can be seen with respiratory muscle weakness and isolated decrease in diffusing capacity of the lungs for carbon monoxide can occur with pulmonary hypertension, hence, imaging is the critical diagnostic study. Response to therapy can be monitored by longitudinal pulmonary function testing obtained at appropriate follow up intervals.

The recommendation for imaging is a non-contrast high resolution chest CT scan with inspiratory and expiratory imaging [51]. In some individuals prone imaging may be appropriate as well. This study provides the optimal image quality for parenchymal lung disease but can also provide information on lung masses and suggestions of pulmonary hypertension (enlarged pulmonary artery in comparison to aorta). However, this study is not adequate to evaluate for pulmonary embolism if this is a concern. Disease extent and response to therapy may also be evaluated by high resolution chest CT scan. As noted in the therapy section, some patients with ASyS will manifest limited interstitial changes but have no clinical symptoms. The clinical significance of these findings in terms of long-term outcome is not currently known.

Once ILD is identified, the clinical impact of disease should be assessed. In ambulatory patients, pulmonary function testing is helpful in this process to classify restriction and diffusing capacity of the lungs for carbon monoxide impairment as mild, moderate or severe. Serial pulmonary function testing is typically used to monitor disease and response to therapy. In the first year of treatment and in more severely impacted patients, pulmonary function testing should be assessed approximately every 3–4 months [52]. The goals of treatment should be defined as improving or stabilizing lung function depending on the individual patient. In addition to pulmonary function testing, ambulatory oxygen saturations performed informally or in the context of a 6-minute walk test are important to assess need for supplemental oxygen as well as providing (in the case of 6-minute walk test) another assessment of response to therapy. For patients with more severe disease who are hospitalized or in the ICU, evaluation of response to therapy may depend on other indicators such as ability to wean from the ventilator or decrease in supplemental oxygen needs. For hospitalized patients, it is also important to assess oxygen requirement with ambulation since oxygen needs at rest may not fully capture the degree of illness.

5. Muscle manifestations

Following this general overview including aspects of serology and pulmonary involvement, a detailed presentation of muscular manifestations was provided. In order to highlight the muscular specificities of ASyS, the organizers proposed dedicating this session to the myopathology. Both presentations had the major aim to describe the myopathological features that can occur in affected muscles in ASyS and to place them into the morphological framework of other types of myositis.

5.1. Myopathology of ASyS: a distinct entity from other myositis - diagnostic criteria

In the next presentation, Werner Stenzel first outlined the aims of his talk, which were to (i) describe the morphological features of ASyS in skeletal muscles, (ii) focus on distinct and shared features of ASyS with other types of myositis, (iii) elaborate on what could be a minimal necessary set of stains to diagnose ASyS by morphological means, and (iv) spotlight possible mimickers and enumeration of 'red flags' in the diagnosis of ASyS.

The morphological features of ASyS in affected muscles first comprise a necrotizing myopathy with individual myofibre necrosis (no areas or whole fascicles of necrosis), mostly occurring in the perifascicular region of muscle fascicles. This feature may not occur in all fascicles but the overall impression is that necrotic myofibres occur predominantly at the edges of fascicles (perifascicular), then at the center or with a diffuse distribution. In this perifascicular area, besides necrotic fibers, atrophic fibers are mixed with fibers of normal size and with hypertrophic rounded fibers often with internalized myonuclei. These features are best evaluated by Gömöri trichrome stains, H&E, non-specific esterase or acid phosphatase.

Next, a characteristic feature of ASyS muscle pathology is the enlargement and fragmentation of the perimysial connective tissue, which occurs typically in a focal rather than an evenly distributed phenomenon. Here, one can observe edema of the perimysium associated with numerous macrophages and activated fibroblasts, fragmentation of the fibrous tissue and enlargement of this area. All those changes can be highlighted by stains such as e.g. alkaline phosphatase in combination with non-specific esterase and acid phosphatase as well as EvG.

Among the enzyme histochemical features, an important 'negative' aspect is the normal perifascicular aspect of myofibers in combined COX-SDH stains. This is in contrast to DM, where a perifascicular pattern (with predominant atrophic rather than necrotic fibers) most often occurs and where a COX-paleness with persistence of the blueish hue exists in many biopsies [53–56].

Most importantly, among the immunohistochemical reactions, there is a combined and strong MHC class I and class II positivity that predominantly occurs on the sarcolemma (and the sarcoplasm) of myofibers in the perifascicular region [56–59]. This aspect can vary in the different fascicles and is best appreciated at low magnification (x40 magnification). The perifascicular myofibers also show a positive and fine granular reaction pattern of the sarcolemma by C5b-9 stains of many fibers. This also often shows a decreasing gradient towards the center of fascicles. In contrast, DM skeletal muscle biopsies show either a sarcolemmal pattern of complement (in anti-Mi2+ DM) or a capillary predominant staining pattern in anti-NXP2 or -TIF1γ DM.

The inflammatory infiltrate, while variable, is often quite prominent and predominantly localized in the perimysium and extending into the adjacent endomysium. In the ASyS, focal and pronounced endomysial collections of infiltrates/lymphocytes and macrophages are atypical. There are also no relevant autophagic features, which would be highlighted by commonly employed

Table 1
Minimal stain sets and typical myopathological pattern for Antisynthetase syndrome (ASyS) and dermatomyositis (DM).

Disease		*MHC class I*	*MHC class II*	*Complement (C5b-9)*	*Gömöri*
ASyS		++ (pf)	+-+++ (pf)	+ (sarc)	pf necrosis
DM	**Anti-Mi2+**	++ (pf)	±	+ (sarc)	pf atrophy and some necrotic fibers
	Anti-NXP2+	+++ (pf)	–	+ (cap & sarc)	pf atrophy or focal ischemic necrosis
	Anti-MDA5+	+	–	±	pf atrophy mild and focal
	Anti-SAE	+	–	±	pf atrophy mild and focal
	Anti-TIF1*γ*	+++	–	+(cap)>sarc	pf atrophy pronounced and ghost fibers and pale COX stain

Legend: pf = perifascicular, sarc = sarcoplasmatic, cap = capillary.

stains such as p62 or LC3, which is in contrast to IMNM and IBM biopsies [55,60,61].

In order to differentiate ASyS cases from DM, and specifically from anti-Mi2 DM, the MxA stain is especially useful. In the vast majority of ASyS biopsies, MxA is negative or exceptionally weak on myofibers while it is positive and highlights perifascicular pathology in DM [62–64]. This is also true for ISG15 and ISG20 stains, but the latter have been validated less well in larger cohorts. Of note, these staining patterns are in good accordance with transcriptomic studies of ASyS muscle biopsies [62,65] (see 7.2).

Next Werner Stenzel described the differences and similarities of ASyS muscle biopsies with muscle biopsy features from anti-Ku, anti-Pm/Scl-, and anti-U1-RNP-positive patients. In anti-Ku and anti-U1-RNP patients, the biopsies are characterized by a diffuse necrotizing pattern and a variable amount of endomysial inflammation, similar to prototypic IMNM. The major difference with the latter entity is the constant positivity of MHC class II on the sarcolemma of myofibers in the former and the negativity in the latter. In ASyS, the MHC class II staining pattern can be both diffuse and patchy or pronounced in perifascicular areas, like ASyS muscles, and there is only rarely MxA positivity on myofibers. Complement (C5b-9) may be positive on some (rather rare) myofibers, but not specifically with a perifascicular distribution. On the ultrastructural level, myonuclear actin aggregates occur in ASyS but not in DM, and, conversely tubuloreticular inclusions reflecting an interferon-related impact on endothelial cells occur in nearly all types of DM but in ASyS less frequently. The quantitative aspect of electron microscopic (EM)-features has not been studied in sufficient detail and is thus a personal observation. Table 1 summarizes this pattern and indicates a minimal set of stains to be used.

Finally, Werner Stenzel also presented the cases of two patients who had lung predominant disease at initial presentation but who nonetheless developed myositis even when their ILD was under good clinical control with Rituximab. Of note, these patients still harboured plasma cells in their muscles while other B cells were not detectable [66].

5.2. Myopathology of ASyS: differences and similarities related to the different autoantibodies

Jantima Tanboon began her talk with the historical pathological description of the ASyS. The ASyS, and especially anti-Jo1 ASyS, was initially described as an “immune myopathy with perimysial pathology (IMPP)”, which is characterized by widened and damaged perimysium, the presence of acid phosphatase (ACP) positive cells and alkaline phosphatase (ALP) activity in perimysium, sarcolemmal deposition of membrane attack complex (MAC, C5b9) on myofibers near the perimysium, and perifascicular myofiber necrosis [59,67,68]. Notably, IMPP pathology is not limited to anti-Jo1 ASyS, but is also described in anti-EJ, anti-PL7, and anti-PL12 ASyS, anti-HMGCR IMNM, and anti-Mi2 DM [68,69]. Perifascicular necrosis (PFN), defined as the presence of myofiber necrosis predominantly in perifascicular areas (>2/3 of the total number), was originally described by Mescam-Mancini et al. as the prominent feature in anti-Jo1 ASyS, being present in 75.8% of the case [58]. However, later studies by Uruha et al. and Tanboon et al. showed that PFN can be present in different ASyS subtypes (anti-Jo1 30.8%, anti-OJ 45%, anti-PL7 55%, anti-EJ 30%, and 1 case of anti-KS) and in anti-Mi2 DM (50%), whereas PFN is not present in anti-PL12 ASyS [59,69,70]. In these later studies, the muscle biopsies with single fiber necrosis within the perifascicular area were not classified as PFN but as having a non-specific pattern. This might explain the difference of PFN proportions observed by different groups in anti-Jo1 muscle biopsies (communication with Prof. Werner Stenzel).

Among the different ASyS subtypes, anti-OJ is associated with the most prominent muscle pathology in the muscle fiber domain, inflammatory domain, and connective tissue domain [70]. Vasculitis, defined by inflammatory cell infiltration within vascular wall, is also more common in anti-OJ. Muscle pathology in anti-EJ ASyS is likely associated with longer disease duration before muscle biopsy. Anti-EJ ASyS shows prominent muscle fiber and connective tissue pathology, more severe vascular depletion, and more common endomysial fibrosis than the other ASyS but not distinct inflammation. The studies by Tanboon et al. and Preuße et al. agree that anti-PL12 is associated with less extent muscle pathology than the other subtypes [70,71]. There is limited information on the muscle pathology of the uncommon/newly identified ASyS subtypes: anti-KS is associated with necrotizing myopathy with PFN while anti-Zo and anti-valyl tRNA synthetase are associated with necrotizing myopathy [5,70,72]. There is no information on muscle pathology of anti-Ha and anti-Ly ASyS [4].

Overlapping pathological features, although less commonly present in ASyS, without immunohistochemical surrogate markers for IFN1 activation (e.g., MxA or ISG15) could disguise ASyS as DM and vice versa. These features include perifascicular atrophy (PFA), MHC class I expression with perifascicular enhancement, capillary C5b9 deposition in perifascicular areas, decreased capillary: myofiber ratio, and presence of tubuloreticular inclusion in EM. Nuclear actin inclusion originally described by Stenzel et al. is a distinctive finding in ASyS identified in EM of 81% ASyS in the original study [57] and 24% by Tanboon et al. (pilot study) but the frequency of the inclusion is vastly different among the cases.

Essential immunohistochemical studies which may help establish the diagnosis of ASyS by muscle biopsy include MHC class I and -class II, C5b-9, and MxA. Positive staining for MHC class I with perifascicular enhancement is commonly present in anti-Jo1 (26.2%–79.0%) [58,70]. MCH class II expression could

be useful for the pathological diagnosis as it is present in 61.2% of ASyS with the sensitivity and specificity of 61.2% and 95.4% after clinical-pathological exclusion of IBM and DM; MCH class II expression with perifascicular pattern is more common in anti-Jo1 [70] (64.1%). Sarcolemmal C5b-9 deposition is present in 47–50% of ASyS [70,71]; its expression in the perifascicular area is present in 30.2% of ASyS [70]. B cells and plasma cells are commonly present in the perimysium of anti-Jo1, anti-PL7, and anti-PL12 ASyS muscle biopsies [71]. The immunohistochemical markers for B cell and plasma cell (e.g., CD20, CD138, BAFF, APRIL) may help tailoring cases suitable for B cell/plasma cell antagonists.

6. Joint involvement in ASyS

Alain Meyer and Margherita Giannini presented the results of a literature review covering the prevalence, characteristics and treatment of rheumatic manifestations of ASyS (ASyS arthritis). They also presented original data from a monocentric cohort of ASyS arthritis (Referral Centre for Systemic Rare Autoimmune Diseases, Strasbourg, France).

Joint involvement is an independent predictor of quality of life in IIM patients [73] and is a hallmark of ASyS as compared with other subsets (i.e. DM, IMNM and IBM) [74]. In several large independent ASyS cohorts, joint involvement has been reported to be more frequent in patients with anti-Jo1 as compared with other anti-ARS [3,11,17].

Clinically, ASyS joint involvement includes inflammatory arthralgia (63–100%), arthritis (20–60%), subluxation (15–20%) and flexion contracture (<5%) [1,75,76]. It mainly involves the hands (wrist, metacarpophalangeal and proximal interphalangeal joints) resembling rheumatoid arthritis (RA). Elbows, shoulders, feet, knees and hips are less frequently involved [1,75,77,78]. Sonographic findings also resemble features seen in patients with RA. It includes synovial hypertrophy in all cases (with or without active Doppler enhancement), effusion and tenosynovitis in the majority of the cases. Erosion can be found [79].

Because the characteristics of hand radiographs have been only reported in uncontrolled studies [76,80], Drs. Giannini and Meyer presented a personal series of hand radiographs of 40 patients with ASyS arthritis (negative for anti-citrullinated protein/peptide antibodies [ACPA]) compared with 45 RA patients (positive for ACPA and negative for anti-ARS) matched for age, sex and disease duration. The presence of radiologic lesions was associated with disease duration. Capsular calcifications and subluxations were more frequent in ASyS arthritis patients while joint narrowing and bone erosion were more frequent in RA, supporting that ASyS arthritis is a peculiar rheumatic disease, part of the syndrome.

ACPA, biomarker of RA associated with severity [81], have been reported in 10 to 29 % of patients with ASyS arthritis [80,82]. Similarly to what has been described in other settings (such as systemic lupus erythematous), ACPA have been associated with severe arthritis, refractoriness to conventional treatments, higher frequency of bone erosions and joint narrowing on hand radiographs [80,83–85], indicating an overlap with RA.

Isolated arthritis (without muscle or lung symptoms) has been reported as the inaugural manifestation in about a quarter of ASyS patients [41,77,78,84,86,87]. This frequent peculiar chronology has been associated with a delayed diagnosis of ASyS [78,88]. Yet, in these patients, the cumulative incidence of the other hallmarks of ASyS (i.e. myositis and/or ILD) gradually increased overtime. In several independent series with a median follow-up of about 7 years, arthritis remained the sole clinical manifestation in less than 10% of the patients [41,84,86,87].

As a consequence of the above, ASyS arthritis can be (mis)diagnosed as other forms of inflammatory arthritis, especially RA (with or without ILD). In accordance with this view, anti-ARS has been reported in up to 6% of patients diagnosed with RA and these patients were characterized by the higher prevalence of ILD [89,90]. Importantly, the distinction between ASyS arthritis and RA might have implications for the management of the patients.

Evidence to guide the treatment of ASyS arthritis is limited to case reports and series in which the response of joint involvement has been heterogeneously assessed. There is no dedicated tool to monitor the activity of ASyS arthritis. The 2016 ACR/EULAR criteria for clinical response in IIM captures articular activity of ASyS through the visual analogue scale for extra muscular disease activity [91] while disease activity score for RA additionally takes into account the number of swollen joints, the number of tender joints and the acute phase response (ESR or CRP level) [92].

Methotrexate (MTX) was the most frequently used drug for ASyS arthritis. Hydroxychloroquine, leflunomide and sulfasalazine were also frequently used [78,84,88,93]. In the Strasbourg cohort of ASyS arthritis, 23% of patients with ASyS arthritis received unconventional treatments - including anti-CD20 (77.8%), JAK inhibitors (22.2%), anti-IL-6 (22.2%) and CD28–CD80/86 inhibitor (11.1%) - during their follow-up because of refractoriness of joint involvement to first line therapies. Efficacy with acceptable tolerance of these drugs in ASyS arthritis has been previously reported [43,83,94–99].

By contrast, the majority of the reported ASyS arthritis patients treated with anti-TNF-α (approved for RA) experienced the development of extra-articular flare of the disease (myositis, ILD and/or skin rash) [100–105]. This is in line with the high incidence of disease flares, associated with increased IFN-α serum levels, reported in an open pilot study of infliximab in patients with refractory IIM [106] and the association between IFN-α serum levels with activity of ASyS (https://www.researchsquare.com/article/rs-14504/v1.) This further highlights the importance of distinguishing ASyS arthritis from RA at the disease onset (including “isolated inaugural arthritis” and “arthritis with ILD”). A definition of “arthritis” as a major symptom for ASyS is given in Table 2.

7. Skin and ASyS

7.1. Skin and ASyS: mechanic hands and dermatomyositis-like rashes

David Fiorentino began his talk by outlining possible mucocutaneous manifestations of ASyS: mechanic hands, “DM-like” inflammatory erythema, calcinosis cutis, and ischemic manifestations. He noted that there are no “pathognomonic” skin lesions in ASyS.

In the discussion of mechanic hands (MH), he noted that the reported prevalence in ASyS ranges from 19 to 56%, with most studies showing a prevalence of 30–40%. He explained that the first description of mechanic hands was in 1979 [107] and was described with the following features: scaly, hypertrophic, fissured, hyperpigmented plaques. The classic areas involved were ulnar surface of the thumb and radial aspect of the fingers, most notably on index and middle fingers. More rarely, the palms, fingertips and feet could be involved. He commented on the differential diagnoses of psoriasis, hand eczema, or contact dermatitis. Points that help indicating MH are the absence of vesicles and itchiness, the symmetric distribution of lesions on both hands, and that there is no history of contact exposure. He also noted that the original description noted possible resistance to topical corticosteroids, which is indeed the case. Overall, MH are associated with ASyS, MDA5 and PM/Scl antibodies.

Regarding association of MH with ILD, David Fiorentino summarized a recent study suggesting that MH are an independent predictor of ILD in ASyS, with an odds ratio of 10 [108]. However,

Table 2
Proposed consensus for the definition of "arthritis" as a major symptom for ASyS. *Mainly involved in osteoarthritis.

	ASyS arthritis (all criteria are required)
Target population	Patients who • have **at least 1 joint** with **definite** clinical AND/OR sonographic **synovitis (swelling)** • with the synovitis not better explained by another disease
Distribution of joint involvement (see def. below)	**≥ 4 small joints (with or without involvement of large joints)**
Duration of symptoms	**≥ 6 weeks**
Definition of joint involvement	Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment*.
Definition of small and large joints	"Small joints" refer to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists "Large joints" refer to shoulders, elbows, hips, knees, and ankles.
Definition of duration	Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status

in other syndromes, such as DM, there are conflicting data regarding the link between MH and ILD. There are also some data that MH signify a better prognosis in ILD [109].

"DM-like" rashes are also found less commonly in ASyS. These include facial erythema, heliotrope rash, Gottron's papules and Gottron's sign, and less commonly truncal rashes such as chest or upper back erythema. Calcinosis cutis occurs in 3–10% of patients with ASyS. These lesions can be localized or widespread, and can involve the superficial dermis of the skin, subcutaneous fat, and come in the form of small nodules or larger sheet-like plaques deep in the fat or even within muscle tissue.

Vasculopathic lesions can also occur in ASyS. These are rarely reported and usually occur in the first 12 weeks of the disease. Interestingly, the most common sites of ischemia are the digital tips, which can result in gangrene of one or more digits. Both arteritis as well as venous thrombosis have been described, and so the contribution of inflammatory "vasculitis" versus non-inflammatory microocclusive vascular disease remains unclear. Additionally, reports of these lesions in either anti-PL7 or anti-PL12 ASyS appear to be more common.

David Fiorentino then discussed data addressing whether different anti-ARS are associated with different cutaneous manifestations. He explained that the AENEAS multi-center collaborative group published one of the largest retrospective studies of 828 ASyS patients, which suggested that the prevalence of MH across Jo1, PL7, PL12, OJ and EJ groups is not significantly different [3]. However, beyond MH, he noted a Japanese study that suggested that "DM-like rashes" are most commonly associated with anti-Jo1, anti-EJ, anti-PL7 and anti-PL12 antibodies, and are rarer in patients with anti-KS or anti-OJ antibodies [13]. However, between these groups, the frequencies of the specific types of DM rashes varied greatly. Recent data from the MYONET registry suggest that heliotrope rash, Gottron's sign and Gottron's papules are the most commonly found manifestations of ASyS, found in approximately 6–20% of patients, again with varied prevalence across subgroups [110]. A longitudinal study from Johns Hopkins suggested that DM-like rashes can occur in up to 72% of anti-Jo1 patients, and only in 9% and 16% in anti PL12 and anti-PL7, respectively [111].

The talk was closed by discussing treatments for skin manifestations of ASyS, noting that there are no prospective studies in this regard. Mainstays include photoprotection and topical corticosteroids. Dry skin care and the use of keratolytics (e.g. lactic acid, urea) can be helpful for the scaliness of MH. Other topical options include calcineurin inhibitors and, more recently, topical JAK inhibitors, although these are both off label uses for these medications. Finally, systemic agents can be used, especially in debilitating cases of painful MH precluding use of the hands. In these cases, antimalarials, traditional immunosuppressive agents (e.g. MMF, MTX) and intravenous immunoglobulins (IVIGs) can be used. More recently, there are case reports of JAK inhibitors being successfully used for MH.

7.2. Skin and ASyS: how to differentiate between DM-rashes

In his second talk, David Fiorentino presented a framework in which he would discuss a comparison of "DM-like" rashes in ASyS (ASyS-DM) versus the more classic rashes in dermatomyositis (DM). He first made the point that this is a difficult comparison, as, even with the DM diagnostic category, there is substantial heterogeneity in both the types of manifestations as well as their prevalence.

He began by presenting data regarding the frequencies of different types of inflammatory erythema in the two groups. He suggested that heliotrope rash and truncal rashes (e.g. chest and back) are found more commonly in DM than the ASyS-DM group [110,112]. MH, on the other hand, tended to be found more commonly in ASyS-DM. He also discussed that, oftentimes, the quality of the erythema found in some DM patients differs from that of ASyS-DM with the former being more "poikilodermatous", with unevenly coloured areas of white and red within the lesions.

He then discussed the concept of MH, noting that there is a spectrum of lesions found in DM that may have different prognostic significance. For example, lesions on the lateral side of the index finger can vary from mildly hyperkeratotic erythema, Gottron-like 2–3 mm umbilicated papules, to frank scaling and fissuring. He noted that more studies are needed to accurately

classify these lesions, as some may be more associated with particular features (such as ILD) than others.

Next, he presented some observations regarding ischemic lesions of DM vs ASyS-DM. Again, even in DM the ischemic lesions can present variably (as ulcers, small crusts, digital gangrene, oral ulcers) and may have differing significance depending on the underlying DM-specific autoantibody. In contrast, from the scant case reports and small series, it appears that ischemic lesions of ASyS-DM tend to be more commonly localized to the acral surfaces, often as either digital gangrene or livedoid erythema.

David Fiorentino ended his talk by discussing what is known about possible varying pathophysiology between skin lesions of DM vs ASyS-DM. He discussed the classic "interface dermatitis" of DM, which consists of dying keratinocytes, vacuolar degeneration at the dermal-epidermal junction, and pigment deposition ("dropout") in the superficial dermis. However, these histology findings are not specific for DM, as they can be seen in cutaneous lupus, graft-versus-host disease, erythema multiforme, photo-induced eruptions, and some kinds of drug eruptions. An interesting study from Japan compared biopsies of fingertip lesions of patients with DM (anti-MDA5 and anti-TIF1γ) with those of ASyS [113]. While all three autoantibodies were associated with interface dermatitis, only the ASyS lesions could also have pathologies that either looked psoriasis-like ("psoriasiform") or, in some case, eczema-like. They also found that Myxovirus resistance protein A (MxA) staining, a manifestation of elevated type I interferon, was lowest in the ASyS lesions. The discussion was finished with multiplexed mass cytometry data from the U Penn group showing that there is not very much evidence for a large difference between the traditional "DM-like" cutaneous manifestations seen in DM vs. ASyS [114]. However, a caveat of this study was the small number of patients and the large heterogeneity of findings within each group.

8. Cardiac manifestations in ASyS

Louise Diederichsen explained that cardiac involvement is a well-known and feared phenomenon in IIM. However, the frequency of cardiac involvement is a matter of debate and even more so when it comes to the different subsets, including ASyS. Most evidence exists regarding clinically evident heart affection and less to nothing is known about subclinical heart involvement in ASyS.

We have some indications of the prevalence of clinical cardiac involvement in ASyS from several registry studies. A major registry-based study from 2017 investigated associations between IIM subtypes and extra-muscular involvement [115]. The study reported 9% of cases from all subsets (156/1715) having clinical cardiac involvement and 10% of patients with ASyS. Heart involvement in this study was defined as the presence of pericarditis, myocarditis or arrhythmias. Looking more closely into clinical myocarditis in ASyS, a French registry study reported a prevalence of 3.4% (12/352) [14]. Myocarditis in these affected cases was not linked to any autoantibody specifically but was always associated with an active myositis. In the latest registry-based study – including adults from the Johns Hopkins Myositis Center Research Registry – 14 patients with IIM were identified with clinical myocarditis, i.e., less than 1% of patients in the database [116]. Most of the affected patients had active myositis (79%). The retrospective design of these studies is an inherent limitation, and patients with subclinical manifestations are likely not included because of a lack of standardized recommendations and cardiac screening algorithms for detection of myocarditis in IIM in general.

Regarding diagnostic procedures for cardiac disease, several case reports describe severe myocarditis in ASyS, with inflammatory infiltrates revealed by endomyocardial biopsy [117,118]. In addition to endomyocardial biopsy, non-invasive diagnostic procedures include a combined diagnostic work-up with measures of troponin levels (TnT/TnI), electrocardiography, and echocardiography [119]. However, cardiac MRI is the primary noninvasive method to confirm a diagnosis of clinical as well as subclinical myocarditis [120].

Treatment of clinically evident myocarditis, which should target both the cardiac disease and the other manifestations of ASyS, requires collaboration with cardiologists. Based on the current literature, immunosuppressive treatment should include high-dose corticosteroids in combination with steroid-sparing immunosuppressant agents. However, there are no controlled studies of this treatment approach and only case reports are available. In addition, Rituximab and IVIG may be useful [14].

In addition to myocarditis, the occurrence of pulmonary hypertension in ASyS can be significant and dramatically worsens the prognosis according to a retrospectively analyzed French ASyS cohort. Pulmonary hypertension was systematically associated with ILD, which might suggest the utility of echocardiographic screening on a regular basis [121].

Dr. Diederichsen closed her talk by emphasizing that prospective studies of patients with ASyS are needed to gain further knowledge on cardiac involvement, including those with subclinical or milder forms of cardiac manifestations to prevent overt heart disease.

9. Pathophysiology of ASyS

9.1. Lung as the trigger of disease

Ingrid Lundberg began her talk by explaining that, from a clinical perspective, the high frequency of ILD in patients with antisynthetase autoantibodies is striking. Furthermore, she reiterated that ILD is often present at the time of presentation of other organ manifestations such as myositis, even though the ILD may be asymptomatic. Moreover, there are case reports indicating that anti-Jo1 autoantibodies may be present before clinical manifestations of ILD or myositis, suggesting that the immune response leading to anti-Jo1 autoantibodies is an early event taking place before the clinical manifestations of the disease. Thus, a critical question is, where does the immune reactivity leading to ASyS autoantibodies take place?

From population-based epidemiological studies, it has been demonstrated that preceding infections in general as well as both upper and lower respiratory tract diseases, are risk factors for developing an IIM [122]. Furthermore, a "dose" response effect was recorded: the more preceding visits with an infection or respiratory tract disease the higher risk to develop IIM. Unfortunately, these registry-based studies did not allow a separate analysis of the ASyS subgroups. Concerning risk factors for patients with ASyS, smoking is one reported risk factor. Together with the HLADRB1$\times$03 genotype, smoking resulted in almost 8-fold increased risk of developing anti-Jo1 autoantibodies compared to non-smoking, HLADRB1$\times$03 negative European cases with IIM [123]. These observations might suggest that the lung has a role in triggering disease.

So why the lung? Most of the data come from the anti-Jo1 subgroup of ASyS and the autoantigen histidyl-tRNA synthetase. Histidyl-tRNA synthetase is ubiquitously expressed in all cells but with some quantitative difference between organs with higher expression in the lungs and in regenerating muscle fibers compared to other organs [124]. Histidyl-tRNA synthetase can be cleaved by granzyme B in the lung which could reveal neoepitopes [125]. Interestingly, histidyl-tRNA synthetase can act as a chemokine attracting CCR5+ cells [126]. During infections or

other inflammatory conditions, the histidyl-tRNA synthetase and possibly neoepitopes could be exposed to the immune system and activate it and, especially in people with high risk e.g. with HLADRB1×03 genotype, activate the adaptive immune system to start producing anti-Jo1 autoantibodies. Then a second hit is needed to target other organs such as the muscles.

Another clinical link with the lungs in patients with anti-Jo1 autoantibodies is the presence of anti-Jo1 autoantibodies of both IgG and IgA isotype in bronchoalveolar lavage (BAL) fluid, including antibodies targeting the major epitope of the histidyl-tRNA synthetase protein, the WHEP domain [127]. In addition, IgG reactivity towards the WHEP domain in BAL fluid correlated with poor pulmonary function [127]. Furthermore, in occasional patients with anti-Jo1 autoantibodies, higher levels of IgG anti-Jo1 autoantibodies were detected in BAL fluid compared to sera, indicating a local production of IgG anti-Jo1 autoantibodies in the lungs [128]. Further support for the local production of autoantibodies in the lungs is the presence of germinal center like structures in the lungs that were not found in other chronic inflammatory lung diseases. In addition, T cells reacting to the histidyl-tRNA synthetase protein, as well as to a peptide from the WHEP domain, were identified in peripheral blood and BAL fluid of patients with anti-Jo1 autoantibodies. Intriguingly, the T cells in BAL fluid had a much stronger reactivity e.g. by producing interferon gamma after stimulation with the HisRS peptide, compared to the cells from peripheral blood [127].

9.2. Transcriptomic studies and role of B cell niche

Corinna Preusse presented data from multiple groups with a focus on B cells and transcriptomic profiles. In ASyS B cells, plasma cells and their associated factors are thought to be involved in the pathogenesis of the disease. This assumption has been substantiated by various studies, which have shown that there are alterations in the B cell compartment in patients with ASyS. In particular, in the blood of patients an increase of naïve B cells is seen; while the more mature, class-switched B cells are detected at a reduced level [71,129–131]. Furthermore, the Jo1 autoantigen-specific B cells exhibit a limited class switch and a reduced capacity to differentiate into antibody-secreting cells [132].

Dr. Preusse showed that changes in the B cell compartment are accompanied by changes in the Treg and T helper cell compartments, with a reduction in Th1 cells and an expansion of Tfh cells [71]. In line with this, a number of specific T- and B cell factors are significantly elevated in the blood of patients. The soluble factors include factors for B cell activation, proliferation and maturation. In particular, B-cell activating factor (BAFF), a fundamental survival factor for B cells, in blood is found to be significantly increased [71]. Functional relevance of the B cells and B cell factors is supported by the fact that treatment of patients with immunosuppressive therapy changes the B cell compartment, reducing naïve B cells and leading to an increase in memory B cells in the blood of patients [129]. A further indicator of this involvement is the response of patients to IVIG treatment, which has been shown in various studies. Of note, patients with ASyS antibodies were included in a recent clinical trial demonstrating the efficacy of IVIG for DM patients. Among the 15 patients with positive ASyS-antibodies, all showed clinical improvement similar to the DM patients [133].

In addition, transcriptomic studies provide further evidence for the role of humoral immunity as the frequency of memory B cells allows the differentiation of patients with active and inactive IIM. However, not only ASyS, but various forms of myositis were investigated in this study [134]. Furthermore, meta-analyses of multiple studies analysing the effect of rituximab in treatment refractory IIMs show very high overall efficacy rates of this B cell depleting medication, reaching 62% in ASyS. Of note, Jo1-positivity can also predict clinical improvement [135].

Immune cells invading either the perimysium or the endomysium of skeletal muscle tissues are well-known features in myositis patients and this is true for ASyS as well. Here, in addition to the high number of CD68-positive macrophages and various T cell subsets (CD4, CD8), a significant number of (memory) B cells and plasma cells is found [71,131]. The immune cells are often found in clusters or in proximity to other cell types, all of them located in the perimysium or invading the adjacent endomysium, thus suggesting close interaction between them. This was further supported by the expression of chemo-attractants on different immune cell types, whereby the chemo-attractants are involved in homing of B cell and plasma cell subtypes. In the context of skeletal muscle in ASyS, Dr. Preusse highlighted expression of CXCL12 and CXCL13 on macrophages, T cells and B cells, whereas BAFF is only expressed by CD20-positive B cells. In contrast, the expression of APRIL, another important survival factor during B cell development, was shown on macrophages and T cells. Furthermore, expression levels of CXCL13 were significantly increased in ASyS when compared to non-disease controls, while neither APRIL, nor BAFF were elevated [71]. This is of interest, since another study showed BAFF levels in blood correlating with antibody levels and disease severity in IIM [136]. Moreover, in bone marrow, integrin-mediated cell contact of memory plasma cells to stromal cells is essential for their survival, but only with additional survival signals from BAFF or APRIL [137]. While the various roles of survival signals in inflamed tissues are still under investigation, they could potentially depend on certain key factors. Yet, a first proof-of-concept study with the IgG1 monoclonal anti-BAFF antibody Belimumab had no significant clinical effect, but lead to a shift in the B cell population, with a decrease in naïve and increase in memory B cells [138].

Interestingly, a correlation between the B cell clones found in skeletal muscle and those detected in blood has been shown, as the dominant BcR clones in muscle tissue can be retrieved in peripheral blood [139].

In addition, Corinna Preusse presented proteomic studies of skeletal muscle tissues from ASyS patients which demonstrated a significant increase in proteins that are necessary for antigen processing and presentation. In particular, proteins of the MHC class I complex are increased. This fits perfectly with the fact that a clear expression of MHC class I in the muscle is detected by immunohistochemistry [71].

Another important aspect in the pathogenesis of ASyS is the expression of various interferon-associated molecules. In a landmark paper by Pinal-Fernandez and colleagues, specific interferon signatures were found by a bulk transcriptomic approach. Using RNA sequencing of muscle biopsy samples from 119 patients with DM, IMNM, ASyS, or IBM and 20 normal muscle biopsies, the different degrees and specific types of activation of type I and type II interferon-associated genes were defined [65]. Whereas DM has a strong type I interferon response (IFN1) highlighted by high expression of 10 prototypic representative genes, ASyS showed a moderate type I and a high type II interferon response (IFN2). Interestingly, expression of IFN1- and IFN2-inducible genes positively correlated with expression of genes associated with inflammatory cells and muscle regeneration [65]. In addition, the expression of seven specific miRNAs in ASyS patients via miRNA profiling using the NanoString nCounter system was demonstrated. The miRNA-mRNA associations predominantly related to inflammation, cell cycle progression, and IFN-related genes [140].

In summary, these studies demonstrate that B cells and plasma cells in ASyS play a crucial role in pathogenesis, and that IFNs play

a decisive role as well. Both pathogenic pathways could potentially be targeted by specific therapeutic approaches.

9.3. Animal models: what can they tell us about pathophysiology

Tobias Ruck began his presentation by reminding the audience that many clinical and scientific questions regarding ASyS remain open. In particular, the pathogenesis of ASyS is still only fragmentarily understood, which hampers the development of specific diagnostic and therapeutic tools. Thus, a deeper understanding of the underlying pathophysiological mechanisms is inevitable to improve patient care.

Animal models have proven to be an essential resource for in-depth mechanistic studies and for drug development in many human diseases [141]. The most prominent advantages of animal models include the circumvention of restricted access to human tissue and the possibility to investigate mechanisms in the context of a complex organism. On the other hand, differences between organisms may result in findings that are not relevant to human disease. Thus, relevance of results from animal models requires some sort of verification to the human system.

The first animal models for IIMs were described around 70 years ago and the number of publications describing animal models for IIMs has been increasing since that time. However, many models lack central histological and phenotypic properties of IIM, show only partial overlap with human pathophysiology (immunological, degenerative alterations), use irrelevant targets for human disease, are monophasic and self-limiting in disease courses, and/or have not led to the identification of novel therapeutic targets.

One important feature that distinguishes between the different IIM animal models is the method used to induce disease. These includes naturally occurring (e.g. in dogs), diet-triggered (e.g. 2% cholesterol in rabbits), infectious (e.g. Coxsackievirus B1, Chikungunya, Trypanosoma), transgenic (e.g. MLC-APP, *H + T+* mice, Syt VII$^{-/-}$) and immunological models [142]. For ASyS, Katsumata and colleagues generated an immunonological mouse model [9]. They injected NOD congenic strains (B6.G7 and NOD.Idd3/5), which are prone to autoimmunity, with purified antigenic peptides derived from histidyl-tRNA synthetase (HisRS, Jo-1) along with CFA. This led to inflammation in the muscles and lungs, albeit with a suboptimal incidence of around 40–60%. Muscle histology revealed inflammation patterns including perimysial/epimysial inflammation in a perivascular distribution, endomysial inflammation, and muscle fibre invasion/degeneration. However, a clinical phenotype that included muscle weakness and respiratory dysfunction were not shown. The immunized mice demonstrated a Jo1 specific CD4 T cell response and Jo1 antibodies. However, mice lacking Jo1-specific B and T cell responses (DO11.10/Rag-2-KO) still showed significant muscle inflammation after immunization [143]. Additionally, mice lacking significant levels of anti-Jo1 antibodies (TLR4-KO) also experienced muscle inflammation when immunized with Jo1, suggesting that an innate immune response, rather than Jo1-specific adaptive autoimmunity, plays a key role in this model of myositis induced by Jo1. Thus, current mouse models of ASyS recapitulate important histological and clinical features of human ASyS. However, there seem to be certain differences to human immunopathophysiology. In particular, skin and joint manifestations, which can be prominent in ASyS, do not occur in the animal model. Nonetheless, despite the aforementioned limitations, this model offers an opportunity for new mechanistic insights, including testing of new therapeutic strategies. Further development (including other tRNA synthetases as targets) and in–depth characterization of the clinical phenotype as well as the spatio-temporal disease mechanisms will be required to improve the utility of this model.

10. ASyS treatments

10.1. ASyS standard treatment: review of clinical trials

Andrew Mammen began his presentation on standard treatments for ASyS by pointing out that there are no FDA-approved medications specifically for this disease that most studies have been retrospective or uncontrolled, and that few studies have compared different therapies. Furthermore, there are no data to support different strategies for treating different autoantibody-defined subtypes of ASyS (e.g., anti-Jo1 vs. anti-PL7). Despite these limitations, one comprehensive review of the topic suggests that the goal should be to target treatment to the most severe manifestations of the disease [144].

In many instances, ILD is the major driver of morbidity and mortality for patients with ASyS. Initial treatment usually involves oral corticosteroids for mild disease and intravenous steroids for more serious disease. Importantly, adding a steroid-sparing agent at diagnosis has been associated with better survival and fewer relapses [145,146]. To date, it's unclear which drug is the optimal steroid-sparing agent in patients with myositis-associated ILD [147]. In one study, azathioprine and mycophenolate mofetil were similarly effective in improving pulmonary function test measures, but the latter had fewer adverse events [148]. In another study, patients treated with tacrolimus or ciclosporin-A had better outcomes then those who received other therapies [149]. In contrast, a more recent study indicated that in those with myositis-associated ILD, intravenous cyclophosphamide resulted in better functional outcomes than other treatments [150].

10.2. ILD treatment: IS, IVIG, biological, anti-fibrotic

Sonye Danoff explained that treatment in ASys-ILD has not been widely studied in RCT; thus, the majority of recommendations for therapy derive from case series or retrospective studies. Most therapies adopted in the treatment of ASyS-ILD are based on immunosuppresive therapies used in treatment of other manifestations of ASyS. The one exception is the use of anti-fibrotics, which will be discussed in detail below.

When considering therapy for patients with ASyS-ILD, it is convenient to classify patients into three categories of disease burden based on PFTs, HRCT and hypoxemia: mild, moderate, severe (Fig. 1) [50]. Mild disease can be described as having preserved PFTs, minimal or no symptoms, $<$10% involvement on HRCT and no oxygen requirement. Moderate disease includes individuals with abnormal PFTs, evidence of disease progression (by PFTs or symptoms or oxygen requirement) or $>$10% involvement on HRCT. Severe disease is characterized by the need for hospitalization or worsening hypoxemia. These categories are dynamic - so an individual with ASyS-ILD of moderate disease could be treated and improve to have mild disease. Conversely, the patient could worsen or experience an acute exacerbation and develop severe disease.

Initial therapy, if indicated, is typically corticosteroids (CS). CS can be oral or intravenous. The benefit is related to rapid onset of action and wide therapeutic range. However, CS have significant risk of side effects and, thus, are typically paired with a steroid sparing agent. While not specifically focused on pulmonary manifestations, Troyanov et al. [151] showed frequent disease relapse in patients tapered to low dose prednisone without addition of a steroid sparing agent. Dosing ranges, side effects and monitoring recommendations for CS as well as other therapeutic agents are included in Table 3 [152].

While there are many options for steroid sparing therapy, azathioprine and mycophenolate mofetil are common first line agents. These agents appear to have similar efficacy for myositis-

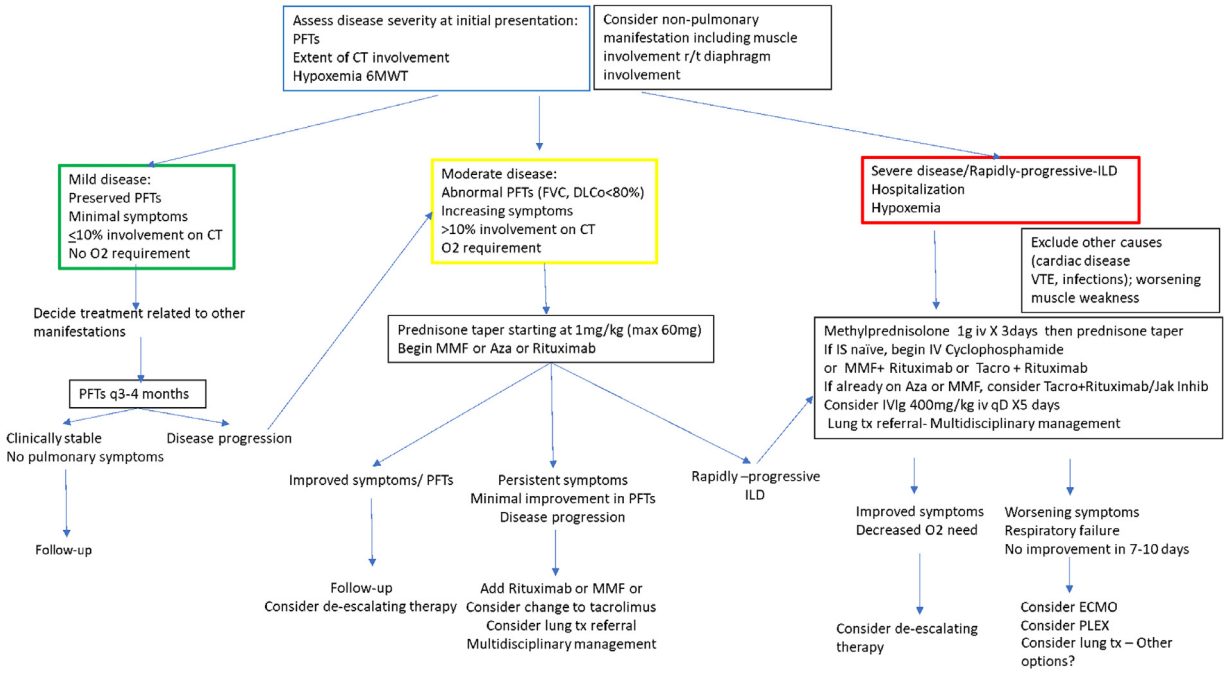

Fig. 1. ASyS-ILD: treatment approach (from [50]) The decision to utilize chronic immunosuppression in patients with myositis-ILD and the agent of choice is influenced by the severity of disease at diagnosis, disease trajectory and response to initial therapy, patient comorbidities and provider familiarity with the treatment modalities available at a given institution.
Note: if muscle involvement consider IVIG earlier General approach and additional interventions that may be necessary from a pulmonological point of view: All patients should receive vaccination; and oxygen if required Patients having clinical deterioration should have echocardiography to avoid misdiagnosis of ILD progression. Preventive care and treatment of opportunistic infections Referral to lung transplantation if no muscle involvement Symptom management (cough, dyspnea, pain, anxiety, depression) Support groups; pulmonary rehab; patient education; diet AZA, azathioprine; IVIG, intravenous immunoglobulin; lung Tx, lung transplantation; MMF, mycophenolate mofetil; PLEX, therapeutic plasma exchange TAC, tacrolimus; RTX, rituximab.

ILD based on retrospective studies. Azathioprine is associated with more side effects, specifically, elevation of liver function tests [148]. Appropriate monitoring for all medications is critical (Table 3).

Calcineurin inhibitors (CNI) (ciclosporin-A and tacrolimus) have also been shown to reduce the risk of progression compared with CS therapy alone [153]. Additionally, CNI have been described in the treatment of rapidly progressive myositis-ILD [154].

If CS and steroid sparing agents are not sufficient to achieve stabilization or improvement in ASyS-ILD, additional agents including IVIG, rituximab or cyclophosphamide can be considered. There are a number of case series supporting the use of IVIG in refractory ASyS-ILD [148,155]. Rituximab was shown to improve FVC and DLCO in a small series of patients with refractory myositis-ILD [156]. A recent randomized control trial compared the effectiveness of rituximab with cyclophosphamide and found the two agents to be equivalent in a mixed autoimmune-ILD population that included 46% myositis-ILD in the cyclophosphamide arm and 43% in the rituximab arm [157].

Recent studies of anti-fibrotics in progressive fibrosis have included patients with autoimmune-ILD which worsened despite adequate disease-specific therapy. Anti-fibrotic therapies were evaluated [158,159] and approved in the US for use in patients with idiopathic pulmonary fibrosis (IPF). The focus of the recent studies is on patients with other forms of ILD (not including IPF) which progress despite disease-appropriate therapy based on one or more of the following criteria over 24 months: 1) relative decline in FVC>10%, 2) relative decline in FVC 5–10% and increased respiratory symptoms/ increased extent of fibrosis on HRCT, 3) worsened respiratory symptoms and increased extent of fibrosis on HRCT. The INBUILD study [160] evaluated 663 patients including approximately 25% autoimmune ILD, although myositis-ILD was not well represented [160]. This study of a mixed patient population showed a slowing of the rate of decline of FVC in participants on nintedanib compared with placebo. Notably, the side effect profile showed diarrhoea in almost 70% of participants. A clinical trial looking at nintedanib specifically in myositis-ILD is currently recruiting (ClinicalTrials.gov ID NCT05799755).

In addition to medical therapy, patients with ASyS-ILD require supportive therapy [161]. This includes assessment for supplemental oxygen needs, pulmonary rehabilitation, age-appropriate vaccination and cancer screening. In addition to these needs, patients with ASyS-ILD should be evaluated for co-morbidities including pulmonary hypertension (pH) and obstructive sleep apnoea. Finally, symptom management can include addressing cough, pain, fatigue, anxiety and depression. This may be facilitated by offering access to support groups as well as patient-centred educational materials.

Lung transplantation may be an option for a selected group of patients with ASyS-ILD which progresses despite other therapy. There are limited reports of lung transplantation in patients with myositis-ILD with no evidence of ILD recurrence after transplant. Survival appears improved in those with clinically amyopathic disease compared to those with overt muscle disease [162]. The risk of systemic manifestations of ASyS continues to be of some concern. Further research will be needed to assess this risk.

10.3. ASyS treatment with CAR T cells

Ioanna Minopulou began by reminding the workshop group that although pathogenetic mechanism of ASyS remains obscure, the presence of circulating autoantibodies [163] as well as the presence of B cells and plasmablasts in the lung [128] and muscle biopsy [71] specimens of patients with ASyS, support the implication of B cells in the development of the disease. The

Table 3
Drug therapies used in the treatment of ASyS-ILD (Adapted from [152]).

Drug	Dose	Monitoring	Adverse effects	Comments
First-line				
Corticosteroids	0.5–1 mg/kg/d prednisone; methylprednisolone 500 – 1000 mg/d for 3 d	Annual bone density scan, glucose monitoring	Osteoporosis, glaucoma, hyperglycemia, insomnia, weight gain, bruising, mood changes, myopathy, risk for PJP	Disease severity dictates dosing. Taper slowly when steroid-sparing agent has reached therapeutic effect.
First Line Steroid-sparing agents				
Azathioprine	2–3 mg/kg/d (generally not higher than 200 mg/d)	TPMT level before initiation; CBC and CMP every 2 wk during dose titration and 4–8 wk thereafter; yearly skin exam	Transaminitis, leukopenia, GI intolerance, increased risk of malignancy	Start at 50 mg/d and titrate by 50 mg/wk if tolerating. May need dose adjustment based on TPMT level. Controls myositis effectively.
Mycophenolate Mofetil	2,000–3,000 mg/d divided in two doses	CBC count and CMP every 2 wk during dose titration and every 4–8 wk thereafter; yearly skin examination	Transaminitis, leukopenia, GI intolerance, increased risk of malignancy, concentration impairment	Start at 500 mg twice a day; titrate slowly over a few wks according to tolerability. Controls skin disease effectively
CNIs				
Ciclosporin A	2–5 mg/kg/d divided in two doses	CBC, CMP, CsA trough levels every wk for first month and every 4 wk thereafter; yearly skin exam and lipid panel	Renal toxicity, hypertension, hyperlipidemia, tremors, hyperglycemia, increased risk of malignancy	Start at 2 mg/kg and titrate dose by 0.5 mg/kg Maintain serum trough level 100–200 ng/mL Controls myositis and joint disease effectively
Tacrolimus	Typical starting dose is 0.5–1.0 mg or 0.075 mg/kg twice a day	CBC count, CMP, TAC trough level every week for the first month and every 4 wk thereafter; yearly skin exam and lipid panel	Renal toxicity, hypertension, hyperlipidemia, tremors, hyperglycemia, increased risk of malignancy	Titrate to a trough of 3–6 ng/mL Controls myositis and joint disease effectively
Adjunct therapy				
Rituximab	1,000 mg day 0 and day 14; repeat about every 6 months	Hepatitis and latent TB screening; immunoglobulin, CBC prior to infusions; CD19/CD20 levels before initiation and sometimes during therapy	Hepatitis reactivation, increased risk of severe SARS-CoV-2 infection, infusion reactions, progressive multifocal leukoencephalopathy	Reserved for severe or resistant disease as an adjunct therapy. Controls myositis and joint disease effectively
Human intravenous immunoglobulin	2 g/kg/mo divided over 2–5 d	Screen for IgA deficiency before initiation	VTE, volume overload, headaches (aseptic meningitis), antibody-mediated cytopenia, anaphylaxis, infusion reactions	Initially prescribed for 6 months before slowly tapering by spacing out therapy every 5–8 wk before withdrawal. Not considered to be immune-suppressive. Reserved for severe and resistant disease as an adjunct therapy. Can be used in acute exacerbations
Cyclophosphamide	2 mg/kg po daily; 500–750 mg/m^2 IV monthly	CBC count and CMP every 2 wk initially, UA monthly, lifelong urine cytologic analysis annually	Myelosuppression, malignancy, hemorrhagic cystitis, infertility	Maintain WBC >3500/mm^3. Dose adjust by 25% according to WBC count of initial IV dose; not to exceed 1000 mg/m^2 Often reserved for acute exacerbation

Abbreviations: CBC, complete blood count; CMP, comprehensive metabolic panel; CsA, ciclosporin A; ILD, interstitial lung disease; IgA, immunoglobulin A; IVIG, IV immunoglobulin; mo, month(s); PJP, Pneumocystis jirovecii pneumonia; TAC, tacrolimus; TB, tuberculosis; TPMT, Thiopurine S-methyltransferase; UA, urinalysis; VTE, venous thromboembolism; WBC, white blood count; wk, week(s).

pathogenetic role of B cells is further highlighted by the success of B cell depletion therapies targeting CD20 in the treatment of ASyS. In particular, a subanalysis of the Rituximab in Myositis trial showed that antisynthetase antibody positivity was a predictive factor for clinical response to rituximab therapy [164], while the decrease in anti-Jo1 levels correlated with clinical outcomes [165]. Nevertheless, a significant proportion of patients does not respond to treatment with rituximab [135]. This limited therapeutic efficacy of rituximab could be either due to the persistence of autoreactive B cells within lymphoid organs [166] and inflamed tissues despite peripheral B cell depletion [167], or due to the pathogenetic role of CD20-negative B cells that could potentially maintain the autoimmune response.

Within this context, autologous T cells [168] genetically engineered to express chimeric antigen receptors that target CD19 (anti-CD19 CAR T cells) could be used to overcome these limitations and achieve a broader B cell depletion. Indeed, three ASyS patients (two males [169] and one female [170]) have already been successfully treated with anti-CD19 CAR T cells. All patients were in their early forties and anti-Jo1 positive. The male patients suffered from both myositis and ILD [169,171], whereas the female patient presented mainly with myositis and polyarthritis [170]. All patients were refractory to several immunosuppressive treatments including anti-CD20 B cell depletion therapies [169–171].

In these patients, immunosuppressive treatment was ceased and glucocorticoids were tapered prior to leukapheresis. After

leukapheresis, autologous T cells were transduced by lentiviral anti-CD19 CAR vectors and expanded. The patients received conditioning therapy with fludarabine and cyclophosphamide followed by a single intravenous infusion of anti-CD19 CAR T cells. Shortly after their administration, CAR T cells expanded *in vivo*, while B cells were rapidly eliminated from the peripheral blood. Prompt and remarkable response to treatment was observed in all patients and the response was maintained for up to 8 months of follow-up [169–171]. All three patients regained muscle strength, muscle enzymes were normalized and inflammatory changes suggestive of myositis resolved on magnetic resonance imaging [169,171] and positron emission tomography [170]. Similarly, pulmonary function tests improved [169,171] and alveolitis completely resolved in the chest CT of one patient post treatment [169]. B cells reappeared in all patients, without any evidence of disease activity. However, anti-CD19 CAR T cell therapy was followed by mycophenolate mofetil in one patient [171] and therefore, potential synergistic effects cannot be excluded. Anti-CD19 CAR T cell therapy was safe and well tolerated. Two patients developed mild, grade 1 cytokine release syndrome [169,171], while one patient experienced mild dizziness interpreted as grade 1 immune-effector cell associated neurotoxicity syndrome [170].

Taken together, these findings suggest that anti-CD19 CAR T cell therapy is feasible in ASyS. Anti-CD19 CAR T cells led to improvement of ASyS manifestations despite cessation of immunosuppressive treatment in two out of the three treated patients [169,170]. Hence, anti-CD19 CAR T cells may represent a therapeutic option in patients with therapy-refractory disease. Nevertheless, future studies are warranted to evaluate the safety and efficacy as well as the cost-effectiveness of the treatment.

10.4. Outcome measures for ASyS and future trials

In this session of the workshop, Olivier Benveniste explained that, based on the data presented, ASyS is a distinct and unique type of IIM [172]. He emphasized that in order to evaluate new treatments in homogeneous groups of patients, outcome measures specific for ASyS should be developed. As ASyS often includes the triad of myositis, arthralgia and ILD, outcome measures for each of these clinical domains need to be incorporated.

Olivier Benveniste reminded the audience that generic outcome measures for IIM were developed by the International Myositis Assessment and Clinical Studies Group (IMACS) in 2011 [173] and updated in 2018 [174]. A consensus was reached in 2016 using absolute percentage change in the 6 core set measures of IMACS with thresholds for minimal (≥30 points), moderate (≥45), and major improvement (≥70) [91] with the development of a web calculator. Some limitations of this tool have been pointed out [175] but it is validated and used in many ongoing clinical trials that include ASyS patients (e.g. NCT05523167, NCT05379634 or NCT05669014). However, the main persisting problem is that many ASyS patients present with an exclusively articular or pulmonary involvement and the IMACS criteria are insufficient to demonstrate improvement in this context. As the IMACS definitions are not relevant for those ASyS patients with prominent ILD and/or arthritis system but not myositis, these patients are usually excluded from clinical trials.

11. Consensus findings

11.1. Consensus for ASyS classification

The workshop participants reached consensus on classification criteria for definite ASyS and probable ASyS.

A patient can be classified as having definite ASyS when they have a positive high confidence antisynthetase autoantibody test (Table 4) and at least one of the following clinical features (Table 5): ILD, myositis, or arthritis (skin symptoms are optional) (Fig. 2).

Table 4
Confidence of ASyS autoantibody testing.

High confidence ASyS autoantibody is defined as follows:
- Any ASyS autoantibody identified by protein or RNA immunoprecipitation.
- An ASyS autoantibody identified as part of a screening anti-ASyS ELISA [26,27]. Of note, these assays do not identify the specific ASyS autoantibody.
- Anti-Jo1, anti-PL12 or anti-PL7 identified using a commercial immunoblot assay if at least two times above the cut-off for positive as defined by the manufacturer (e.g. Euroimmune Myositis lineblot with signal strength >2+.
- Positive results for antisynthetase antibodies identified using a commercial immunoblot assay that do not fulfil the aforementioned criterion but are confirmed in an alternative assay.

Medium confidence ASyS autoantibody is defined as follows:
- Any other ASyS autoantibody (i.e., non-Jo1, -PL12 and -PL7) identified using a commercial immunoblot assay if at least two times above the cut-off for positive as defined by the manufacturer.

Patients should not have another MSA (e.g. signal strength >2+ for Euroimmune lineblot 16S), ACPA or another CTD-specific autoantibody. Anti-ASyS should still be considered in those cases where a false positive MSA result is strongly suspected e.g. PmScl 100 or 75 in isolation or anti-Mi2 α or β in isolation. Where possible an alternative assay/additional testing should be used to confirm a suspected false positive result. MAA may be present (e.g. Ro52, Ro/La) - except anti-U_1RNP, anti-PmScl or anti-Ku: the presence of these antibodies would suggest mixed CTD or an overlap CTD syndrome.

In contrast, a patient can be classified as having probable ASyS if they have (i) a positive medium confidence antisynthetase autoantibody test (Table 4), (ii) no other known myositis-specific autoantibody and, (iii) either ILD or two of the following: myositis, arthritis, and/or cutaneous features (Table 5).

11.2. Consensus for autoantibody detection in ASyS

Antisynthetase autoantibodies are differentially integrated in the definition of definite and probable ASyS. Since large studies that compare distinct assays are lacking, reliable data on test-characteristics for the individual antisynthetase antibodies are not available. Obviously, this is most apparent for the rare antibodies. Therefore, pragmatic definitions for high and medium confidence antisynthetase antibody positive results were formulated based on existing literature and expert opinion.

For definite ASyS a high confidence antisynthetase antibody positive result is required (Table 4). First, a positive result for any antisynthetase antibody detected by immunoprecipitation is considered highly reliable. Although different protocols may be applied, immunoprecipitation is considered the gold standard for the detection of antisynthetase antibodies. However, this technology is only available in expertise centers in research settings. As a consequence, the results reported are generally not included in the accreditation scope of a clinical laboratory. Second, a positive result for antisynthetase antibodies detected by an available screen ELISA has been shown to have very high concordance with immunoprecipitation [26,27]. This screen ELISA contains a mixture of Jo1, PL7, PL12, EJ, and KS antigens. Results will not reveal which of these antigens is recognized by autoantibodies. Furthermore, other antisynthetase antibodies and also other MSA relevant for the diagnostic workup of a patients suspected of idiopathic inflammatory myopathy will be missed. Third, a result for anti-Jo1, anti-PL7, and anti-PL12, obtained by line or dot immuno-assay (LIA/DIA), is considered sufficiently reliable if the result is at least two times above the cut-off for being positive as defined by the manufacturer. The prevalence of the other antisynthetase antibodies is too low and, as a consequence, reliable

Table 5
Clinical features of ASyS.

1. ILD	Defined according to American Thoracic Society guidelines [35].
2. Myositis	Defined as having two or more of the following (typically all are present): • Elevated muscle enzymes • Proximal muscle weakness • Muscle edema on MRI • Irritable myopathy on EMG • Skeletal muscle biopsy showing perifascicular and perimysial pathology characteristic of ASyS*
3. Arthritis	Defined as having all of these features: • Symmetric arthritis of multiple small joints (>/= 4) • One swollen joint based on clinical or sonographic examination • Longer than 6 weeks duration
4. Cutaneous features	Defined as having both of these features: • Two of these: Gottron's papules, or Gottron's sign, heliotrope rash • Skin biopsy showing interface dermatitis

* See Table 6.

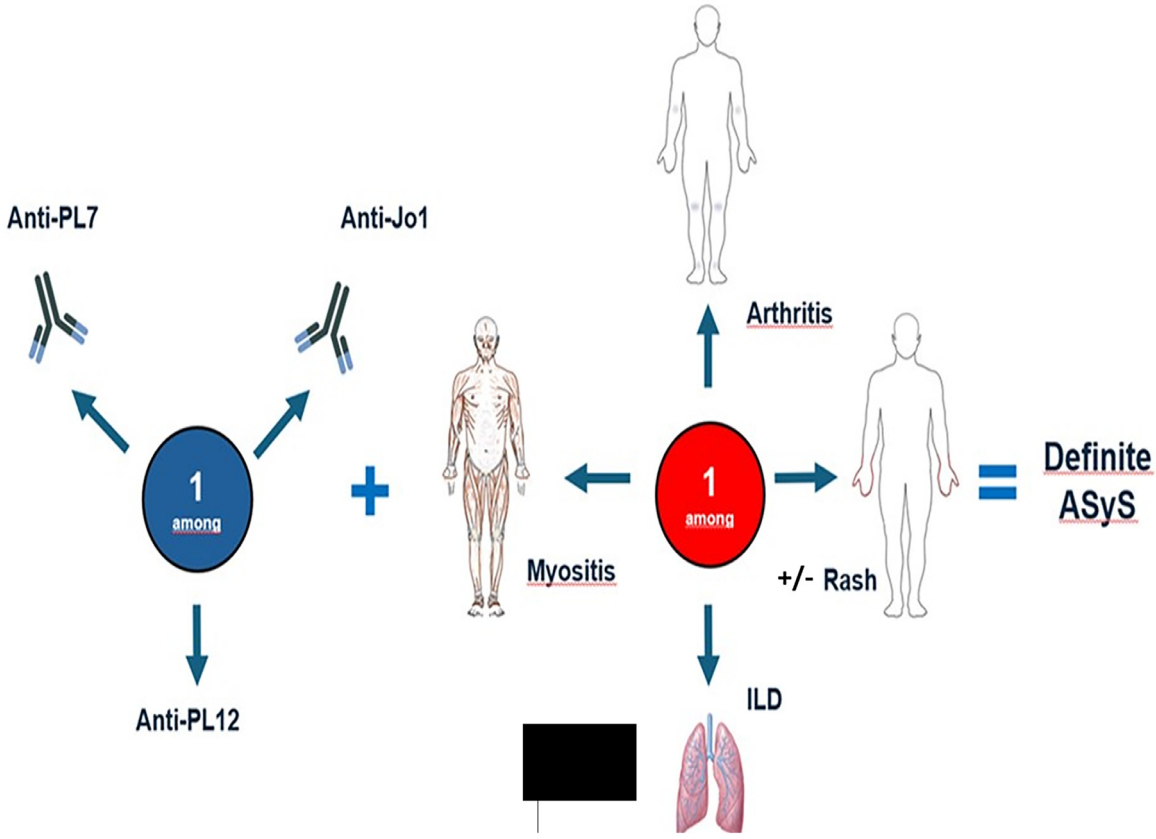

Fig. 2. Classification of definite ASyS can be achieved with a positive high confidence antisynthetase autoantibody test and at least one of the following clinical features: ILD, myositis, or arthritis. Presence of rash is optional.

data on test characteristics are lacking. Fourth, if a positive result for antisynthetase antibodies detected by LIA/DIA, but not fulfilling the aforementioned criterion, is confirmed in an alternative assay, this can be considered as a high confidence positive result. Importantly, the result has to be unequivocally positive in both assays. For some antisynthetase antibodies an alternative assay is currently not available.

For probable ASyS, a medium confidence antisynthetase antibody positive result is required (Table 4). Such a medium confidence positive result involves a positive result for any antisynthetase antibody (other than anti-Jo1, anti-PL7, and anti-PL12) obtained by DIA/LIA if at least two times above the cut-off for positive as defined by the manufacturer. In addition, caution is warranted if patients also have another MSA or MAA as determined by immunoprecipitation or, in particular DIA/LIA. If determined by DIA/LIA, results of such non-antisynthetase MSA and MAA are considered positive if at least two times above the cut-off for positive as defined by the manufacturer or are confirmed in an alternative assay. With respect to MAA, anti-PM-Scl, anti-Ku, and anti-U_1RNP are considered relevant. The presence of these antibodies suggests mixed connective tissue disease or on overlap connective tissue disease. Anti-Ro52/TRIM21 is to be excluded as MAA in this context because these antibodies may often co-occur with antisynthetase antibodies and are prognostic for more severe pulmonary involvement. Furthermore, the presence of antibodies to the distinct entities of PM-Scl (75 and 100) and Mi2 (α and β) in isolation may anyway require confirmation.

Table 6
Muscle biopsy criteria (short version).

Characteristic features	
1. Perifascicular pathology	With all pathological features/hallmarks typically decreasing towards the centrofascicular region
2. Perimysium	Is often enlarged, edematous with cellular infiltrates
3. MHC class I and class II stains	With strong perifascicular to centrofascicular gradient
4. Complement	On the sarcolemma of non-necrotic fibers with a fine punctate pattern usually in the perifascicular region Sarcoplasmic positivity is non-specific and shows some necrotic fibers (but not all of them)

11.3. Consensus on myopathological features

The workshop group did not develop muscle biopsy classification criteria that could be used to classify ASyS in the absence of serologic or clinical features. The workshop group did reach consensus regarding skeletal muscle biopsy features that characterize ASyS. Those criteria can be used to classify ASyS even if serologic or clinical features are unknown or unclear. For those cases, please refer to Supplemental Table 1. A short version of myopathological features that suffices for daily routine and that can be combined with clinical and serological parameters is given in Table 6.

11.4. Consensus for treatment of musculo-skeletal dominant disease

The workshop group reached consensus on some of the treatment recommendations for the Musculo-skeletal dominant disease.

11.5. Consensus on treatment of thoracic domain

The workshop group proposes the possible treatment algorithm shown in Fig. 1 to address ILD manifestation in ASyS.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Werner Stenzel: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization. **Andrew L Mammen:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Laure Gallay:** Writing – review & editing, Methodology, Data curation. **Marie-Therese Holzer:** Writing – review & editing, Data curation, Conceptualization. **Felix Kleefeld:** Writing – review & editing, Validation, Investigation, Conceptualization. **Olivier Benveniste:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Yves Allenbach:** Writing – review & editing.

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Supplementary materials

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