frontiers | Research Topics

Into the heart of systemic autoimmune diseases

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Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-8325-4682-6
DOI 10.3389/978-2-8325-4682-6

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Into the heart of systemic autoimmune diseases

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Citation

Franceschini, F., Brucato, A., Fredi, M., Piantoni, S., eds. (2024). *Into the heart of systemic autoimmune diseases*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4682-6

Table of contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 18 March 2024
DOI 10.3389/fmed.2024.1392487

OPEN ACCESS

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RECEIVED 27 February 2024
ACCEPTED 04 March 2024
PUBLISHED 18 March 2024

CITATION
Fredi M, Piantoni S, Brucato A and Franceschini F (2024) Editorial: Into the heart of systemic autoimmune diseases.
Front. Med. 11:1392487.
doi: 10.3389/fmed.2024.1392487

Editorial: Into the heart of systemic autoimmune diseases

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KEYWORDS

autoimmunity, cardiovascular risk, inflammation, biomarkers, cardiovascular assessment

Editorial on the Research Topic
Into the heart of systemic autoimmune diseases

Autoimmune rheumatic diseases are further burdened by cardiovascular complications (1). The amount of risk for each patient, who deserve personalized monitoring for cardiovascular comorbidities, is related to two main factors: the effects of the underlying inflammatory mechanisms of the disease, which have a direct action on the cardiovascular system (2) and the lengthening of life expectancy due to new therapeutic interventions (3). In fact, the traditional cardiovascular risk factors, which act in the general population, are also largely found in our patients (3). Currently, the main pillars of cardiovascular risk reduction are the pharmacological and non-pharmacological management of the modifiable risk factors, as well as the tight control of disease activity (4).

The aim of this Research Topic of manuscripts was to give an update about the physio-pathological and clinical aspects of the primary or secondary cardiovascular manifestations in systemic autoimmune diseases.

Due to the lack of specific prediction models, clinicians should use scores validated for the general population to screen for cardiovascular risk in autoimmune diseases (4). This Research Topic has been addressed by Mandel et al. in their review. They proposed new surrogate markers of cardiovascular risk, such as arterial stiffness and the parameters obtained from cardiovascular imaging techniques, or soluble markers that were demonstrated to be disease-related. Moschetti et al. focused their review on endothelial dysfunction, which is considered the first inflammation-induced pathogenic event triggering vascular remodeling, at the basis of microangiopathy. They focused on systemic lupus erythematosus and on systemic sclerosis, in which endothelial dysfunction is the main event of pre-clinical atherosclerosis or a key pathogenetic factor at the basis of the disease itself, respectively. Some experience in the use of some of these techniques for the evaluation of cardiovascular risk in autoimmune diseases was described by our group in one of the papers of the collection. Piantoni et al. described the use of adaptive optics, a new tool for the evaluation of retinal arterioles, which represents a good arterial compartment for the study of microcirculation. They demonstrated the reduction of an index, which is a sign of microvasculature alteration, after 12 months of therapy with abatacept, proposing a possible new scenario in the use of biological disease modifying anti-rheumatic drugs (bDMARDs) in rheumatoid arthritis. Lazzerini et al. proposed a specific interesting topic in their review: the role of autoimmunity in the pathogenesis of cardiac arrhythmias. In

particular, they underlined that increasing evidence was being reported on the role of the anti-Ro/SSA antibodies in affecting the ventricular repolarization. This is an important point considering the prevalence of these autoantibodies in patients with autoimmune diseases, but also in the general population.

Systemic vasculitis is a heterogenous group of autoimmune diseases, some of which have the cardiovascular system as one of the primary target organs. Two reviews in this Research Topic were dedicated to summarizing novel therapies used in Takayasu arteritis (TAK) and Eosinophilic granulomatosis with polyangiitis (EGPA). As revised by Uzzo et al., TAK is one of the vasculitis with the most frequent heart involvement. The whole aorta and all the aortic branches can be affected, but also cardiac manifestations can appear. High dosage of steroids remains fundamental, but adverse events and possible relapses require the introduction of alternative treatment. Several new therapeutic approaches with bDMARDs and targeted synthetic DMARDs have showed promising results, with high efficacy and acceptable safety profile, although most of the available data are obtained from cohort studies. These results led to the inclusion of anti-TNF alpha therapies as first line therapies in the most recent ACR guidelines (5).

Similarly, also for EGPA in the last decades several new treatments were available, as revised by Regola et al. Cardiac involvement in EGPA is a rare complication of a rare disease, but remains one of the most serious and main causes of death. Historical treatments include high dosage of steroids combined with several conventional DMARDs. However, as revised by the authors, in 2017 the first biological treatment, the anti-IL5 mepolizumab, was approved for EGPA. For both TAK and EGPA several RCTs are ongoing, targeting molecules/cell types involved in the pathogenesis of the diseases.

In the era of the COVID-19 pandemic infection, a small percentage of children have been reported to have developed a serious condition with multi-system organ disfunction, increased inflammatory biomarkers, that was called Multisystem Inflammatory Syndrome in Children (MIS-C). Panaro et al. underlined in their review how this new condition shares several similarities with Kawasaki disease (KD), one of which is cardiac involvement. Moreover, the authors summarized clinical features, pathogenesis, and available treatments as first-line treatment or for refractory patients. As mentioned, cardiac involvement is one of the most severe clinical features of KD, with an increased risk of coronary artery aneurysm or cardiovascular events. Buda et al. reviewed the available treatment guidelines and summarized the standard second-line treatment and drugs used in non-responsive or high-risk patients.

Finally, Ammirati et al. focused their review on two cardiological manifestations that can occur in the context of autoimmune diseases or as isolated forms: acute myocarditis and recurrent/acute pericarditis. Additionally, the authors provide an overview of the available treatments for both conditions and how evolving technologies may guide the use of these treatments.

In conclusion, we believe that the papers included in this Research Topic are excellent examples of the complex and heterogeneous involvement of the cardiovascular system in the field of systemic autoimmune diseases.

Author contributions

MF: Writing—original draft. SP: Writing—original draft. AB: Writing—review & editing. FF: Writing—review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers in Medicine

REVIEW
published: 06 September 2021
doi: 10.3389/fmed.2021.730161

Anti-Ro/SSA Antibodies and the Autoimmune Long-QT Syndrome

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Autoimmunity is increasingly recognized as a novel pathogenic mechanism for cardiac arrhythmias. Several arrhythmogenic autoantibodies have been identified, cross-reacting with different types of surface proteins critically involved in the cardiomyocyte electrophysiology, primarily ion channels (autoimmune cardiac channelopathies). Specifically, some of these autoantibodies can prolong the action potential duration leading to acquired long-QT syndrome (LQTS), a condition known to increase the risk of life-threatening ventricular arrhythmias, particularly Torsades de Pointes (TdP). The most investigated form of autoimmune LQTS is associated with the presence of circulating anti-Ro/SSA-antibodies, frequently found in patients with autoimmune diseases (AD), but also in a significant proportion of apparently healthy subjects of the general population. Accumulating evidence indicates that anti-Ro/SSA-antibodies can markedly delay the ventricular repolarization *via* a direct inhibitory cross-reaction with the extracellular pore region of the human-ether-a-go-go-related (hERG) potassium channel, resulting in a higher propensity for anti-Ro/SSA-positive subjects to develop LQTS and ventricular arrhythmias/TdP. Recent population data demonstrate that the risk of LQTS in subjects with circulating anti-Ro/SSA antibodies is significantly increased independent of a history of overt AD, intriguingly suggesting that these autoantibodies may silently contribute to a number of cases of ventricular arrhythmias and cardiac arrest in the general population. In this review, we highlight the current knowledge in this topic providing complementary basic, clinical and population health perspectives.

Keywords: anti-Ro/SSA antibodies, long QT syndrome, autoimmune cardiac channelopathies, hERG potassium channel, Torsades de Pointes, sudden cardiac death

OPEN ACCESS

Edited by:
Micaela Fredi,
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Reviewed by:
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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *24 June 2021*
Accepted: *11 August 2021*
Published: *06 September 2021*

Citation:
Lazzerini PE, Laghi-Pasini F, Boutjdir M and Capecchi PL (2021) Anti-Ro/SSA Antibodies and the Autoimmune Long-QT Syndrome. Front. Med. 8:730161. doi: 10.3389/fmed.2021.730161

INTRODUCTION

The long QT-syndrome (LQTS) is a cardiac electric disorder characterized by an abnormal prolongation of the heart rate-corrected QT interval (QTc) on the electrocardiogram (traditionally >440 ms; currently, >470 ms for men, and >480 ms for women) (1) which predisposes to life-threatening ventricular arrhythmias (VAs), specifically Torsades de Pointes (TdP) (1–3). TdP is a polymorphic ventricular tachycardia presenting with a typical pattern of

twisting points that can rapidly degenerate into ventricular fibrillation (VF) and cause sudden cardiac death (SCD) (1). The more the QT prolongs the more the risk of TdP increases, it becoming high for QTc > 500 ms, very high for QTc > 600 ms (1). In addition, accumulating evidence indicates that when QT interval prolongs as the result of the specific lengthening of the terminal component of the T wave, from the peak to its end (Tpeak-Tend interval, Tp-Te), the risk to develop TdP is particularly important (4, 5).

The QTc on the electrocardiogram (ECG) is commonly used in the clinical practice as a proxy of the average action potential duration (APD) in ventricular cardiomyocytes, in turn determined by the sequential activation of several ion channels mediating inward depolarizing (sodium, Na^+ and calcium, Ca^{++}) or outward repolarizing (potassium, K^+) currents, respectively (6). Whenever a dysfunction of one or more of these channels occurs leading to a net inward shift in the balance of currents (i.e., an increase of Na^+ or Ca^{++} currents and/or a decrease of K^+ currents), APD prolongs and, therefore, the QTc (6, 7). A wide number of etiologic factors can be responsible for LQTS, classically categorized as congenital, due to mutations in genes encoding for K^+, Na^+, or Ca^{++} channels and related regulatory proteins, or acquired (8, 9). While inherited forms are relatively rare, with an estimated prevalence of ∼1:2,000 of apparently healthy newborns (8, 10), acquired LQTS is a quite frequent finding (11, 12), more commonly due to medications blocking the human *ether-à-go-go* related gene K^+ channel (hERG-K^+) carrying the rapidly activating component of the delayed outward-rectifying current (I_{Kr}), or electrolyte imbalances (hypokalaemia, hypocalcaemia, hypomagnesemia) (1, 9). Other well-defined causes of acquired LQTS include structural heart diseases, bradyarrhythmias, liver and endocrine diseases, nervous system injuries, starvation, hypothermia, and toxins (9, 13). Although wide, this list of "conventional" risk factors is not able to account for all cases of LQTS/TdP occurrence (and recurrence) in the clinical practice (4) and for this reason in the recent years, intensive investigations were undertaken to identify previously unrecognized risk factors. As a result, an increasing number of novel, "non-conventional" QT-prolonging risk factors for acquired LQTS have been recently recognized, including human immunodeficiency virus infection (14), male hypogonadism (15, 16), heart failure with preserved ejection fraction (17), QT-prolonging foods (18), inflammation and autoimmunity (19, 20).

Regarding the autoimmune LQTS, the most investigated form is associated to the presence of circulating anti-Ro/SSA-antibodies, responsible for one of the first identified arrhythmogenic autoantibody-induced channelopathies (*autoimmune cardiac channelopathies*) (19, 21, 22). In fact, accumulating evidence exists that anti-Ro/SSA-antibodies exert significant electrophysiological effects on the heart *via* an inhibitory cross-reaction with the extracellular pore region of the hERG-K^+ channel (23–27), leading to a higher propensity of developing LQTS (28–31) and VAs/TdP (24, 32, 33) in anti-Ro/SSA-antibody positive adults and newborns subjects. In this review, we highlight the current knowledge on this autoimmune associated LQTS form providing complementary basic, clinical and population health perspectives.

ANTI-Ro/SSA-ANTIBODIES

Anti-Ro/SSA-antibodies, comprising the anti-Ro/SSA-52kD and anti-Ro/SSA-60kD sub-specificities, result from an autoimmune response against the two subunits of the intracellular ribonucleoprotein Ro (Ro52-kD and Ro60-kD) (34). They are polyclonal antibodies, usually of the IgG class, commonly found in patients with autoimmune diseases (AD) and beyond (34–36). In particular, anti-Ro/SSA-positivity is frequent in connective tissue diseases (CTD), primarily Sjögren's syndrome and systemic lupus erythematosus (SLE) (34). In these disorders, anti-Ro/SSA-60kD sub-specificity has a more established direct pathogenic role than anti-Ro-52kD in the development of classical autoimmune manifestations (37, 38), also being associated with a higher prevalence of extraglandular features, especially vasculitis, and greater systemic activity (39, 40). Indeed, large studies have demonstrated that anti-Ro/SSA-antibodies can be also detected in a significant proportion of subjects of the general population (0.5-2.7%) (41–43), who are in most cases (60%) asymptomatic for AD (43), particularly when anti-Ro/SSA-52kD positivity occurs alone (44).

Large evidence exists that the trans-placental passage of anti-Ro/SSA-antibodies from the mother to the fetus causes the autoimmune-congenital heart block (aCHB) (45), a paradigmatic form of passively acquired autoimmunity (46, 47). Although the pathogenesis of this disorder is complex and only in part elucidated, many clinical and experimental data have demonstrated that an inhibitory cross-reaction between anti-Ro/SSA-antibodies and the L- and T-type Ca^{++}-channels in fetal conduction system cardiomyocytes plays a key mechanistic role (48–54).

In the clinical practice, several laboratory methods are available for anti-Ro/SSA-antibody detection, the more commonly used being immunoenzymatic tests (ELISA, FEIA) and line-blot immunoassay (LIA), all based on recombinant Ro antigens use as substrate (34, 36, 55, 56). However, increasing evidence indicates that immuno-Western blot (iWB), using the native Ro antigen, is the most sensitive technique to reveal anti-Ro/SSA-positivity in the general population (36) as well as arrhythmogenic autoantibodies in aCHB (57), more frequently being identified as the anti-Ro/SSA-52kD subtype (57).

ANTI-Ro/SSA-ASSOCIATED LONG-QT SYNDROME

Clinical Data

The first studies showing an association between anti-Ro/SSA-antibodies and LQTS were performed in children in the early 2000s (**Table 1**). Cimaz et al. (28) reported that newbors/infants without aCHB from anti-Ro/SSA-positive mothers had longer QTc than anti-Ro/SSA-negative controls. Moreover, the same authors demonstrated that such alteration normalized spontaneously during the first year of life together with the

TABLE 1 | Clinical studies showing an association between anti-Ro/SSA antibodies and QTc/TdP.

References	Study population	Anti-Ro/SSA+ (*n*)	Anti-Ro/SSA– (*n*)	Main results
Newborns/children				
Cimaz et al. (28)	Newborns of CTD mothers	21	7	Mean QTc significantly longer in anti-Ro/SSA-positive subjects; QTc prolongation > 440 ms in 42% of cases (vs. 0% in controls)
Gordon et al. (58)	Children of CTD mothers	38	7	Mean QTc significantly longer in children of anti-Ro/SSA-positive mothers
Cimaz et al. (59)	Children of anti-Ro/SSA-positive mothers	21	-	Concomitant disappearance of QTc prolongation and acquired maternal antibodies at 1 year follow-up
Jaeggi et al. (60)	Newborns/children of anti-Ro/SSA-positive mothers	116	-	Transient QTc prolongation > 440 ms in 15% of cases
AlTwajery et al. (61)	Children with SLE	16	25	Anti-Ro/SSA-positive patients showed higher prevalence of ECG abnormalities, particularly QTc prolongation > 450 ms
Friedman et al. (62)	Newborns/children of anti-Ro/SSA-positive mothers	45	-	QTc prolongation > 2 SD above historical healthy controls in 11% of cases
Duke et al. (63)	Newborn of an anti-Ro/SSA-positive mother	1	-	QTc prolongation and ventricular tachycardia
Wang et al. (64)	Child of an anti-Ro/SSA-positive mother	1	-	QTc prolongation and TdP
Mizuno et al. (65)	Child of an anti-Ro/SSA-positive mother	1	-	QTc prolongation and TdP
Adults				
Lazzerini et al. (29)	CTD	31	26	Mean QTc significantly longer and prevalence of QTc prolongation > 440 ms significantly higher in anti-Ro/SSA-positive subjects (58 vs. 0%)
Lazzerini et al. (32)	CTD	26	20	Mean QTc significantly longer and prevalence of QTc prolongation > 440 ms significantly higher in anti-Ro/SSA-positive subjects (46 vs. 5%); QTc prolongation significantly associated with the presence of complex ventricular arrhythmias
Bourrè-Tessier et al. (30)	SLE (two studies)	57 113	93 165	5.1-12.6-times higher risk of QTc prolongation in anti-Ro/SSA positive vs. negative group. The risk of QTc prolongation directly correlated with anti-Ro/SSA concentration
Lazzerini et al. (66)	CTD	25	24	Mean QTc significantly longer and prevalence of QTc prolongation ≥ 460 ms significantly higher in anti-Ro/SSA-positive subjects (48 vs. 17%); significant correlation between anti-Ro/SSA-52kD concentration and QTc duration
Pisoni et al. (67)	AD	55	18	Anti-Ro/SSA positivity significantly more frequent among CTD patients with QTc prolongation ≥ 440 ms (all patients with QTc prolongation were anti-Ro/SSA positive, 20 vs. 0%)
Sham et al. (68)	SLE	47	53	Mean QTc significantly longer in anti-Ro/SSA-positive subjects
Nakamura et al. (33)	TdP	1	-	QTc prolongation and TdP in an anti-Ro/SSA-positive woman without AD
Lazzerini et al. (24)	TdP	25	-	High prevalence of anti-Ro/SSA-52kD in unselected TdP patients (60%)
Perez-Garcia et al. (69)	SLE	66	-	Anti-Ro/SSA and anti-Ro/SSA-52kD levels significantly higher in patients with QTc prolongation, and linearly correlated with QTc duration
Tufan et al. (70)	CTD	15	39	QTc max, Tp-e and Tp-e/QT ratio higher in anti-Ro/SSA-52kD-postive vs. negative CTD patients (and HC, $n = 22$);Tp-Te duration strongly correlated with anti-Ro/SSA-52kD titer

(Continued)

TABLE 1 | Continued

References	Study population	Anti-Ro/SSA+ (*n*)	Anti-Ro/SSA– (*n*)	Main results
Mostafavi et al. (71)	SLE	150	-	Anti-Ro/SSA positivity significantly associated with QTc prolongation > 440 ms
Hu et al. (72)	SLE	299	-	Anti-Ro/SSA positivity identified as one of the most important independent variables associated with QTc prolongation > 450 ms
Lazzerini et al. (31)	US Veterans	612	6,727	QTc prolongation (>470 ms in males/>480 ms in females) in 10% of anti-Ro/SSA-positive vs. 6.2% of negative subjects (marked QTc prolongation, >500 ms, 3.1 vs. 1.0%). Anti-Ro/SSA positivity independently associated with a 2-times higher risk of marked QTc prolongation (>500 ms; OR 2.27, 95%CI 1.34-3.87)

CTD, connective tissue disease; AD, autoimmune disease; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; HC, healthy controls; QTc, corrected QT interval; TdP, torsades de pointes; Tp-e, interval from the peak to the end of the T wave; Tp-e/QT ratio, interval from the peak to the end of the T wave/QT interval ratio.

disappearance of maternally-acquired anti-Ro/SSA-antibodies, thereby pointing to a functional and reversible interference on ventricular repolarization (59). Later, four independent groups provided data further supporting this association (**Table 1**). Gordon et al. (58) demonstrated that QTc was significantly prolonged in children from anti-Ro/SSA-positive mothers when compared to those from anti-Ro/SSA-negative mothers, with a more marked prolongation in siblings of a child with aCHB. Then, Jaeggi et al. (60) reported that in a Canadian cohort 116 anti-Ro/SSA-positive newborns/infants without aCHB, transient QTc prolongation was rather frequent, it being present in 15% of cases (60). Consistent data were more recently obtained by Friedman et al. (62) who analyzed the ECGs of 45 infants without aCHB born from anti-Ro/SSA-positive mothers and found that QTc prolongation > 2 standard deviations above historical healthy controls was present in 11% of subjects. Moreover, AlTwajery et al. (61) demonstrated that among 41 children affected with SLE, anti-Ro/SSA-positivity was associated with a higher prevalence of ECG abnormalities, particularly QTc prolongation > 450 ms. Finally, three cases of marked QTc prolongation complicated with ventricular tachycardia/TdP in infants from anti-Ro/SSA-positive mothers with aCHB are reported (63–65).

In agreements with these findings, several studies demonstrated an increased prevalence of QTc prolongation and VAs in adults with circulating anti-Ro/SSA-antibodies (**Table 1**). Our group was the first to provide evidence that anti-Ro/SSA-positive adults with CTD frequently show QTc prolongation (>440 ms in ∼45-60% of cases) (29, 32), persisting throughout the 24 h and correlating with the risk of complex VAs (32). Later on, Bourré-Tessier et al. (30) conducted two consecutive studies on a larger cohort of SLE patients where anti-Ro/SSA-positivity was found to be associated with a 5-12-times higher incidence of QTc prolongation, with a correlation with autoantibody levels. This latter finding was confirmed and refined by our group, by demonstrating that only the serum concentration of the anti-Ro/SSA-52kD subtype significantly and specifically associated with QTc duration (66).

After these seminal studies, many other authors provided clinical evidence supporting the existence of a relationship between anti-Ro/SSA-antibodies and QTc prolongation risk in adults (**Table 1**). Pisoni et al. (67) demonstrated that among 73 AD patients, the prevalence of QTc > 440 ms was significantly higher in anti-Ro/SSA-positive (20%) vs. –negative subjects (0%). Consistent results were obtained by four subsequent studies, all conducted in SLE patients. Sham et al. (68) reported that mean QTc was longer in SLE subjects with, rather than without circulating anti-Ro/SSA-antibodies, while Mostafavi et al. (71) and Perez-Garcia et al. (69) found that anti-Ro/SSA-antibodies were more commonly detectable and at a higher concentration when SLE patients with QTc prolongation were compared to those with a normal QTc. Moreover, in a study using machine learning in 299 patients with SLE, Hu et al. (72) identified anti-Ro/SSA positivity as one of the most important independent variables associated with QTc prolongation > 450 ms in these subjects. Regarding the specific role of the anti-Ro/SSA-52kD subtype, Tufan et al. (70) reported increased QTc maximum and Tp-Te values in anti-Ro/SSA-52kD-positive CTD patients in comparison to negative patients and healthy controls. In addition, Perez-Garcia et al. (69) and Tufan et al. (70) found that anti-Ro/SSA-52kD levels were significantly associated with QTc and Tp-Te duration in SLE and CTD patients, respectively.

Further studies provided evidence that anti-Ro/SSA-antibodies, regardless of the presence or absence of a clinically evident CTD/AD, are *per se* associated with LQTS/TdP (**Table 1**). This is a very important point, as it intriguingly suggests that these autoantibodies may represent a concealed risk factor possibly contributing to life-threatening VAs/SCD events in the general population (21). In fact, after the early case report by Nakamura et al. (33) of recurrent TdP episodes in an otherwise healthy anti-Ro/SSA-positive woman with circulating anti-Ro/SSA-antibodies, our group more in general demonstrated that circulating anti-Ro/SSA-antibodies are silently found in a significant proportion of unselected patients presenting with TdP. By analyzing a prospective cohort of 25 TdP subjects consecutively collected from the general population, we found the presence of anti-Ro/SSA-52kD-antibodies in over 50% of patients, in most cases without a history of AD (24). In agreement with what was observed in children with aCHB (57) and patients with CTD (66), also in this case iWB was demonstrated to be the most sensitive laboratory technique in revealing arrhythmogenic autoantibodies. Strong support for these data is provided by

TABLE 2 | Basic mechanisms of anti-Ro/SSA-associated LQTS: data from experimental studies.

References	Effect on hERG-K^+	Effect on IKr	Effect on APD	Effect on QT interval
Nakamura et al. (33)	direct binding in HEK293-hERG cells incubated with purified IgGs from an anti-Ro/SSA-positive TdP patient	chronic inhibition in HEK293-hERG cells incubated with sera/purified IgGs from an anti-Ro/SSA-positive TdP patient	-	-
Yue et al. (23)	1. direct binding in HEK293-hERG cells incubated with purified IgGs from anti-Ro/SSA-positive CTD patients with LQTS 2. direct binding in HEK293-hERG cells and guinea-pig ventricular tissue incubated with anti-Ro/SSA-positive sera from Ro52kD-immunized guinea-pigs 3. cross-reactivity with a 31–amino acid peptide corresponding to the pore-forming region (segment S5-S6) incubated with sera from anti-Ro/SSA-positive CTD patients with LQTS	1. acute inhibition in HEK293-hERG cells and/or guinea-pig ventricular myocytes incubated with sera/purified IgGs/affinity-purified anti-Ro/SSA-52kD antibodies from anti-Ro/SSA-positive CTD patients with LQTS 2. acute inhibition in HEK293-hERG cells incubated with anti-Ro/SSA-positive sera from Ro52kD-immunized guinea-pigs	prolongation in guinea-pig ventricular myocytes incubated with purified IgGs from anti-Ro/SSA-positive CTD patients with LQTS	prolongation at the surface ECG in Ro52kD-immunized guinea-pigs
Lazzerini et al. (24)	1. direct binding in HEK293-hERG cells incubated with purified IgGs from anti-Ro/SSA-positive TdP patients 2. cross-reactivity with a 31–amino acid peptide corresponding to the pore-forming region (segment S5-S6) incubated with sera from anti-Ro/SSA-positive TdP patients	acute inhibition in HEK293-hERG cells incubated with purified IgGs from anti-Ro/SSA-positive TdP patients	-	-
Fabris et al. (26)	-	acute inhibition in HEK293-hERG cells and guinea-pig ventricular myocytes incubated with sera from guinea-pigs immunized with a 31–amino acid peptide corresponding to the hERG pore-forming region (E-pore peptide) and cross-reacting with sera from anti-Ro/SSA-positive CTD patients with LQTS	prolongation in guinea-pig ventricular myocytes incubated with sera from E-pore peptide-immunized guinea-pigs	prolongation at the surface ECG in E-pore peptide-immunized guinea-pigs
Szendrey et al. (27)	1. direct binding to the extracellular S5-pore linker in HEK293-hERG cells incubated with commercial anti-Ro/SSA-52kD antibodies 2. decreased expression with enhanced endocytic degradation in HEK293-hERG cells incubated with commercial anti-Ro/SSA-52kD antibodies	chronic inhibition in HEK293-hERG cells and neonatal rat ventricular myocytes incubated with sera from anti-Ro/SSA-52kD-positive CTD patients or commercial anti-Ro/SSA-52kD antibodies	prolongation in neonatal rat ventricular myocytes chronically incubated with commercial anti-Ro/SSA-52kD antibodies	-

hERG-K^+, human ether-à-go-go related gene potassium channel; HEK293-hERG, human embryonic kidney-293 cells stably expressing hERG-K^+; I_{Kr}, rapidly activating component of the delayed outward-rectifying current; APD, action potential duration; CTD, connective tissue disease; IgGs, immunoglobulins G; LQTS, long-QT syndrome; TdP, torsades de pointes; ECG, electrocardiogram.

a very recent population study conducted in a large cohort of 7339 US Veterans, including 612 anti-Ro/SSA-positive (31). In these subjects, circulating anti-Ro/SSA-antibodies were independently associated with a ∼2-times higher risk of marked QTc prolongation (>500 ms), regardless the presence or not of history of CTD. Moreover, stepwise multivariate logistic regression analysis demonstrated that anti-Ro/SSA positivity was one of the most important contributors to marked QTc prolongation, with a significant synergy with most of the concomitant traditional QT-prolonging risk factors, including antimalarials (31). In fact, accumulating evidence demonstrates that this class of drugs, commonly used for the treatment of CTD patients, can inhibit the hERG-K^+-channel (73–75) and promote LQTS development (76). Nevertheless, by stratifying Veterans according the antimalarials use, it was demonstrated that even in the absence of these drugs subjects who were anti-Ro/SSA-positive showed a prevalence of QTc > 500 two-fold higher than in those who were anti-Ro/SSA-negative (31).

Besides the aforementioned studies, it should be noted how other authors reported that adult or pediatric anti-Ro/SSA-positive patients showed increased QTc duration and/or QTc prolongation prevalence with respect to negative controls. However, such differences approached but did not reach the statistical significance, most likely because of the undersized samples used. This is the case of four additional studies reporting slightly longer mean QTc (Gordon et al., $p = 0.06$; Motta et al., $p = 0.06$) (77, 78) or higher proportion of QTc prolongation (Nomura et al., $p = 0.08$; Bourré-Tessier et al., wide 95%CI) (79, 80) in the presence of circulating anti-Ro/SSA-antibodies.

While this large body of data provides robust evidence for a clinically significant association between anti-Ro/SSA-antibodies and LQTS risk, some studies involving children (81, 82) or adults (83–87) reported apparently conflicting results. Several factors may account for these discrepancies, also possibly contributing to the significant variability in anti-Ro/SSA-associated QTc prolongation frequencies even reported by positive association studies (∼10-60%) (25, 66). Firstly, given that the QT-prolonging effects seems to be specifically due to the anti-Ro/SSA-52kD subtype, and in a concentration-dependent manner (23, 24, 27, 66, 69, 70), it is likely that patients in these cohorts did not present circulating levels of this autoantibody sufficient to produce measurable electrocardiographic changes. Indeed, among different CTDs, a wide variability exists in terms of anti-Ro/SSA-52kD concentrations (for example, in systemic sclerosis patients the antibody level is typically low) (85, 88, 89), and in most of the negative association studies specific subtype assessment was not executed. In addition, most of these studies were retrospective and utilized different cutoffs to define QTc prolongation, these factors also potentially contributing to inconsistencies. This is the case, for example, of the study by Teixeira et al. (83) in which the QTc was considered as prolonged when >500 ms. As a result, only 10 out of 317 SLE patients showed QTc prolongation, a sample size that is underpowered for any statistical comparison between anti-Ro/SSA-positive (4/111, 3.6%) and -negative subjects (83). Data from the recent population study on US Veterans provide new important details, which provide support to the above considerations (31). In fact, in this large cohort, where only the qualitative data of anti-Ro/SSA-positivity was considered (no information on antibody subtypes and related concentrations available) the overall prevalence of QTc prolongation > 470 ms (males)/480 ms (females) in anti-Ro/SSA-positive subjects was 10% (vs. 6.2% in anti-Ro/SSA-negative, $p < 0.001$) (31), a percentage underestimated since several individuals without/with low levels of anti-Ro/SSA-52kD subtype were certainly present among those labeled as anti-Ro/SSA-positive. Notably, 3.1% of the subjects with circulating anti-Ro/SSA-antibodies (vs. 1.1% in anti-Ro/SSA-negative) showed QTc prolongation > 500 ms, proportions in part similar to those found by Teixeira et al., (83) but in this case very different from a statistical point of view ($p < 0.001$) due to the adequate power of the sample size (31).

Finally, as discussed in more details in the following section "Experimental Data," anti-Ro/SSA-antibodies can concomitantly inhibit multiple cardiac ion channels, resulting in conflicting effects on APD, thereby on QT interval duration on the surface ECG (21, 90). Such a multifaceted impact on cardiomyocyte electrophysiology, along with the inherent (genetic and acquired) variability in cardiac ion channels reserves among different individuals (91, 92), may also significantly contribute to the reported discrepancies among clinical studies (21, 90).

Experimental Data

Accumulating data from experimental studies based on *in-vitro*, *ex-vivo,* and *in-vivo* models (**Table 2**) (23, 24, 26, 27, 33) demonstrated that the QT-prolonging effect of anti-Ro/SSA-antibodies, specifically the anti-Ro/SSA-52kD subtype, is due to a specific cross-reaction with the cardiac hERG-K^+ channel leading to an inhibition of the related current, I_{Kr} (21). The direct electrophysiological nature of such an effect can well explain why circulating anti-Ro/SSA-antibodies are *per se* associated with an increased risk of QTc prolongation/TdP in the clinical setting, regardless of the presence or not of an overt AD (24, 31).

Specifically, our group demonstrated that incubation of human embryonic kidney-293 cells stably expressing the hERG-K^+-channel (HEK293-hERG) or guinea-pig ventricular myocytes with serum, purified IgGs, or affinity-purified anti-Ro/SSA-52kD obtained from CTD patients with LQTS was associated with an acute (minutes), concentration-dependent and reversible I_{Kr} inhibition (23). Moreover, the development of high levels of circulating anti-Ro/SSA-52kD antibodies in guinea-pigs immunized with the Ro52 antigen was associated with an evident prolongation of the APD measured in ventricular myocytes, as well as of the QTc measured at the surface ECG (23). Furthermore, by combining WB and ELISA experiments, we also provided evidence that anti-Ro/SSA-antibodies can directly cross-react with the hERG-K^+-channel, specifically with the S5-S6 segments of the extracellular loop of the pore region where a significant sequence homology with the Ro52 antigen was demonstrated (23). Consistently, the immunization of guinea-pigs with a 31-amino acid peptide corresponding to this region of the hERG-K^+-channel resulted in high levels antibodies able to block I_{Kr}, prolong APD and QTc, in the absence of any structural change at the pathology examination of the myocardium (26). In addition, a recent Canadian study provided further mechanistic insights into anti-Ro/SSA-associated QTc prolongation, explaining its long-lasting persistence as observed in the clinical setting (27). In fact, these authors demonstrated that prolonged incubation of HEK293-hERG cells with anti-Ro/SSA-52kD-positive sera from patients with rheumatic diseases significantly decreased I_{Kr} compared to cells treated with autoantibody-negative patients' sera (27). Moreover, they showed that anti-Ro/SSA-52kD antibodies chronically facilitated hERG endocytic degradation by targeting the extracellular S5-pore linker region of the channel, and that these changes were associated with persistent I_{Kr} reduction and APD prolongation in neonatal rat ventricular myocytes (27).

The same mechanisms are implicated in anti-Ro/SSA-positive subjects who develop TdP, despite the absence of a manifest AD (**Table 2**). The first evidence was provided by Nakamura et al. (33) who demonstrated that serum and purified IgGs from an otherwise healthy anti-Ro/SSA-positive woman presenting with marked QTc prolongation and recurring TdP, cross-reacted with the hERG-K^+-channel and chronically blocked I_{Kr} in HEK293-hERG cells. Our group confirmed and refined these findings in a prospective cohort of 25 consecutive TdP patients, including 15 (60%) with circulating anti-Ro/SSA-52kD antibodies, in most cases (13/15, 87%) without a history

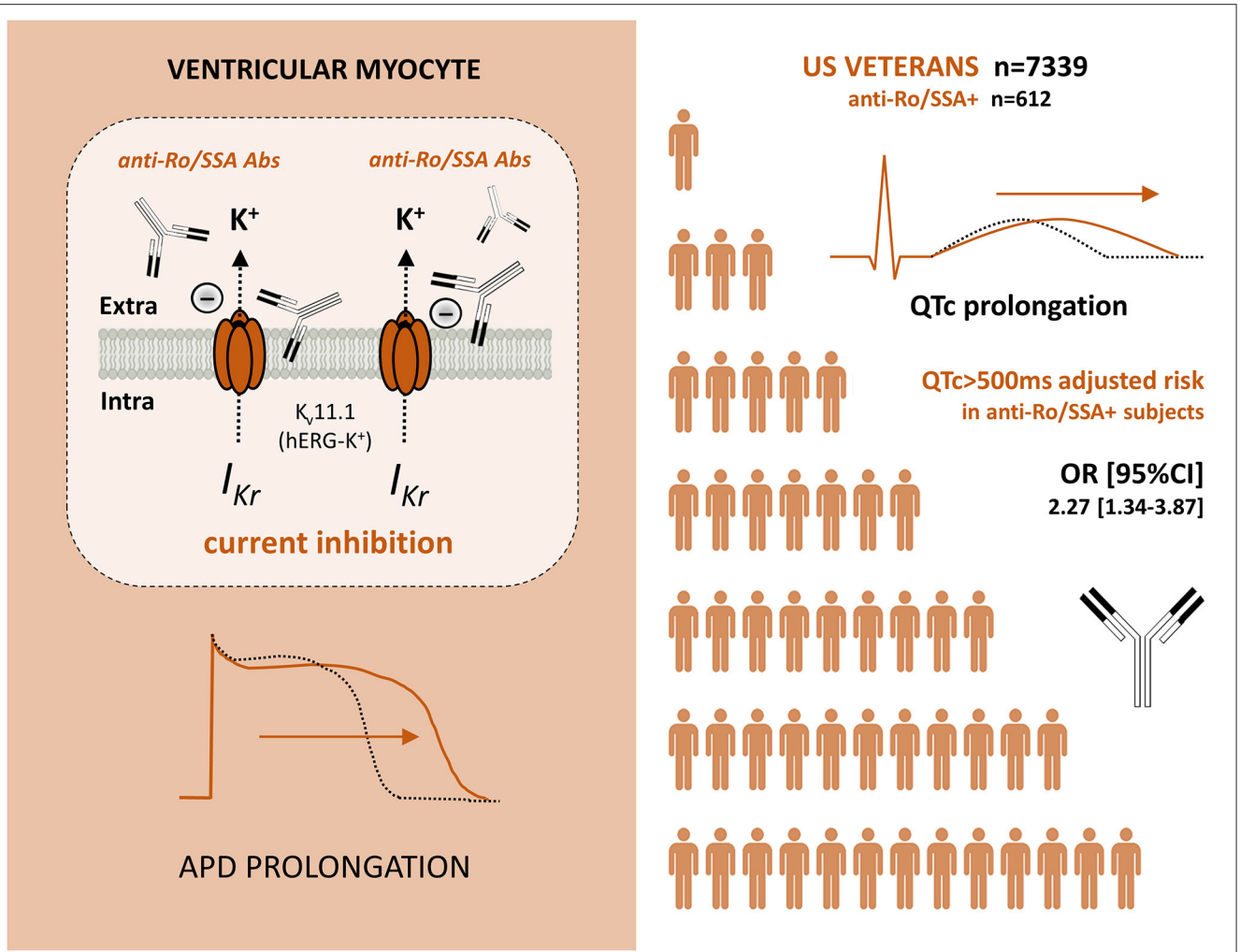

FIGURE 1 | Anti-Ro/SSA antibodies, which can inhibit the I_{Kr} current by directly recognizing hERG potassium channel (brownish), are independently associated with an increased risk of marked QTc prolongation in a large cohort of Veterans (white). Abs, Antibodies; I_{Kr}, rapidly activating component of the delayed outward-rectifying K^+ current; hERG-K^+, human *ether-à-go-go* related gene potassium channel; APD, Action potential duration; QTc, heart rate-corrected QT-interval; OR, odds ratio; CI, confidence interval.

of AD (24). Again, sera and IgGs from anti-Ro/SSA-52kD-positive subjects significantly reduced I_{Kr} in HEK293-hERG cells, but also in guinea-pig ventricular myocytes, and recognized the hERG-K^+-channel by specifically interacting with the S5-S6 segment of the extracellular loop of the pore-forming region (24).

Altogether, these data robustly support the hypothesis that a direct hERG-K^+-channel blockade is the molecular mechanism underlying QTc prolongation and TdP observed in anti-Ro/SSA-positive subjects. However, it should be noted that anti-Ro/SSA-antibodies can also cross-react with and block cardiac Ca^{++}-channels (48, 50–52, 54), responsible for opposite effects on APD. This view is supported by a mathematical modeling study which demonstrated how a simultaneous anti-Ro/SSA-associated inhibition of I_{CaL} during the plateau phase partly counterbalances the APD prolonging effect due to I_{Kr} decrease (26). Based on this evidence, it is likely that the inherent ion channel reserve which characterize each single subject (92) may significantly influence the overall impact of anti-Ro/SSA-antibodies on the duration of the QTc on the surface ECG, thereby contributing to explain the inconsistencies among clinical studies on the association of anti-Ro/SSA-antibodies and QTc prolongation (25). However, given that I_{Kr} physiologically activates after the T wave peak on the ECG (6, 93), a specific evaluation of the Tp-Te might represent a more accurate method to assess in the clinical setting, the discrete impact of anti-Ro/SSA-antibodies on this current. This also in consideration of the particularly important prognostic role that Tp-Te prolongation seems to have in predicting TdP risk (4, 5). In agreement with such premises, Tufan et al. (70) demonstrated that in anti-Ro/SSA-52kD-positive CTD patients Tp-Te was significantly prolonged when compared to anti-Ro/SSA-52kD-negative patients and healthy controls, even in those in whom the whole duration of the QTc was normal.

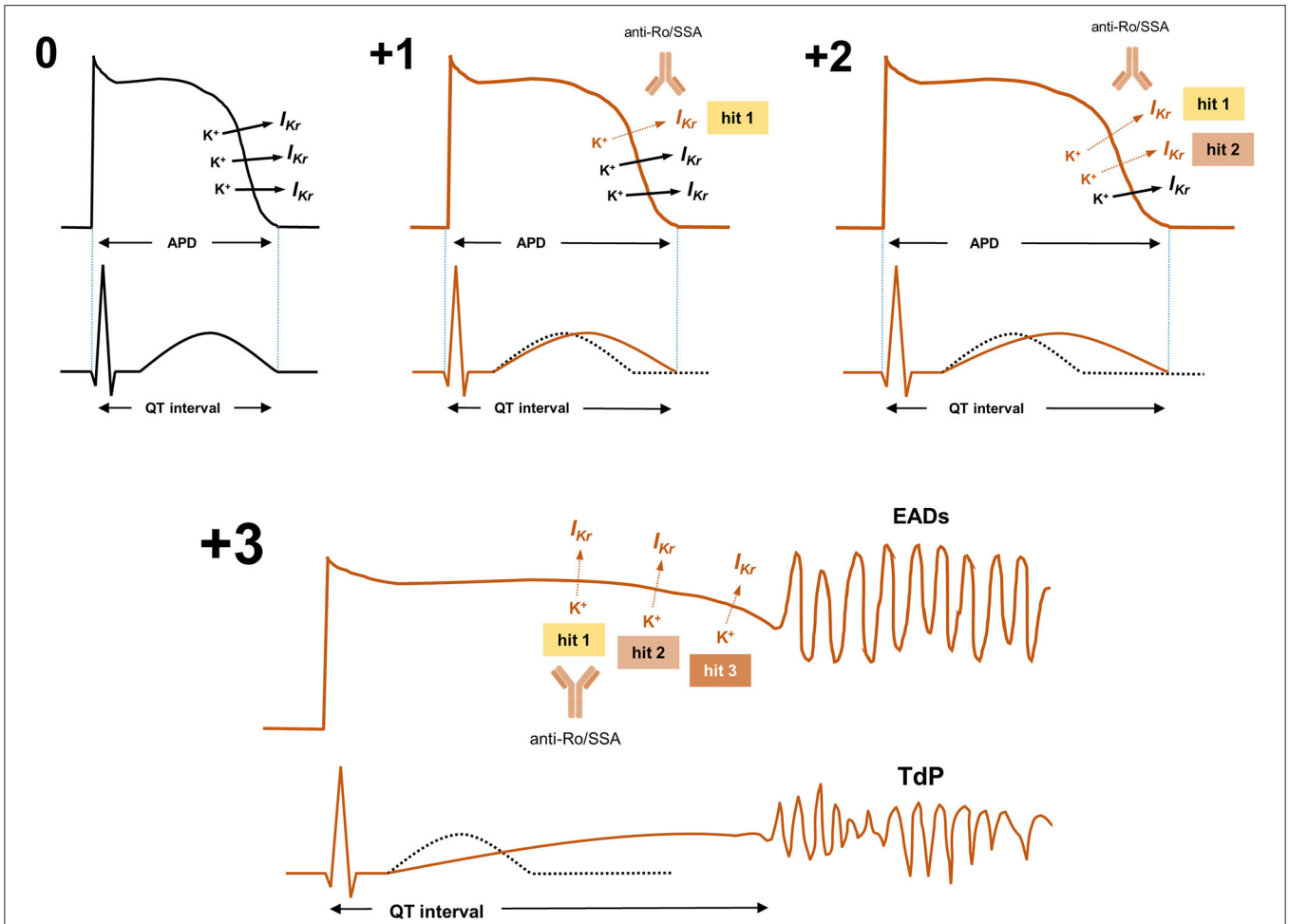

FIGURE 2 | Anti-Ro/SSA-antibodies and the multi-hit theory of long-QT syndrome: given that manifold often-redundant ion channel mechanisms physiologically preserve ventricular APD, hence QTc length (repolarization reserve), many QT-prolonging risk factors ("hits") need to be concomitantly present in a single patient to induce the marked disruption of ventricular repolarization necessary to the occurrence of life-threatening arrhythmias such as TdP. In the absence of QT-prolonging factors (0), I_{Kr} is preserved, and APD and QTc are normal. In subjects with the sole presence of arrhythmogenic anti-Ro/SSA antibodies partially inhibiting I_{Kr} (+1/hit 1), APD/QTc usually slightly/moderately prolongs (or even remains in the normal range, depending on pre-existing genetically-determined repolarization reserve). In these subjects, only the concomitant presence of other genetic or acquired QT-prolonging risk factors further inhibiting I_{Kr} and/or other key ion currents (such as drugs, electrolyte imbalances, etc.: +2/hit 2, +3/hit 3, etc.), can induce the marked APD/QTc prolongation critically required for TdP occurrence. I_{Kr}, rapidly activating component of the delayed outward-rectifying K^+ current; APD, Action potential duration; EADs, early afterdepolarizations; QTc, heart rate-corrected QT-interval; TdP, Torsades de Pointes.

CONCLUSIONS

Mounting evidence from clinical and experimental studies indicates that anti-Ro/SSA-antibodies can markedly affect the ventricular repolarization via a direct inhibitory cross-reaction with the extracellular pore region of the cardiac hERG-K^+-channel, resulting in an increased predisposition to LQTS/TdP in anti-Ro/SSA-positive patients. Notably, recent data demonstrate that such a risk is increased independent of a history of overt AD, intriguingly suggesting that these autoantibodies may also silently contribute to a number of cases of VAs and cardiac arrest in the general population (**Figure 1**).

In fact, although anti-Ro/SSA-antibodies alone cannot usually prolong QTc in a so critical manner to induce TdP development (similarly to all the other better recognized determinants of LQTS) (91, 94), nevertheless they can reduce the ventricular repolarisation reserve (92), thereby enhancing the arrhythmic risk when other conventional QT-prolonging factors (drugs, electrolyte imbalances, genetic mutations, etc.) are concomitantly present (*multi-hit theory*) (**Figure 2**) (24, 91, 95–99).

Based on these considerations and in some way referring to the existing guidelines on the approach to aCHB (100), it is recommended that anti-Ro/SSA-positive subjects receive serial ECGs and specific counseling about medications and management of other risk factors that may critically enhance the risk for QT-associated malignant arrhythmias. On the other hand, patients with "idiopathic" rhythm disturbances should be considered for specific anti-Ro/SSA testing (iWB technique is recommended for detecting arrhythmogenic anti-Ro/SSA subtypes), regardless the presence or not of a manifest

AD, given that the demonstration of circulating antibodies may lead to innovative therapeutic opportunities. Indeed, in agreement with current recommendations for incomplete forms of aCHB (100) (and with several case reports showing the reversing effects of immunosuppressive therapy in anti-Ro/SSA–associated atrioventricular blocks in adults) (101–104), preliminary data from anti-Ro/SSA-positive CTD patients suggest that a short course immunomodulating treatment with corticosteroids is associated with a significant QTc shortening (104, 105). Larger studies are warranted to confirm these intriguing findings. Moreover, given that anti-Ro/SSA-antibodies prolong APD/QTc by directly reacting with a specific amino acid sequence of the hERG-K^+ channel, a peptide-based therapy serving as a decoy to prevent autoantibody-channel binding may be another innovative approach, as preliminarily supported by *ex-vivo* data on sera from anti-Ro/SSA-positive TdP subjects (24).

AUTHOR CONTRIBUTIONS

PL: conception and design of the work and drafting the work. PL, FL-P, MB, and PC: final approval of the version to be published. FL-P, MB, and PC: revising the draft of the work critically for important intellectual content and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by: 1) Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR), Progetti di Rilevante Interesse Nazionale (PRIN), and Bando 2017, protocollo 2017XZMBYX; and 2) Biomedical Laboratory Research and Development Service of Veterans Affairs Office of Research and Development (Merit Review Grant I01 BX002137 to Dr. Boutjdir).

ACKNOWLEDGMENTS

We thank Dr. Andrea Francioni for his contribution to the **Figure 2**.

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frontiers in Medicine

REVIEW
published: 28 October 2021
doi: 10.3389/fmed.2021.747190

The Spectrum of Manifestations of Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV2) Infection in Children: What We Can Learn From Multisystem Inflammatory Syndrome in Children (MIS-C)

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OPEN ACCESS

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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *25 July 2021*
Accepted: *29 September 2021*
Published: *28 October 2021*

Citation:
Panaro S and Cattalini M (2021) The Spectrum of Manifestations of Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV2) Infection in Children: What We Can Learn From Multisystem Inflammatory Syndrome in Children (MIS-C). Front. Med. 8:747190. doi: 10.3389/fmed.2021.747190

Multisystem Inflammatory Syndrome in Children (MIS-C) is defined as a clinically serious condition requiring hospitalization with fever, multi-system organ disfunction, inflammatory biomarkers increase. The syndrome develops in the context of a probable or ascertained Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV2) infection, but other possible etiologies should be ruled out for definitive diagnosis. On the clinical side, along with the multi-system involvement, myocarditis with heart failure and shock is the most striking feature. Capillary leak is another fundamental feature of MIS-C. In fact, shock and hemodynamic compromise in MIS-C can occur also in the absence of laboratory evidence of myocardial inflammation, with preserved cardiac function and rapid reversibility. Since the first observations of MIS-C patients, it was evident that there is a delay between the peak of adult cases of Coronavirus disease 19 (COVID-19) and the MIS-C peak. Moreover, SARS-Cov2 isolation in children with MIS-C is not always possible, due to low viral load, while positive serology is far more commonly observed. These observations lead to the interpretation of MIS-C as a post-infectious disease. Although the exact pathogenesis of MIS-C is far from being elucidated, it is clear that it is a hyperinflammatory disease with a different inflammatory response as compared to what is seen in acute SARS-CoV-2 infection and that the disease shares some, but not all, immunological features with Macrophage Activation Syndrome (MAS), Kawasaki Disease (KD), Hemophagocytic Lymphohistiocytosis (HLH), and Toxic Shock Syndrome (TSS). Different mechanisms have been hypothesized as being responsible, from molecular mimicry to antibody dependent enhancement (ADE). Some evidence has also been collected on the immunological profile of patients with MIS-C and their difference from COVID-19. This review is focused on critical aspects of MIS-C clinical presentation and pathogenesis, and different immunological profiles. We propose a model where this

hyperinflammatory disease represents one manifestation of the SARS-CoV2 spectrum in children, going from asymptomatic carriers to the post-infectious MIS-C, through symptomatic children, a low number of which may suffer from a severe infection with hyperinflammation (pediatric Hyper-COVID).

Keywords: SARSCoV-2, MIS-C, children, COVID-19, myocarditis

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an outbreaking pandemic, threatening public health from at least September 2019. Until now we count at least 127 Million cases through the Globe, with 2,79 Million deaths, as stated by World Health Organization (WHO) (1). Children are less likely to be infected by SARSCoV2 and, even if so, usually develop a mild disease characterized by low-grade fever, abdominal pain and diarrhea and mild upper respiratory tract involvement (2–5). Soon after the first peak of SARSCoV2 in Italy, Verdoni et al. reported on an unusual peak of children presenting with some manifestations of Kawasaki Disease (KD), but with atypical features, as older age at onset, high incidence of cardiogenic shock and myocarditis and abdominal symptoms. In the weeks after, as the SARSCoV2 spread across Europe first and U.S. thereafter more reports came of this hyperinflammatory syndrome possibly related to SARSCoV2 (6–17). This syndrome is nowadays called Multisystem Inflammatory Syndrome in Children (MIS-C) or Pediatric Inflammatory Multisystem Syndrome temporally associated with SARSCoV2 (PIMS-TS) and different case definition criteria have been proposed (11, 18, 19). MIS-C is a serious condition with systemic inflammation, always requiring hospitalization and whose main features are fever, multiorgan dysfunction, elevated acute phase reactants. The syndrome develops in the context of a probable or ascertained SARS-CoV2 infection, but other possible etiologies should be ruled out for definitive diagnosis, as the disease mimics KD shock syndrome (KSS), but also sepsis and Toxic Shock Syndrome (TSS) (20). The epidemiology of MIS-C is still unclear, although it appears to be a relatively rare condition, with an incidence of <1% in SARS-CoV2-infected children (9). As the number of cases reported is rising, it is not clear which exact mechanism links SARSCoV2 infection to MIS-C, and whether there is clinical overlap between acute severe COVID-19 (Hyper-COVID), MIS-C, and K D. In the lack of controlled trials, the treatment I usually based on the combination of immunoglobulins i.v. (IVIG), systemic steroids and, in the more severe cases anti-cytokine treatments. A literature search through Medline/Pubmed was carried out with different key-words: "SARSCoV2," "COVID-19," "MIS-C," "PIMS-TS," "Kawasaki Disease SARSCoV2," "Kawasaki coronavirus," "Kawasaki like disease," "SARSCoV2 shock," "Severe SARSCoV2," "Severe COVID-19" with and without the filter "children." We included original studies, reviews, case reports if written in English.

PATHOGENESIS

Although a definite model for MIS-C is far from being elucidated, some preliminary evidence is now available.

The Superantigen Theory vs. Antibody-Dependent Enhancement (ADE)

Epidemiological data showing a peak of MIS-C cases soon after the peak of SARSCoV2 infection in the general population, and the observation that the majority of patients with MIS-C have negative nasopharyngeal swabs but positive serology for SARS-CoV2 point to a post-infective event, whose pathogenesis is still far from clear (10, 21, 22). The disease seems to arise from a dysregulated immune response, leading to a hyperinflammatory state, and endothelial dysfunction, which ultimately causes a capillary leak and multiorgan failure. Notably, there is a clinical and laboratory overlap with some other hyperinflammatory conditions, such as KD (KD), Kawasaki Shock Syndrome (KSS), Haemophagocytic Lymphohistiocytosis (HLH), Macrophage Activation Syndrome (MAS), and Toxic Shock Syndrome (TSS) (23). One hypothesis to explain how SARS-CoV2 may elicit this systemic response is through molecular mimicry, but also a superantigen may play an important role in the process, triggering self-reactive T cells (24). There is evidence that cells from unexposed individuals can respond to S-protein epitopes from SARS-CoV2, supporting the hypothesis of cross-viral immunity from other strains of coronavirus. It is also known that SARS-CoV-2 encodes a superantigen motif that is very similar to another superantigen, known to cause TSS which, as already pointed out, shows remarkable clinical overlapping with MIS-C; the presence of a superantigen domain in selected viral strains of SARS-CoV2 could also explain why MIS-C has been seen in Europe and North America, but not in Asian countries (25). On the other hand, Antibody-Dependent Enhancement (ADE) is another mechanism possibly involved in the pathogenesis of both COVID infection and MIS-C, with crucial clinical implications (26). ADE could explain the clinical overlapping between MIS-C and severe Dengue disease and why some patients, developing high titers of virus-specific antibodies, have a worse clinical outcome (26, 27). ADE has been demonstrated for other coronaviruses: elevated levels of SARS-CoV-1 IgG antibodies in critical SARS cases and anti-S IgG neutralizing antibody responses developed more rapidly after the onset of clinical symptoms in fatal forms compared with recovered cases, leading some to attribute the enhanced tissue damage to ADE (28). One mechanism regarding ADE for coronavirus suggests

that the Fc Receptor-antibody complex mimics the viral receptor in mediating viral entry, although this effect seems to be highly dependent on antibody dosage (29). Patients with MIS-C carry higher anti-spike antibodies, compared to children infected by SARS-CoV2 but not developing MIS-C (26). It has also been speculated that, in infants, ADE deriving from maternally acquired SARS-CoV-2 antibodies bound to mast cells can be the triggering mechanism of MIS-C (30). Another possibility is that antibodies directed toward one strain might be not neutralizing or sub-neutralizing for viral infections of other strains and lead to ADE (26). Finally, dissemination of the virus has been demonstrated in children who died from severe COVID-19, with and without MIS-C, suggesting that direct virus replication in different organs has a role in the most severe cases (31).

The Role of Endothelial Damage

From a pathological point of view, endothelial damage is one of the main features of the disease. This damage may lead to the overexpression of some molecules pivotal for inflammation, such as Toll-Like Receptors (TLRs), IL-1, IL-6, TNF-alpha (32). The role of endothelial damage is suggested by the finding of Burr cells and schistocytes in patients with MIS-C and severe COVID-19, and may explain the occurrence of renal failure and thrombotic microangiopathy seen in some patients successfully treated with eculizumab (20, 33, 34).

The Role of Dendritic Cells

Dendritic cells can likely be one pivotal target of COVID infection. They are largely distributed in the respiratory tract and through the gut. They are classified as plasmocytoid dendritic cells, producing IFN I, crucial in antiviral response, and classical dendritic cells, interacting with T lymphocytes for priming. A new role for CD147, expressed on lymphocytes, macrophages, and dendritic cells have been suggested for COVID infection. Indeed spike protein can interact with CD147 on dendritic cells and allow virus entry (35). Dendritic cell-specific intracellular adhesion molecule-grabbing non-integrin (DC-SIGN) enhances immune response during viral infections (36). Expression of DC-SIGN or liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN) alone has no impact on infection by SARS-CoV2, but amplifies infection of already-permissive cells, i.e., ACE2 expressing cells (37). SARS patients carrying the DC-SIGN promoter−336 G variant, which leads to reduced DC-SIGN protein expression, had lower risk of having severe SARS-CoV2 infection (38). Plasmacytoid dendritic cell-derived type I IFNs is crucial for viral clearance in humans (36, 39). Reduction in the percentage of dendritic cells, mainly of the plasmocytic phenotype, in the peripheral blood of severe patients in both acute and convalescent phases of SARS-CoV-2 infection was also observed (average of 13 and 30 days after symptoms onset, respectively), suggesting a possible defect in type I interferon response as a possible factor for severe disease (40). Further, the percentage of conventional dendritic cells have been found decreased in the resolution phase of MIS-C and dendritic cells also had decreased HLA-DR and CD86, which could indicate impaired antigen presentation to $CD8^+$ T cells and priming of naive helper T cells (41).

Autoantibodies

Preliminary evidence suggests that specific autoantibodies may be responsible for the systemic and organ-specific manifestations of MIS-C. For instance, anti-endoglin (a glycoprotein expressed by endothelium) antibodies were found in several patients affected by MIS-C, but their role is still undefined. There is also preliminary evidence that antibodies against common cold-Coronaviruses could give some protection for MIS-C. IgG antibodies to human coronavirus HKU1 and beta-coronavirus 1 were commonly observed in COVID patients, healthy volunteers and Kawasaki-disease children, but lacked in MIS-C patients. The relevance of this should be still determined but it is possible that the presence of IgG antibodies against common coronaviruses modulates the immune response to SARS-CoV-2 infection and plays a role in the pathogenesis of MIS-C (42).

Differences in antibodies production between children with MIS-C and adults with COVID-19 have been postulated by Weisberg et al. In their study they found distinct antibody profiles in MIS-C, COVID-19, and convalescent plasma donors. MIS-C patients display a restricted antibody response, largely limited to anti-Spike antibodies, with the overall lowest neutralizing activity. On the other hand, patients with ARDS caused by primary infection show the highest overall levels and the most prominent neutralizing activity. The MIS-C cohort lacked anti Nucleocapsid (Anti-N) antibodies, letting one think to a weaker immune response in this subset of patients, almost not neutralizing, although these results are not definitive (43).

CLINICAL PRESENTATION

General Features

MIS-C is believed to occur 2–6 weeks after SARS-CoV2 infection, although definite demonstration of a preceding infection in children with MIS-C is not always possible. Data from the first case series showed that the majority of patients with MIS-C are positive only for SARS-CoV2 serology, almost a third of the patients resulted positive on both serology and nasal swabs polymerase chain reaction (PCR), while a minority of patients (around 5%) are negative on both SARS-CoV2 antibodies and PCR (6, 10, 11, 22, 44). This delay between SARS-CoV2 infection and MIS-C onset is testified also by the delay that has been described in different countries between COVID-19 peaks in the general population and MIS-C occurrence and may justify the higher viral cycle thresholds of MIS-C patients compared to severe COVID cases observed (10, 20, 22).

MIS-C usually has an abrupt onset with high spiking fever and signs and symptoms of systemic involvement. The clinical picture may be wide, in terms of organ manifestations and severity. In a recent systematic review, the commoner symptoms were gastrointestinal (71%), with the occurrence of abdominal pain (36%), diarrhea (27%), and vomiting (25%), followed by mucocutaneous manifestations (skin rash, strawberry tongue, dried-cracked lips, conjunctivitis) (33, 34). Patients may present with shock in a high percentage of cases, from 30 to 70%, depending on different case series published. Shock is most probably determined by a concurrence of heart failure and capillary leak syndrome and requires intensive care treatment

(7, 16, 45, 46). Heart involvement is, indeed, one of the most striking features of MIS-C as further discussed. Neurological involvement is quite common, described in up to 20% of affected patients, with varying severity, from irritability and meningismus to severe encephalopathy (47). Patients may also present with kidney failure. Most notably, respiratory symptoms are seldomly described, and this may help to differentiate MIS-C from severe COVID-19, as further discussed (48).

Cardiovascular Involvement

Cardiac involvement is very common in MIS-C, and one of the key features to distinguish this disease from severe COVID-19. Myocarditis seems to be the most common cardiac manifestation: more than a third of reported patients in the literature showed depressed cardiac function with variable severity, and this was the main cause for ICU admission (48). Mild to moderate mitral regurgitation and tricuspid regurgitation, but also some localized contractility defects are the most common findings on echocardiography. In those cases where MRI was performed, it showed myocardial edema with late-gadolinium enhancement (46). Markers of cardiac involvement, namely Troponin-T, brain natriuretic peptide P (BNP) and N-terminal-prohormone BNP (NT-proBNP) were raised in up to 77% of patients (46). Different mechanisms have been proposed to cause heart involvement, from myocardial edema to myocardial stunning, but also myocardial necrosis (49, 50). An intense inflammatory state, but also transitory ischemic states secondary to the hypoperfusion and hypotension (in the most severe MIS-C cases) may be possible determinants of the cardiac stunning. MIS-C with myocarditis shows a stronger inflammatory profile compared to patients without myocarditis. Eleven cytokines and chemokines, including CSF2, CCL2, IL-6, CXCL10, FLT3L, 177 VEGF, TGF-A, IL-1RA, PD-L1, CX3CL1, TGF-B1, were found to be higher in MIS-C with severe myocarditis (51). In contrast, a slightly higher expression of IFN-α2 and IL-17A was found in MIS-C without myocarditis (51). Another possible mechanism of damage is the direct invasion of SARS-CoV2 in the heart: post-mortem biopsy analysis in few children showed myocarditis, endocarditis, pericarditis with necrosis of cardiomyocytes; the presence of viral particles in endothelium, myocardium, myocardial macrophages, together with lung and kidney microthrombi (16, 52).

Other less common findings in children with MIS-C are pericarditis, in up to 20% of patients, and coronary artery aneurisms (CAAs), which are reported in up to 25% of patients (44, 46). The occurrence of CAAs in patients with MIS-C is intriguing, as CAAs are a classic feature of Kawasaki Disease (KD) and, together with some mucocutaneous manifestations that are present in some patients, are the main feature suggesting common pathogenesis, if not a continuum of disease, between MIS-C and KD. Interestingly, the incidence of CAAs was the same among three different categories of patients with MIS-C, where two of the three categories had no other common manifestations of KD (11). The outcome of cardiac manifestations seems to be very good in patients with MIS-C, as the majority of cases show resolution within few weeks, probably for the aggressive treatment the majority of patients receive (53).

Laboratory Findings

Lab tests in patients with MIS-C testify the systemic inflammation. The most common findings are leukocytosis with neutrophilia, elevated inflammatory markers (CRP, ESR, fibrinogen, procalcitonin), mild anemia, in up to 90% of patients. Some patients may exhibit lymphopenia, although lymphocyte levels are usually higher than in patients with acute COVID-19. Finding high Troponin T and/or B natriuretic peptide (BNP) or N-terminal pro-BNP (NT-pro-BNP) may suggest cardiac involvement (48). Liver enzymes may be found elevated and the most inflamed patients may satisfy Macrophage Activation Syndrome criteria, with thrombocytopenia, markedly elevated ferritin, hypofibrinogenemia, elevated lactate dehydrogenase (6, 22). Hypoalbuminemia and prolonged PT and aPTT may be found. Higher inflammatory markers and markers for cardiac involvement seems to correlate with a poorer prognosis (54).

As already pointed out, evidence of infection from SARS-CoV2 is present in almost 60% of cases, through positive PCR on nasopharyngeal swabs or, more commonly, positive serology. Noteworthy, as per case definitions, in case PCR o serology is negative, patients must have a positive history of SARS-CoV2 exposure.

Imaging

Hameed et al. described a case series of 35 children with MIS-C. Chest radiography can be negative or can show peri-bronchial cuffing and perihilar interstitial thickening (34%), perihilar airspace opacification (31%). Interestingly, these findings associate with cardiac dysfunction (12). In some cases, a focal perihilar consolidation at admission, as well as small bilateral pleural effusions and atelectatic changes, changing within days from site to site were described (55).

Thoracic computer tomography (CT) imaging was performed when embolism was suspected, due to raised values of D dimer and fibrinogen. Basal consolidation with collapse (39%) and pleural effusions (30%) were the most common findings (55).

As MIS-C has some overlapping features with KD, cardiac CT was performed in case of myocardial dysfunction in 30 of the 35 children (80%) and showed abnormal coronary artery aneurysms in 6 (20%). Aneurysms ranged from very mild single coronary artery dilatation (e.g., left anterior descending artery diameter of 4.3 × 4.1 mm and z score of +2.7) to large aneurysms affecting more than one coronary artery (left anterior descending artery diameter of 6.5 × 7.7 mm and *z* score of +13.9 in one child) (55). Heart magnetic resonance imaging (MRI) showed signs of diffuse myocardial edema and hyperemia with no focal myocardial necrosis or fibrosis (40, 55).

Outcome

Although MIS-C may have an abrupt onset requiring intensive care management, the global outcome is generally favorable. According to a systematic review, the duration of hospitalization was 4–13 days (median, 7 days), and intensive care was required in 68% of patients. Inotropic support was required in 40%, mechanical ventilation was required in 15%, and ECMO was required in 2.7%. The fatality rate was reported to be 1.7%

in the US and 1.4% in Europe (56). Among the studies that reported outcomes at discharge (13) or during follow-up, almost all patients with cardiac involvement experienced nearly full recovery of left ventricular function and normalization of cardiac inflammatory markers except for mild cardiac dysfunction observed in nine patients at discharge in one study (57–60).

When compared with classic KD, MIS-C patients had a worse left ventricular systolic and diastolic function. The strongest predictors associated with myocardial injury in MIS-C patients were globulin longitudinal strain (GLS), global circumferential strain (GCS), left atrial strain (LAS), and longitudinal strain of the right ventricular free wall (RVFWS), with an odds ratio: 1.45 [95% confidence interval (CI): 1.08–1.95], 1.39 [95% CI: 1.04–1.88], 0.84 [95% CI: 0.73–0.96], 1.59 [95% CI: 1.09–2.34], respectively) (61). Higher inflammatory markers and markers for cardiac involvement seems to correlate with a poorer prognosis (54).

TREATMENT

To date, the majority of patients with MIS-C have been treated with a combination of systemic corticosteroids and high-dose i.v. immunoglobulins (IVIG). This is most certainly due to the clinical overlap between MIS-C and KD. On this basis, some scientific societies proposed guidance for management and treatment of MIS-C and, although with slight differences among them, they generally suggest tailoring the treatment on the patient clinical picture and general management with the use of IVIG alone in patients with less severe disease, adding systemic corticosteroids (1–2 mg/kg/day i.v.) in patients with evidence of shock (62–64). Pulse methylprednisolone is considered an option for the most severe patients by some societies. Finally, as for KD itself, Anakinra has been proposed for the treatment of refractory cases, or on top of corticosteroids and immunoglobulins at disease onset in the most severe patients (i.e., patients needing ICU admittance, with signs or symptoms of secondary HLH) (65). Few data are available to address the real efficacy of different treatments. In a recent study by Son et al., the initial treatment with IVIG plus glucocorticoids was associated with a lower risk of new or persistent cardiovascular dysfunction than IVIG alone, while McArdle et al. found no evidence that recovery from MIS-C differed after primary treatment with IVIG alone, IVIG plus glucocorticoids, or glucocorticoids alone (53, 66). These discrepancies are most probably due to the retrospective nature of the studies and to patients heterogeneity. Tocilizumab has also been used in different case series but no specific trials are available (44, 49).

Other ancillary treatments regarding thrombotic risk and inotropic support must be evaluated case by case. Acetylsalicylic acid should be given in case of coronary abnormalities, as it is for KD (64). Prophylactic low molecular weight heparin should be considered in children with MIS-C, given the high inflammatory state and stratifying the thrombotic risk based on D-Dimer levels and the presence of other pro-thrombotic risk factors (67). Inotropic support is another important issue because of capillary leak complicating MIS-C (68). Finally, therapy with eculizumab should be considered in case of acute kidney failure and evidence of microangiopathy (34).

HOT-TOPICS FOR DISCUSSION

MIS-C, KD, or Severe COVID-19? The Spectrum of Clinical Manifestations of SARS-CoV2 Infection in Children

The clinical picture of MIS-C is quite obvious in the majority of cases; still, the disease has many overlaps with other conditions, such as KD, Toxic Shock Syndrome (TSS), and sepsis (see **Table 1**). Case definition criteria have been proposed for the prompt identification and treatment of suspected cases, but their sensitivity has never been evaluated. There is now evidence that patients with MIS-C may satisfy also Kawasaki Disease criteria and that some patients with acute COVID-19 may have severe disease, with some features of systemic inflammation, and possibly satisfying criteria for MIS-C (here referred to as Hyper-COVID) (22, 69). To close the loop, clear-cut Kawasaki Disease cases, without the classic features of MIS-C (such as shock and myocarditis) and positive nasopharyngeal swabs for SARS-CoV2, have been described (70).

Although pediatric COVID-19 is generally a benign disease a minority of patients require ICU admittance for severe manifestations (15, 71). Bhumbra et al. reported on few patients with severe COVID-19, requiring ICU admission, and compared their characteristics with less severe patients. Patients requiring ICU were older, with longer disease duration before admittance, worse respiratory parameters and evidence of intense systemic inflammation with lower WBC, platelets, and higher inflammatory markers (69). When reviewing MIS-C cases in the US during the march to July 2020 period, Godfred-Cato et al. were able to distinguish three different categories of patients: a third of the patients had a higher incidence of multiorgan involvement, with the cardiovascular and intestinal systems being almost constantly affected. Those patients had also the highest incidence of shock, higher inflammatory markers, and more commonly showed increased Troponin, BNP, or pro-BNP. SARS-CoV2 serology was positive in almost all the children in this category. The second category of patients, encompassing almost a third of the studied population, included younger children, with a higher incidence of mucocutaneous manifestations and lower incidence of multisystem involvement. Children from this category had also a lower incidence of shock, myocarditis, and most commonly satisfied Kawasaki criteria. SARS-CoV2 serology was positive in 2/3 of the patients, with a third having also PCR positivity. Finally, the remaining third of the patients had a higher incidence of respiratory symptoms and severe respiratory involvement, with higher fatality rates and SARS-CoV2 swabs were positive in a significantly higher percentage of children. The authors themselves hypothesize that this third category of patients most probably comprises children with acute severe COVID-19, satisfying also MIS-C criteria (11). Feldstein et al. recently published their research where they recruited more than 1,100 children with SARS-CoV2-related diseases, comparing those with MIS-C (as per CDC

TABLE 1 | Main clinical and laboratory differences between MIS-C, Hyper-COVID, KD, TSS, and MAS (macrophage activation syndrome).

	MIS-C	Hyper-COVID	KD	TSS	MAS
Age	Older children (>8 y/o)	Adolescents	Young Children (<5 y/o)	Older children (>10 yrs)	Variable
Sex	Male	Equal	Male	Equal	Equal
Ethnicity	Non-African black	Equal	Asian	Equal	Equal
Skin involvement	KD-like >50% cases	Uncommon (rash)	Typical	Very common (erythema)	None
G.I. involvement	Severe abdominal pain/diarrhea common	Abdominal pain/diahhrea possible	Uncommon	Common	Uncommon
Respiratory Involvement	Uncommon	Severe low respiratory tract Common	Uncommon	Uncommon	Uncommon
Neurologic involvement	Irritability/ meningismus Very common to common	Uncommon	Irritability very common Meningismus uncommon	Common	Severe Common
Kidney Involvement	Possible AKI	Uncommon	Uncommon	Common	Uncommon
Hepatosplenomegaly	Common	Uncommon	Possible	Uncommon	Very common
Lymphadenopathy	Possible	Uncommon	Very common	Uncommon	Very common
Heart Involvement	Very common Myocarditis/pericarditis/CAAs	Uncommon	Common CAAs	Common (myocarditis)	Uncommon
Shock	Common	Uncommon	Possible	Common	Uncommon
Lymphocyte count	Normal to low	Very low	Normal	Normal to high	Normal to low
Neutrophil count	Normal to high	Normal	Normal to high	high	Normal to low
Platelets	Normal to low	Normal to low	Normal	Normal	Normal to low
LFTs	Normal to high	Normal to high	Normal to High	Normal	High to very high
Triglycerides	Normal	Normal to high	Normal	Normal	High
Fibrinogen	Normal to low	Normal to low	Normal to high	Normal to high	Very low
Ferritin	Normal to high	Normal to high	Normal to high	Normal to high	Very high
CRP	High to very high	High to very high	High	high	High to very high

criteria) from those with severe acute COVID-19 (as per a pre-defined set of criteria). Patients with MIS-C were significantly younger, with a higher incidence of non-Hispanic Black ethnicity, lower incidence of comorbidities, higher incidence of cardiovascular involvement without respiratory involvement, and higher incidence of mucocutaneous manifestations. Patients with MIS-C also had higher inflammatory markers than patients with acute COVID-19 (44).

Taken together all this evidence seems to suggest that SARS-CoV2 may determine in children a spectrum of diseases: on one end of the spectrum, there are the majority of children, that remain asymptomatic or, develop a very mild disease, which is clinically characterized by a low-grade fever and mild gastrointestinal and respiratory involvement. A lower number of ACE2 receptors in the high respiratory tract of children, but also the so-called "trained immunity" theory have been recalled as responsible for this benign course of SARS-CoV2 infection (72–74). Moving through the spectrum of diseases there is then the minority of children that develop Hyper-COVID. These are usually older children, usually with comorbidities. Severe respiratory involvement, often requiring ICU treatments and high fatality rates are the main characteristics of these children, which usually have positive nasopharyngeal swabs for SARS-CoV2. At the other end of the spectrum, there is the post-infection disease, called MIS-C. High incidence of shock, cardiac and gastrointestinal involvement, very intense systemic inflammation, and racial predisposition are key features of this form. Patients with MIS-C may have some features of KD, mainly the mucocutaneous manifestations and CAAs formation so that some of them, usually the younger ones, satisfy KD criteria. Finally, there is also the possibility to have *bona-fide* KD triggered by SARS-CoV2 (70). The high incidence of CAAs in the cohort of patients studied, underlie that the two diseases share some common pathogenetic mechanisms.

Many attempts are directed toward the identification of different immunological profiles to distinguish MIS-C and severe COVID (**Table 2**). According to a recent study, in MIS-C marked thrombocytopenia, neutrophilia, a higher neutrophils/lymphocytes ratio and higher levels of Myeloperoxidase (MPO) were found compared to COVID-19 (75). C-Reactive Protein plasma levels were found to be higher in MIS-C rather than in COVID-19 children (75). In MIS-C an important reduction of plasmocytoid dendritic cells was found, with a proinflammatory cytokine profile characterized by high levels of IL-6, CXCL8, CCL2, CXCL9, and CXCL10 (75). Acute COVID disease cytokine profile is characterized by high IFNα levels with respect to MIS-C, while IFN-γ was

TABLE 2 | Merging knowledge from different studies.

MIS-C	COVID
***MIS-C with severe myocarditis**	
High levels of CSF2, CCL2, IL-6, CXCL10, FLT3L, 177 VEGF, TGF-A, IL-1RA, PD-L1, CX3CL1, TGF-B1 (51)	High IFNα (75)
Neutrophilia (75)	
Decreased NF-KB inhibitors* (51)	
Thrombocytopenia (75)	
Higher ratio neutrophils/lymphocytes (75)	
Increased S-100 proteins* (51)	
High MPO expression (80)	
Increased NF-KB expression* (51)	
Low plasmocytoid dendritic cells (75)	
Decreased MHC II expression* (51)	
High levels of IFNγ, IFNα2, IL-17A, TNF-α, IL-10, Granzyme B (51)	Lower levels of IFNγ, IFNα2, IL-17A, TNF-α, IL-10, Granzyme B compared to MIS-C (51)
High levels of IL-10 + TNF-α (20)	
High levels of Hypoxia induced response (HIF1-α)* (51)	
High levels of IFNα2 and IL-17A* (51)	
High SARS–CoV2 RT-PCR cycle threshold (20)	Low SARS–CoV2 RT-PCR cycle threshold (20)
Restricted antibody response, mainly anti-Spike antibodies, lower neutralizing activity (43)	Higher levels of specific antibodies with the most prominent neutralizing activity (43)
Lack of anti-N antibodies (43)	Presence of anti- N antibodies (43)
Overexpression of interferon induced genes (51)	

**means MIS-C with severe myocarditis.*

undetectable in both processes. This data is congruent with a reduction in plasmocytoid dendritic cells encountered in MIS-C, as they produce large amounts of IFNα; nevertheless, others found an increase in IFN-γ (20, 75). The role of many molecules, including IFN-γ, was studied by Smith et al. (76). They found an important role for CXCL9, a monokine induced by IFN-γ. Increased levels of CXCL9 were correlated with the severity of MIS-C (76). Indeed, CXCL9 could possibly allow distinguishing MIS-C from KD. The optimal CXCL9 value to distinguish MIS-C from KD was determined to be 535 pg/mL with a sensitivity of 93% and specificity of 100% (76). Furthermore, CXCL9 followed the clinical picture, as it decreased after administration of immunomodulators (76). Other authors pointed out that the sum of IL-10 and TNF-α levels allowed to distinguish MISC from severe COVID-19 presentations, but not between severe and mild MIS-C (20). SARS–CoV2 reverse transcription polymerase chain reaction (RT-PCR) cycle thresholds were found to be low in COVID patients and high in MIS-C patients (20). Soluble C5b-9 (sC5b-9), instead, has been suggested to be useful to distinguish severe COVID-19 from mild COVID-19, but not severe COVID-19 from MIS-C (20).

Post-vaccine Myocarditis: Is There a Link With MIS-C?

Some reports have been published since May 2021 on the occurrence of myocarditis and pericarditis in adult patients receiving the mRNA vaccines. Myocarditis seems to occur mainly in people under 30 years of age and is usually very mild (77). More recently, Marshall et al. reported on seven adolescents (from 14 to 19 years old) who developed myocarditis soon after SARS-CoV2 vaccine (78). Although there was no evidence of a causal relationship between SARS-CoV2 vaccination and the occurrence of myocarditis, the observation that myocarditis is one of the main features of MIS-C whose pathogenesis may be linked to the production of autoantibodies, there have been concerns that SARS-CoV2 vaccines may be related to MIS-C. This is in contrast with the funding that all seven adolescents lately reported had no evidence of acute SARS-CoV-2 infection and did not fulfill criteria for MIS-C, also, myocarditis was generally mild and all patients recovered without sequelae. Myocarditis has been linked to other vaccines, smallpox in particular, but a possible link between SARS-CoV2 vaccine and MIS-C could not be excluded by now and only further understanding of MIS-C pathogenesis would lead to final conclusions (79). It is crucial to underline that, at the time Marshall et al. reported on the 7 adolescents with myocarditis, more than 2.5 million doses of the Pfizer/BioNTech vaccine had been delivered to adolescents 12–15 years old and 4 million doses were given to 16–18 years since FDA EUA approval. As 4 million COVID-19 cases have been diagnosed in children under 18 in the US that resulted in over 15,000 hospitalizations and between 300 and 600 deaths, it is clear that, by now, the benefits of vaccination far exceed the risks of rare adverse events (80).

CONCLUSIONS

MIS-C is a post-infectious severe disease occurring in children with a SARSCoV2 previous contact. As the definition and clinical characteristics may overlap with severe acute SARSCoV2 infection (referred here as HyperCOVID), but also with other hyperinflammatory conditions (such as KSS, sHLH, TSS) the careful evaluation of both clinical features and laboratory markers are needed before a final diagnosis is established. To date, the best treatment strategy seems to rely on the variable association of systemic corticosteroids, IVIG and anti-IL-1 treatments, tailored on an individual basis depending on the disease severity. Future research should be focused on a better definition of the therapeutic strategy, possibly with randomized trials. A very crucial point to further explore is the pathogenesis of the disease, and in particular of the possible role of anti-SARSCoV2 antibodies, also to rule/out the possibility of vaccine-induced MIS-C or MIS-C like manifestations.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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frontiers in Medicine

REVIEW
published: 02 November 2021
doi: 10.3389/fmed.2021.754434

Novel Targets for Drug Use in Eosinophilic Granulomatosis With Polyangiitis

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Eosinophilic Granulomatosis with Polyangiitis (EGPA) is a rare autoimmune disease characterized by medium and small vessels inflammation. Cardiac vasculitic involvement is one of the most severe manifestations with a significant impact on patients' long-term prognosis: anyway, a specific therapeutic approach for heart involvement in EGPA has not been explored yet. Current regimen consists of a long-term therapy with high dose of glucocorticoids, causing the well-known related-adverse events; immunosuppressive drugs are used in patients with severe manifestations, with some limitations. New therapeutic approaches are needed for patients with refractory disease or contraindications to conventional therapies. The quest for the ideal therapy is going toward a more and more personalized approach: on the one hand, efforts are made to use already existing therapies in the most appropriate way; on the other hand, new insights into EGPA pathogenesis allow the discovery of new targets, as demonstrated by mepolizumab and rituximab, targeting eosinophils, and B-cell compartments. This review summarizes the emerging therapies used in EGPA, focusing on the most recent studies on biologics and analyzing their efficacy and safety.

Keywords: Eosinophilic Granulomatosis with Polyangiitis, heart involvement, novel therapies, biologics, rituximab, mepolizumab, omalizumab

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *06 August 2021*
Accepted: *11 October 2021*
Published: *02 November 2021*

Citation:
Uzzo M, Regola F, Trezzi B, Toniati P, Franceschini F and Sinico RA (2021) Novel Targets for Drug Use in Eosinophilic Granulomatosis With Polyangiitis. Front. Med. 8:754434. doi: 10.3389/fmed.2021.754434

INTRODUCTION

As classified by the 2012-revised Chapel Hill consensus conference, Eosinophilic Granulomatosis with Polyangiitis (EGPA, formerly Churg Strauss syndrome) is a rare systemic necrotizing vasculitis of small and medium size vessels, characterized by asthma and blood and tissue eosinophilia; among all kind of vasculitis, EGPA can have an impressive heart involvement (1). It is a rare disease with a prevalence ranging between 7.3 and 17.8 per million and an annual incidence of 0.9–2.4 per million (2–4).

EGPA belongs to the anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis group (AAV), including granulomatosis with polyangiitis (GPA), and microscopic polyangiitis (MPA). However, only 30–40% of patients are ANCA positive and ∼70% with myeloperoxidase (MPO) specificity: the ANCA positive disease is characterized by vasculitic features and shares pathogenetic mechanisms with the other AAV; the ANCA negative disease seems to share the pathogenetic mechanisms observed in eosinophilic syndromes instead (5). A recent genome-wide association study confirmed the existence of those two phenotypes from a genetical point of view: the former is

linked with HLA-DQ, the latter with genes involved in mucosal responses (6). The natural history of the disease involves three steps: asthma or allergy related symptoms, followed by eosinophilia and lung infiltrates and finally, after a mean of 9.3 years, vasculitic manifestations (7).

CV INSIGHTS

Heart involvement is one of the most severe manifestations in EGPA and its primary cause of death (31%), due to myocardial infarction, cardiac insufficiency, or arrhythmia. Myocardial involvement is associated with a higher mortality risk rate according to the Five Factor Score (FFS), a prognostic tool: as described in the FFS published in 1996, cardiac insufficiency showed a marked risk of mortality in EGPA [HR 2.8; 95% CI 0.15–0.9; $P = 0.03$; (8)]. The pathophysiological mechanisms underlining CV involvement are coronary vasculitis, extravascular granulomas and eosinophilic interstitial infiltrate, causing eosinophilic myocarditis, pericarditis, hypertension, valvulopathy, and congestive heart failure (9, 10).

Cardiac involvement in EGPA is often related to a specific clinical phenotype, characterized by ANCA negativity and eosinophilic infiltrates. The ANCA positive phenotype is characterized by vasculitic features, with peripheral neuropathy, purpura, renal involvement, and biopsy-proven vasculitis; less frequently, heart involvement can be present [5.7% in the ANCA positive phenotype vs. 22.4% in the ANCA negative one, $P = 0.042$; (11)]. While the ANCA -positive phenotype is associated with more frequent relapses (35.2 vs. 22.5%, $P = 0.01$), the ANCA-negative one is characterized by a worst prognosis with a higher mortality [5.6 vs. 12.5%, $P < 0.5$; (9)].

Despite clinical and prognostic differences, current EGPA treatment is not based specifically on heart involvement nor on the ANCA-phenotype, while emerging strategies aim at a more personalized approach. Screening strategies aiming at the early recognition and treatment of EGPA patients with cardiac involvement are still not defined (12). From 62 to 90% of patients in disease remission have cardiac changes; furthermore, from 50 to 65% of EGPA patients have late gadolinium enhancement (83% sensitivity, 56% specificity) on cardiac MRI during active disease. In this context, echocardiography could be a cheap and safe method to investigate CV involvement (83% sensitivity and 80% specificity in EGPA): according to the recently published American College of Rheumatology (ACR) guidelines, an echocardiogram at the time of diagnosis is recommended for all patients, even in the absence of cardiac symptoms (13). New studies are warranted to compare different imaging tests and define a common screening program (14–16).

THE THERAPEUTIC CHALLENGE IN EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

The establishment of a proper treatment plan in EGPA is a challenging decision and a risk benefit balance of the aimed treatment should be considered. The new published guidelines for EGPA reflect the evolving management of the disease: on the one hand, new therapeutic approaches are needed to treat relapsing and/or refractory disease; on the other hand, novel therapies aim at reducing GC exposure over time and therefore its drug-induced toxicity (13).

Eosinophilic Granulomatosis With Polyangiitis: Current Therapy

EGPA is an extremely rare disease: its current treatment is mainly based on studies involving the others, more frequent AAV.

Topical drugs should be preferentially used to treat asthma and ENT manifestations: these symptoms, which greatly impact on the patient's quality of life, could be easily alleviated by local therapies and their course is usually independent from the vasculitic systemic manifestations (17).

EGPA therapy with systemic involvement, as well as of MPA and GPA, needs to be staged in an induction and a maintenance phase (18).

According to Comarmond et al., although almost 90% of EGPA patients achieved remission, 25.3% relapsed, and 18% experienced asthma or ENT flares, justifying prolonged use of GC (9).

Therapeutic strategies differ according to disease severity, defined as life-threatening manifestations, and/or organ impairment associated with long-term poor prognosis. EGPA severity can be assessed with the Five Factor Score (FFS), a prognostic tool published in 1996 and revised in 2011 in a broader population of patients. The 2011 version included 5 items: age (>65 years), heart involvement, GI involvement (hemorrhage, infarction, or pancreatitis), renal insufficiency with a stabilized peak creatininemia (>150 μmol/L) and lack of ENT manifestations (19).

While the definitions of severe and non-severe EGPA used in the recent published ACR guidelines were not based on the FFS, the tool was found to be useful in clinical practice to facilitate treatment decisions. Anyway, its applicability to newer therapies is still unknown (13).

For active and severe EGPA, defined as FFS ≥1 or other life-and organ threatening manifestations (alveolar hemorrhage, eye involvement, and fulminant mononeuritis multiplex), IV pulses (7.5–15 mg/kg/day) or high dose oral GC should be administered as initial therapy. Prednisone should be taken at 1 mg/kg/day for 2–3 weeks, followed by gradual tapering (ideally down to 0.3 mg/kg/day after 3 months and 0.15 mg/kg/day after 6 months), to the minimal effective dose.

Duration and tapering of the GC therapy have not been defined yet in RCT for EGPA: the optimum daily dose to avoid adverse events should be <7.5 mg (18); however, the 85% of patients need a long-term GC therapy to control severe asthma and ENT manifestations. Potential GC toxicity is a major concern, especially in young patients needing treatment for several years or often for the whole life (9).

The adjunction of a cytotoxic agent is recommended to treat severe EGPA: cyclophosphamide (CYC) has been commonly used as the first line induction regimen. Data on the use of CYC as induction regimen comes from studies regarding others

AAV: according to the CYCLOPS trial, bolus-IV CYC appeared to be the safest induction therapy as compared to the oral-daily administration because of the reduced cumulative dose allowing less adverse events (20, 21); the CORTAGE trial included 14 patients with EGPA and proposed a low dose IV CYC regimen (0.5 mg/m^2 every 2–3 weeks until remission), showing no differences and fewer adverse events as compared to the standard regimen (22). The actual dose is 0.5–0.7 g/m^2: 3 infusions every 2 weeks and 3 infusions every 3 weeks (a total of 6 infusions). According to the new published ACR guidelines, either CYC or RTX may be prescribed for remission induction in severe EGPA, thanks to the increasing experience with others AAV. Given the higher clinical experience, CYC should be preferentially used in patients with active cardiac involvement and a worst prognosis. Anyway, the comparative effectiveness of CYC and RTX as induction regimen in EGPA is still unknown. Regardless of the induction regimen used, RTX is also recommended as induction regimen in relapsing disease, in order to reduce the dose related CYC toxicity (13).

Without maintenance therapy relapse rates range between 73.8 and 85.7%, depending on the CYC therapy duration (6 or 12 months) (23): maintenance therapy should be started 2 weeks after last CYC administration. Once again, no RCT has compared the available maintenance therapy in EGPA, neither has defined its duration: the azathioprine and methotrexate equivalence and the superiority to mycophenolate mofetil in other AAV has been stated in RCT (24, 25). Azathioprine is administered at a dose of 2 mg/Kg/day and methotrexate at a dose of 0.25 mg/Kg/week. Despite the lack of data, a 18–24 months maintenance therapy after remission is recommended (13).

In patients with non-severe EGPA (FFS = 0) the addiction of an immunosuppressive to the GC therapy was previously not recommended (26–28); anyway, according to the new published ACR guidelines, the addition of an adjunctive immunosuppressive regimen is recommended to lower GC toxicity. Particularly, treatment with mepolizumab is the first line choice, as its efficacy was recently stated in a RCT; on the other hand, the well-known methotrexate, azathioprine, and mycophenolate mofetil have not been assessed in RCTs for this purpose. Switch between immunosuppressive treatments is recommended for relapsing disease. GC alone can be used in appropriate patients (mild asthma, allergic symptoms, pregnancy) (13).

Further Therapies

The use of plasmapheresis (PLEX) in EGPA is based on data coming from other AAV studies: PLEX is considered for patients with rapidly progressive glomerulonephritis or interalveolar hemorrhage (29). The long-term follow-up did not show a sustained effect of PLEX in terms of risk reduction of the composite outcome of death and ESRD (30). Furthermore, a small RCT involving 14 EGPA patients did not show any benefit in adding PLEX to the ongoing therapy (31).

Data on the intravenous immunoglobulins (IVIg) role in EGPA is scarce: according to case reports, IVIg can be used in a case-by-case evaluation as a second line therapy, especially in patients with myocardial or neural involvement (32, 33).

Interferon a (IFNa) has a cytoreductive action on eosinophils and seems to induce EGPA remission in several case reports. Anyway, it has limited efficacy on major relapses and has important AEs (34, 35).

Eosinophilic Granulomatosis With Polyangiitis: New Targeted Therapies

Since EGPA shares features with systemic AAV, eosinophilic disorders and asthma, new biologics for non-responding, and relapsing disease are needed for induction and maintenance of vasculitis remission and asthma targeting therapies.

A deep knowledge of EGPA pathogenesis allows the identifications of new targets for drug use: the emerging role of eosinophils and the Th2 interleukins (ILs) activation pathway led the way to the identification of new therapies targeting eosinophils biology, such as the anti-IL-5 mepolizumab; the role of B cell compartment in EGPA has not been completely cleared, but the anti-CD20 monoclonal antibody rituximab has been extensively studied in the others AAV and its use in EGPA is currently under investigation.

Anti-B-cells: Rituximab (Anti-CD20)

Rituximab (RTX) is a chimeric anti-CD20 monoclonal antibody: CD20 is a B cell membrane specific antigen involved in B cell differentiation and B-T cell stimulation; it is expressed by all lineage of B cells except for pro-B cells and plasma cells (36). Because of its B cell depletion activity, RTX was firstly approved in 1997 for treatment of B cell lymphoma and in 2005 for rheumatoid arthritis (37, 38).

One case report in 2001 firstly motivated the study of RTX as an induction regimen for GPA and MPA, reporting the efficacy of peripheral CD20 positive B cells depletion and a satisfactory clinical response (39). RTX is now approved as both induction and maintenance regimen in GPA and MPA treatment, thanks to RCT suggesting non-inferiority compared to CYC in general AAV, the reduction of costs due to the availability of biosimilars and the better safety profile compared to CYC. Anyway, no EGPA patients were included in those studies (40, 41).

RTX use in AAV is based on the pathogenicity of ANCA antibodies: the clear pathogenetic role of MPO- ANCA in EGPA was shown *in vitro* and mouse models (42, 43).

The depletion of progenitors of ANCA producing plasma-cells is not the only explanation for RTX efficacy: other B cells functions are involved in AAV pathogenesis (44).

IL-5 production in B-T cell interaction plays a key role in eosinophils maturation and survival, showing a strong association with clinical parameters of EGPA activity [Birmingham Vasculitis Activity Score (BVAS), eosinophilia] (45). Higher levels of CD80+, CD27+, CD95+ B cells, and lower levels of CD20+ B cells were described in patients with frequently relapsing EGPA (46). Despite only 30% of EGPA patients are ANCA-positive, overlap exists between the two phenotypes: particularly, IgG4 immune response is always present. IgG4 serum concentration, as a surrogate of B-lymphocyte activation, reflects disease activity, and IgG4 B cells massively infiltrates the organs involved (47, 48). Furthermore, the activity of CYC on B

cells confirms their pathogenetic role (49). Differently from CYC, RTX acts directly toward its target, preserving other functions of the immune system. The recommended dose for AAV remission induction is 375 mg/m^2/week for 4 weeks; for rheumatoid arthritis the standard dose is 1 g every 2 weeks, approved for AAV treatment too. The maintenance dose is 500 mg every 6 months for almost 18 months (50, 51).

Stated the role of B immune response in EGPA pathogenesis, many case reports, and open label studies suggested the efficacy of RTX in EGPA treatment: in a recent retrospective collaborative study, 63 patients with relapsing/refractory EGPA treated with RTX were collected from different centers around Europe. In 87% of patients RTX was administered for a vasculitis flare mainly characterized by peripheral neuropathy, skin and renal manifestation; the 83% of patients had active asthma at the treatment start. The BVAS declined from a median of 8.5 (IQR 5–13) at start to 1 (IQR 0–4.5) at 6 months and to 0 (IQR 0–2) at 12 months; remission rate, partial response, treatment failure, and adverse events requiring treatment stop were 49, 24, 24, and 3%, respectively. Remission rate was better in ANCA-positive patients compared to ANCA negative, without a statistically significant difference. The sparing GC effect was significant but not complete, from a median of 20 mg/day (IQR 15–37.5) at baseline to 7.5 mg/day (IQR 5–10) both at 6 and 12 months, due to the asthma long term therapies (52).

In another retrospective single-center study, two groups of 14 patients, matched for sex, and age, were treated with RTX or CYC as induction regimens and followed-up for 3 years. The 86% of RTX treated patients had a relapsing/remitting disease. Remission rates were not statistically significant between the two treatment groups (OR = 1.39; 95% CI: 0.28–6.84; $P = 0.404$). RTX was also associated with GC sparing from a median of 22.5–5 mg in 12 months. Furthermore, in the RTX group there was a trend toward more ANCA-positive patients achieving remission compared to ANCA-negative (45 vs. 23%) (53). Those data are concordant with previous studies, showing high rates of improvement and reduced need of GC after RTX therapy, especially in ANCA-positive patients, with a lower effect on asthma and ENT symptoms (54, 55).

Furthermore, in a AAV RCT, a RTX based maintenance therapy was associated with a lower risk of relapse than azathioprine, including renal relapse. In the 5-year extended follow-up, RTX was still superior, although in the context of a high relapse risk after therapy withdrawal (50). Concerning EGPA, a scheduled maintenance RTX therapy significantly reduced the relapse rate, as compared to on demand administration (56).

RTX has a similar safety profile as described in RCT for other AAV (27, 28, 50, 54). Two phase 3 RCT are currently ongoing to evaluate a RTX based induction (REOVAS) and maintenance regimen (MAINRITSEG) compared to conventional therapeutic strategy in patients with newly diagnosed or relapsing EGPA (ClinicalTrials.gov Identifier: NCT02807103; ClinicalTrials.gov Identifier: NCT03164473).

In conclusion, RTX effectively induced and sustained remission in patients with a new diagnosis or a refractory/remitting EGPA; it also worked as a GC sparing agent, with a potential benefit among ANCA-positive patients. Further RTCs are needed to confirm these data.

Anti-Th2-ILs

Mepolizumab (Anti-IL-5)

Cytokines and chemokines have a key role in the regulation pathway of other cells of the immune system. Particularly, IL-5 is the major cytokine responsible for eosinophils activation and survival: it is produced both by Th2 and innate lymphoid cells, regulating innate immunity (57).

Mepolizumab is an anti-IL-5 humanized monoclonal IgG1 antibody which prevents IL-5 binding with the a-subunit of its receptor, mainly expressed on eosinophils. It was firstly approved for the treatment of severe eosinophilic asthma (58).

Thanks to its involvement in the allergic pathway, mepolizumab was investigated in many allergic-related diseases, such as hypereosinophilic syndromes, atopic dermatitis, and chronic rhinosinusitis. EGPA shares the pathogenetic mechanisms observed in eosinophilic syndromes, too. The eosinophils proliferation in EGPA causes massive tissue toxicity due to eosinophils products: eosinophils cationic protein and eosinophils peroxidase are found at high levels in sera, urine and tissues (59). Furthermore, EGPA eosinophils seems to have a lower expression of pro-apoptotic genes (BLCL13, CASP2, CARD4) and defective CD95 (Apo-1 Fas)-mediated apoptosis (60, 61). As seen in asthma, the Th2 lymphocyte phenotype is activated: EGPA sera and bronchoalveolar lavage (BAL) fluid contains high concentrations of IL-5, IL-4, and IL-13 (62). The elevated concentration of IL-5 in EGPA patients suggests this cytokine as a potential target of therapy.

After some open-label pilot studies showing a potential benefit of mepolizumab in EGPA (63, 64), a double-blinded RTC involving 136 patients with uncontrolled non-severe disease (asthma and/or ENT manifestations or mostly non-severe systemic vasculitis), compared the addiction of mepolizumab or placebo to the target therapy. All patients were taking GC and a half were using other immunosuppressive therapy. Mepolizumab was injected subcutaneously at a dose of 300 mg monthly up to week 48, three times the dose approved for asthma. Remission was defined as a BVAS of 0 and no more than 4 mg of prednisone/day; active asthma was considered a feature of relapse. Only 10% of patients were ANCA positive. In the mepolizumab arm 28% of patients obtained a sustained remission for at least 24 weeks, as opposed to 3% of placebo-arm patients (OR 5.91 (95%CI 2.68–13.03; $p < 0.001$). Relapses were less common in the mepolizumab group at 52 weeks (56 vs. 82%, HR 0.32 (95%CI 0.21–0.5; $p < 0.01$), with fewer flares involving both vasculitic and asthma features. Asthma symptoms regressed, without changing in lung-function-tests. Forty-four percent of subjects treated with mepolizumab were able to taper prednisolone or prednisone to <4 mg/day, compared with 7% of subjects who received placebo during weeks 48 through 52. No differences were found between the two arms according to adverse events, previously transient, and not severe (65).

This trial led to the FDA approval of mepolizumab in 2017 as the first specific drug for EGPA. Mepolizumab is now considered a potential treatment for non-severe relapsing/refractory EGPA,

with limited data on life-threatening manifestations; particularly, the specific impact on vasculitic features is still unclear, as symptoms were combined with asthma and ENT manifestations.

Long term efficacy is currently under evaluation in an extension phase of the first trial: patients who previously required a dose of prednisolone (or equivalent) of ≥5 mg/day for adequate control of their EGPA were included (104 patients are enrolled), receiving subcutaneously administered mepolizumab at a dose of 300 mg every 4 weeks (ClinicalTrials.gov Identifier: NCT03298061).

While mepolizumab was approved at the dosage of 100 mg/month subcutaneously for the treatment of severe eosinophilic asthma, the approved FDA dose for EGPA is 300 mg/month. The use of mepolizumab in Europe at this dosage is currently off-label: real-life data are scares and its optimal dosage and route of administration is still unclear. While previous studies reported higher doses of IV mepolizumab (750 mg/month) (66, 67), recent data report positive results with low-dose mepolizumab (100 mg/month): low-dose mepolizumab showed clinically relevant benefit in exacerbation rates, asthma symptoms, GC, and immunosuppressive use in EGPA patients (68).

In a retrospective European collaborative study, remission rates at 12 months in patients receiving MEPO at a dose of 100 and 300 mg were 76 and 82%, respectively, with a comparable GC sparing effect: low-dose MEPO could be used as a first line therapy, with the possibility to increase to 300 mg monthly in cases with inappropriate response, since 10% of patients showed improvement after dose escalation. Anyway, further studies are needed to validate the low-dose and standard-dose benefits for the control of systemic and respiratory symptoms in EGPA (68).

Future Therapies: Anti-IL-5, Anti-IL-4, and Anti-IL-13

Further anti-IL-5 therapies are currently under investigation: after the successful completion of phase 3 RCTs in asthma, reslizumab (anti-IL-5), and benralizumab (anti-IL-5 a receptor) were investigated in phase 2 trials. Reslizumab was well-tolerated and resulted in a significant reduction in daily oral GC ($P < 0.05$). Benralizumab was also well-tolerated, allowing an oral GC reduction, and reduced exacerbations in EGPA. Larger controlled trials are warranted to evaluate the role of both therapies in EGPA (69, 70).

All Th2 ILs are involved in EGPA pathogenesis: IL-4 and IL-13 are also involved in Th2 activation. Recent and ongoing trials for asthma may open new possibilities for EGPA treatment (58): dupilumab is a fully human monoclonal antibody that binds to the alpha subunit of the IL-4 receptor, inhibiting the activity of both IL-13 and IL-4. It is currently approved for uncontrolled moderate-to-severe atopic dermatitis, moderate to severe asthma, and inadequately controlled chronic rhinosinusitis with nasal polyposis.

Anti-IgE: Omalizumab

IgE are involved in the allergic pathway shared by EGPA. Omalizumab is a monoclonal IgG antibody which recognizes free circulating IgE, preventing the binding to its specific high-affinity receptor and interfering with mast cells and basophils degranulation. The beneficial effect of omalizumab in EGPA concerns the downregulation if the IgE receptor, lowering mast cells activation and their interaction with eosinophils. Furthermore, the IgE-mediated antigen presenting processes and the Th2 amplification of inflammatory reactions is inhibited (71). Omalizumab is currently used

TABLE 1 | Main biological therapies in EGPA (36–68, 71–79).

Drug	Pathogenetic basis	Evidence in EGPA	Dose	Clinical use
Rituximab (36–56)	*Anti-CD20—B cell differentiation and B-T cell stimulation* ■ ANCA pathogenetic antibodies ■ Eosinophils maturation and survival promoted by IL-5 (B-T cell interaction) ■ IgG4 (a surrogate of B-lymphocyte activation) infiltration of the organs involved	■ Case reports and open label studies ■ Previous AAV studies (not involving EGPA) ■ Two phase 3 RCT ongoing in EGPA: - *REOVAS* (RTX as induction therapy) (NCT02807103) - *MAINRITSEG* (RTX as maintenance therapy) (NCT03164473)	■ Induction: 375 mg/m^2/week for 4 weeks or 1 g every 2 weeks (AAV treatment) ■ Maintenance: 500 mg every 6 months for almost 18 months	*RCTs ongoing:* ■ New diagnosis or refractory/remitting disease ■ GC sparing agent
Mepolizumab (57–68)	*Anti-IL-5—eosinophils activation and survival* ■ Eosinophils products: massive tissue toxicity ■ EGPA eosinophils: lower expression of pro-apoptotic genes and defective apoptosis	■ *FDA approval in 2017 as the first specific drug for EGPA* (RTC involving 136 patients with uncontrolled non-severe disease) ■ Long term efficacy: ongoing RCT (NCT03298061)	■ 300 mg/month (FDA approved) ■ 100 mg/month (severe eosinophilic asthma, under evaluation in EGPA)	■ Non-severe relapsing/refractory disease
Omalizumab (71–79)	*Anti-free circulating IgE* ■ Lower mast cells activation and interaction with eosinophils ■ Inhibition of Th2 and IgE mediated antigen presenting processes	*Evidence contradictory and scarce:* ■ Positive results in EGPA with asthma resistant to GC ■ Scarce information about vasculitic involvement ■ Possible trigger factor for EGPA	■ Subcutaneously every 2–4 weeks	■ Maintenance therapy in patients with uncontrolled and severe asthma/ENT symptoms but with a complete control of non-allergic symptoms

for the treatment of severe asthma with elevated IgE, chronic spontaneous urticaria and allergic rhinitis (72–74). The drug is injected subcutaneously every 2–4 weeks, based on body weight and serum IgE, with a good safety profile.

Available information on its use in EGPA is contradictory and scarce. First, the drug was mostly used in patients with severe EGPA associated asthma resistant to GC: while some reports showed positive results, with a GC sparing effect, information about vasculitic involvement is scarce. The dose used was different among studies, without a scheduled regimen.

The available evidence supports the use of omalizumab as maintenance therapy in EGPA patients with uncontrolled and severe asthma/ENT symptoms but with a complete control of non-allergic symptoms. Anyway, in one discordant case report, two patients suffered from asthma exacerbations (75). Furthermore, other studies suggested an association between omalizumab intake in severe asthma and the onset of EGPA: while a role of omalizumab as a trigger factor of EGPA cannot be excluded, the steroid sparing could reveal a hidden disease (76–79).

CONCLUSIONS

The quest for the ideal regimen in EGPA is going toward a more personalized approach, looking for new therapies as well as tailored regimens adapted to different subsets of patients (divided according to disease severity, age, organ involvement, and predictable outcomes) (7). A deep knowledge of the pathogenesis of EGPA allows the identifications of new targets for drugs use (**Table 1**). The recent guidelines highlight gaps in our knowledge for the treatment of EGPA: new studies are warranted to find more reliable markers and indicators of disease activity, to identify the best use (dose, duration, long-term safety) of actual therapies and to investigate new targeted therapies, with steroid sparing activity.

AUTHOR CONTRIBUTIONS

FF and RAS conceived the study. MU and FR conducted a review of the literature and drafted the manuscript. MU, FR, BT, PT, FF, and RAS reviewed and edited the manuscript and support the study. All authors checked the final version of the manuscript.

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Conflict of Interest: RAS has received consulting fees from GSK, Roche, and Otsuka.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Medicine

REVIEW
published: 30 November 2021
doi: 10.3389/fmed.2021.738850

Anti-inflammatory Treatment of Kawasaki Disease: Comparison of Current Guidelines and Perspectives

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Kawasaki disease (KD), an acute, generalized vasculitis, is associated with an increased risk of coronary heart disease and is the most common cause of acquired heart disease in childhood. The incidence of KD is increasing worldwide. There are numerous international treatment guidelines. Our study aims to perform the first one so far comparison of them. While the gold standard therapy remains still the same (intravenous immunoglobulins and aspirin), there is currently a lack of evidence for choosing optimal treatment for high-risk patients and refractory KD. In this review, we also discuss the treatment of complications of KD and Kawasaki-like phenotypes, present an anti-inflammatory treatment in the light of new scientific data, and present novel potential therapeutic targets for KD.

Keywords: Kawasaki disease, vasculitis, guidelines, anti-inflammatory treatment, coronary artery aneurysm, SARS-CoV-2, PIMS-TS, MIS-C

OPEN ACCESS

Edited by:
Silvia Piantoni,
ASST-Spedali Civili and University of Brescia, Italy

Reviewed by:
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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *09 July 2021*
Accepted: *04 November 2021*
Published: *30 November 2021*

Citation:
Buda P, Friedman-Gruszczyńska J and Książyk J (2021) Anti-inflammatory Treatment of Kawasaki Disease: Comparison of Current Guidelines and Perspectives. Front. Med. 8:738850. doi: 10.3389/fmed.2021.738850

INTRODUCTION

Kawasaki disease (KD) is an acute, systemic, vasculitis, most commonly occurring in children under 5 years of age. KD, firstly described in Japan in 1967 is the present most common cause of acquired heart disease in childhood (1, 2). The incidence ranges from 138–322/100,000 in Asia, to 4.5–25/100,000 in Europe and the United States of America (3–6). In Great Britain, the number of new cases has doubled in recent years and is now 8.1/100,000 (7, 8). The immunopathogenic mechanism for KD is not completely understood. The epidemiological observations suggest that in genetically predisposed children an environmental agent causes an abnormal hyperactivation of the immune system which results in damage of vascular endothelial cells and systemic vasculitis (8). Many genes responsible for susceptibility to KD have been identified through genome-wide association studies, however they differ within populations (9–15).

The diagnosis of KD should be considered in any child with a febrile exanthematous illness and presence of inflammation, particularly if it persists longer than 4–5 days. The diagnosis of KD is based on clinical criteria, established by the Japanese Ministry of Health Research Committee and adopted by the American Heart Association (16–18). Classic KD is defined as the presence of fever of $\geq$5 days plus at least $\geq$4 of the following diagnostic criteria: oral mucosal changes, non-suppurative conjunctival injection, polymorphous skin rash, peripheral changes, including erythema and/or edema of hands and feet, and cervical lymphadenopathy. The incidence of atypical form is increasing. It is more common in infants younger than 6–12 months, the only clinical sign could be fever and abnormalities in laboratory tests, which can cause diagnostic errors. Currently, the diagnosis of KD is only based on clinical and laboratory criteria, interestingly, Wright V et al. found that molecular patterns could enable earlier diagnosis and treatment of KD and reduce inappropriate treatment in those with other diagnoses (19). Although the acute

febrile and exanthematous illness may be self-limiting, some patients develop serious complications that are associated with an increased risk of coronary heart disease. The main complication of the disease are coronary artery abnormalities (CAL), however extra coronary complications can occur. The coronary artery aneurysms occur in around 20–30% of untreated cases (19, 20). Coronary artery events (thrombosis, stenosis, intervention, myocardial infarction, death) occurrs in 1–48% of patients with CAL, the incidence depends on the aneurysm *Z* score <10 and on the absolute dimension (21). Up to 4% of cases of untreated KD with CAL will progress to sudden death during the acute phase of the illness as a result of aneurysmal thrombosis formation, myocardial infarction or dysrhythmia (22). In properly treated patients, the risk of permanent changes in coronary arteries decreases significantly (4%) (20, 23, 24). Patients without coronary artery abnormalities have no symptoms or events during follow-up. Medium to long term prognosis after Kawasaki disease is excellent (25). Recurrence of KD has been previously described. It varies between 0.8% in the united states of America to 3% in Japan (5, 26). The proportion of patients suffering from a recurrence increases with age, majority of recurrence occurs within 2 years of the initial presentation (26). In rare cases (0.2%), patient can suffer multiple recurrences (26).

The preliminary understanding of immunogenetic influences the disease susceptibility has already led to treatment with various regimens. The main goal of therapy is to reduce systemic inflammation as early as possible to prevent coronary artery damage.

There are many diagnostic and therapeutic strategies, the aim of this paper is to compare current guidelines and to discuss anti-inflammatory treatment of KD, complications of KD, Kawasaki-like phenotypes and to discuss new potential targets based on new scientific data.

TREATMENT GUIDELINES

There are differences in the scope of the procedure, depending on the recommendations of individual countries.

Most of them are listed below:

- (2014) Guidelines for medical treatment of acute Kawasaki disease: report of the Research Committee of the Japanese Society of Pediatric Cardiology and Cardiac Surgery (2012 revised version) (17).
- (2017) Scientific Statement, which serves as an update to the 2004 American Heart Association guidelines for the diagnosis, treatment, and long-term management of Kawasaki disease (16).
- (2018) European consensus-based recommendations for the diagnosis and treatment of Kawasaki disease—the Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) initiative (27).
- (2021) Revised recommendations of the Italian Society of Pediatrics about the general management of Kawasaki disease (28).

The comparison of various treatment regimens is shown in **Tables 1**, **2**.

There are also guidelines on the long-term management of patients who have vascular complications of KD. This therapy is individualized, it usually consists of medicines for heart conditions (antithrombotic therapy, statins, beta-blockers, interventional cardiology, cardiac surgery), though this topic exceeds the aim of this paper.

- (2020) Japanese Circulation Society Working Group 2020 Guideline on Diagnosis and Management of Cardiovascular Sequelae in Kawasaki Disease (29).
- (2020) Expert consensus statement "Lifetime cardiovascular management of patients with previous Kawasaki disease" (30).

It is also worth to mention that in 2020 Japan Pediatric Society presented the revision of guidelines for Kawasaki disease (6th revised edition) but only in the context of the diagnosis.

- (2020) Japan Pediatric Society: Revision of diagnostic guidelines for Kawasaki disease (6th revised edition) (18).

STANDARD TREATMENT OF KAWASAKI DISEASE

All above mentioned management guidelines are consistent with the first-line treatment. Treatment of acute illness with intravenous immunoglobulin (IVIG) and acetylosalic acid (ASA) is now the gold standard recommendation. Differences concerning aspirin dose are presented in **Table 1**.

Intravenous Immunoglobulins (IVIG)

Currently, the most effective anti-inflammatory treatment for KD is an early transfusion of intravenous immunoglobulins. Randomized clinical trials performed in the 1980s suggested that IVIG reduced the prevalence of persistent coronary artery lesions (CAL) (21, 31). The systematic review by the Cochrane Collaboration states that CAL development can be reduced by a single dose of 2 g/kg IVIG given before the 10th day after onset, thus, high-dose IVIG is still the first-line treatment of KD according to all current guidelines (**Table 1**) (24).

The molecular mechanisms of IVIG for anti-inflammation in KD remain unclear. Potential mechanisms include the blockade of the Fc receptor, neutralization of the pathogenic products of unknown infectious agents, immune-modulatory effects, stimulation of suppressor activity, and modulation of the cytokines (9, 14, 32–34). Multiple studies show that ∼10–28% of patients are resistant to first-line treatment (no resolution of fever, recurrent fever, no / slight decrease in inflammation parameters) (20, 34). The definition of IVIG resistance varies according to different recommendations (**Table 1**). Many studies have been conducted to identify predictive factors of resistance to IVIG therapy. Xuan Li et al. performed a meta-analysis of 4,442 children with KD and identified the clinical features and laboratory factors: the initial administration of IVIG ≤4.0 days after the onset of symptoms, increased erythrocyte sedimentation rate (ESR) and decreased hemoglobin and platelet counts,

TABLE 1 | Comparison of guidelines for the treatment of Kawasaki disease.

	AHA 2017	SHARE 2018	ISP 2021	JPS 2014
IVIG	High-dose IVIG (2 g/kg given as a single infusion) within 10 days of illness onset but as soon as possible after diagnosis	IVIG (2 g/kg given as a single infusion)	IVIG (2 g/kg), preferably given within the 10th day, better if within the 7th day of illness, but as soon as possible after diagnosis	IVIG—single use (2 g/kg per day) or IVIG—modified single use (1 g/kg per day for 1 or 2 days continuously) or IVIG –divided dosing (200–400 mg/kg per day, over 3–5 days)
IVIG resistance—definition	Persistent or recrudescent fever at least 36 hours and <7 days after completion of first IVIG infusion	Ongoing fever and/or persistent inflammation or clinical signs ≥ 48 h after receiving IVIG as a single dose of 2 g/kg. Laboratory values that can be important in assessing risk stratification for IVIG resistance: low sodium, raised bilirubin, raised ALT, low platelet count, high CRP, low albumin	Failure in the response to IVIG—recrudescent fever reoccurring or persisting 36–48 h after IVIG infusion	Persistent fever after 48 h of starting IVIG
High-risk patients—definition	Not defined criterias for high-risk children outside Japan. In Japan patients at high risk for non-response to IVIG are defined by scoring systems (Kobayashi, Sano)	Patients with severe KD: IVIG-resistant (see above), Kobayashi score ≥5, features of HLH, shock, children under the age of 1 year, children with coronary and/or peripheral aneurysms	Children <12 months or those having CRP higher than 200 mg/l, severe anemia at disease onset, albumin level below 2.5 g/dl, liver disease, overt coronary artery aneurysms, macrophage activation syndrome or septic shock	According to representative scoring systems for evaluating potential IVIG resistance (Kobayashi, Egami, Sano)
ASA moderate-high dose	Administration of moderate (30–50 mg/kg) to high-dose (80–100 mg/kg) ASA is reasonable until the patient is afebrile, although there is no evidence that it reduces coronary artery aneurysms. There are no data to suggest that either dose of ASA is superior	All patients diagnosed with KD who are treated with IVIG should be treated with aspirin at a dose of 30–50 mg/kg/day until fever has settled for 48 h, clinical features are improving, and CRP levels are falling	Treatment of KD is completed by ASA given at a daily dosage of 30–50 mg/kg in the acute phase of KD until 48 h after the disappearance of fever, then switched to the anti-platelet dose (3–5 mg/kg once daily). When GCS are given in patients classified as high risk, ASA is given in low dose (3–5 mg/kg)	Febrile period: oral dose of 30–50 mg/kg/day, in 3 divided doses
ASA low dose	Reducing the ASA dose after the child has been afebrile for 48–72 h. Other clinicians continue high-dose ASA until the 14th day of illness and at least 48–72 h after cessation of fever	The dose of aspirin should subsequently be reduced to an antiplatelet dose of 3–5 mg/kg once daily when fever and inflammation have subsided	Low-dose ASA must be continued until 6–8 weeks in children without CAL and continued in children with CAL until the resolution of coronary artery lesions	48–72 h after defervescence, dosage can be reduced to one dose of 3–5 mg/kg per day

ALT, alanine aminotransferase; ASA, aspirin; CAL, coronary artery abnormalities; CRP, C-reactive protein; HLH, hemophagocytic lymphohistiocytosis; IVIG, intravenous immunoglobulins; KD, Kawasaki disease; AHA, American Heart Association; SHARE, The European Single Hub and Access point for pediatric Rheumatology in Europe; ISP, Italian Society of Pediatrics; JPS –Japan Pediatric Society.

oral mucosa alterations, cervical lymphadenopathy, swelling of extremities, and polymorphous rash (35). Yan et al. in their systematic review and meta-analysis confirmed that gender, IVIG resistance, IVIG treatment beyond 10 days of onset of symptoms and increased C-reactive protein (CRP) level are all significant risk factors for CAL (36). Zheng et al. performed the first meta-analysis that revealed the strongest association between the incidence of CAL and IVIG resistance (37). There is currently no universally accepted classification system to evaluate KD severity. Many predictive models that were designed to evaluate the possibility of IVIG resistance were proposed (38–47). Scoring systems (Kobayashi, Sano, Egami) most commonly used in clinical practice include following parameters: hyponatremia, prolonged illness duration, elevated C-reactive protein, aspartate transaminase, alanine transaminase (ALT), bilirubin, neutrophil ratio, low count of platelets. The problem is that there are no such predictive instruments or scores outside Japan, the effectiveness of such scores has not been confirmed in large-scale prospective cohort studies or meta-analyses. Kuo et al. used a novel approach by conducting a genomewide association analysis to develop a risk score for IVIG resistance (48). However, it is unknown whether one universal prediction model can be developed for all populations or population-specific prediction models will be required (49). Recently, Piram et al. identified predictors of IVIG resistance and presented a new score with good sensitivity and acceptable specificity in a non-Asian population (50). Predictors of secondary treatment after initial IVIG were hepatomegaly, ALT level ≥30 IU/L, lymphocyte count <2,400/mm3 and time

to treatment <5 days. These findings have not yet been used to current guidelines.

The development of CAL despite IVIG treatment ranges from 19 to 42% (51, 52). A genetic contribution to CAL is likely as before effective therapy with IVIG was introduced, only 25–30% of affected children developed CAL (22, 53). Many genes and chromosomal regions have been identified through genome-wide association studies to have an association with KD and CAL formation (10, 14, 53, 54). Genes responsible for susceptibility and CAL formation may be different between populations. The neutrophil antigen 1 allotype in the extracellular domain 1 of *Fcγ R3B* has been identified as a major risk factor for IVIG refractoriness and persistent CAL (32). In the future, risk scores may include genetic testing for high-risk small nucleotide polymorphisms (SNPs).

Acetylsalicylid Acid (ASA)

Aspirin (ASA, aspirin) inhibits platelet function through irreversible inhibition of cyclooxygenase (COX) activity and blocks the synthesis of prostaglandins. The mechanism of action of aspirin depends on dosage, medium-high doses are usually given to obtain the anti-inflammatory effect, low doses inhibit platelet aggregation. ASA has been used in the treatment of KD for many years and is approved for all patients with KD. High-dose (80–100 mg/kg) and medium dose (30–50 mg/kg) acetylsalicylic acid have been recommended as standard treatment during the acute febrile phase by the American Heart Association and Japanese Society of Pediatric Cardiology and Cardiac Surgery, respectively (16, 18). The optimal dose of ASA remains controversial, however. Although high-dose aspirin shortens fever duration, researchers of many recent studies found that the use of medium- or higher-dose ASA in acute Kawasaki disease did not prevent CAL (54–58). Considering the risk of drug toxicity and the lack of evidence for prevention of CALs, the role of aspirin in the acute phase of KD needs to be reassessed and a future randomized controlled trial is needed to determine the optimum dose of ASA. Clinical trials comparing the efficacy of IVIG alone and IVIG plus high-dose aspirin in KD are ongoing. The duration of high-dose ASA administration varies across institutions. Some physicians recommend conversion to an antiplatelet dose of ASA after the child has been afebrile for 48–72 h. Others continue high-dose ASA until the 14th day of illness. Low-dose ASA is continued until the patient has no evidence of CAL by 6–8 weeks after onset of fever. For children who develop CAL, ASA may be continued indefinitely (16).

It is unclear what dose (anti-inflammatory vs. anti-platelet) of aspirin should be used with simultaneous supply of glucocorticosteroids (GCS) and whether to give aspirin at all (since GCS are anti-inflammatory and the combined use of both drugs increases their side effects).

Interestingly, only Italian guidelines indicate that patients treated with GCS as a first-line treatment need to be treated simultaneously with low dose ASA instead of high-dose ASA. Such strategy is reasonable but some authors concluded that in the absence of comparative studies, it is practiced to use both drugs.

SECOND-LINE TREATMENT

Patients who are at increased risk of CAL, unresponsive to IVIG may be treated with second dose of IVIG, glucocorticosteroids, infliximab or other immunosuppressive agents. To date, there have been no robust clinical trials comparing second-line treatment options for IVIG resistant KD. Treatment choice varies according to different recommendations (**Tables 1**, **2**).

Glucocorticosteroids (GCS)

GCS inhibit the transcription of most pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, interferon-γ, and tumor necrosis factor-α) (59). They also inhibit the proliferation of T and B lymphocytes, Langerhans cells, decrease adhesive molecule expression. Because of their effects on a broad range of innate and adaptive responses and effect on multiple types of immune cells, GCS are remarkable helpful in managing many of autoinflammatory and autoimmune diseases (60, 61). Corticosteroids are usually administered in all vasculitides due to their anti-inflammatory effect, but the use of GCS in children with KD is still controversial and varies depending on individual recommendations (16, 27, 28). In 2007, a multi-center prospective randomized, placebo-controlled, double-blinded study found no significant difference in coronary z scores or in the duration of fever in those treated with corticosteroids in addition to IVIG (62). Subsequent Japanese studies have shown that the addition of corticosteroids significantly decreases the risk of CAL; however, these studies included only patients classified as patients with a high risk of IVIG resistance based on Asian risk scores (63–66). In 2016 meta-analyses showed that the frequency of CAL was significantly lower in children that received GCS with IVIG than IVIG therapy only (67). Sixteen comparative studies were analyzed. It is worth noting that most included studies were conducted in Japan. Whether these results are applicable to other countries remains to be elucidated. Others found that long-term steroid treatment should be considered in all children diagnosed with the disease (68). Yang et al. stated that GCS treatment, combined with IVIG, reduces the incidence of coronary aneurysms, but only in Japanese patients, which was not observed in other nations' patients (69). Thus, these studies' conclusions should not be extrapolated to non-Asian populations due to the possible influence of various environmental, genetic, and economic factors on the effects of therapy (70). The current American Heart Association guidelines do not recommend routine use of adjunctive corticosteroids, but rather consideration for high-risk patients. The administration of a longer course of corticosteroids together with IVIG and ASA may be considered for treatment of high-risk patients, when they can be classified before initiation of treatment. Administration of high-dose pulse steroids may be considered as an alternative to the second infusion of IVIG or for retreatment of patients with KD who have had recurrent or recrudescent fever after additional IVIG (16). According to the SHARE guidelines, adjunctive primary GCS treatment should be given to children: who are IVIG resistant, have a Kobayashi score ≥4 or developed MAS/HLH and/or shock. The panel of experts defined additional 'high-risk groups' who might benefit from primary

TABLE 2 | Treatment options for IVIG-resistant KD patients and refractory KD.

	AHA 2017	SHARE 2018	ISP 2021	JPS 2014
IVIG resistance—treatment	IVIG or IVIG + GCS or Infliximab It is reasonable to administer a second dose of IVIG (2 g/kg) to patients with persistent or recrudescent fever at least 36 h after the end of the first IVIG infusion	GCS +/– IVIG A second dose of IVIG is at the discretion of the treating physician	IVIG or IVIG + GCS In non-responder patients with KD treatment requires a second infusion of IVIG and—in case of failure—pulses of methylprednisolone (30 mg/kg/day) for 3 consecutive days, followed by oral prednisone (2 mg/kg/day, then gradually tapered)	IVIG + GCS IVIG in combination with either prednisolone or methylprednisolone
High-risk patients—first line treatment	IVIG + ASA +/− GCS. Administration of a longer course of corticosteroids (e.g., tapering over 2–3 weeks), together with IVIG 2 g/kg and ASA, may be considered for treatment of high-risk patients with acute KD, when such high risk can be identified in patients before initiation of treatment	IVIG + GCS + ASA Corticosteroid treatment should be given to patients with severe KD. Treatment should not be delayed while awaiting echocardiography. Two regimens would be reasonable (see below)	IVIG + GCS + ASA In high-risk patients with KD initial treatment should include: IVIG + single intravenous pulse of methylprednisolone (30 mg/kg/day) + low-dose aspirin (3–5 mg/kg/day). In case of failure treatment should be implemented with a further infusion of IVIG and three pulses of intravenous methylprednisolone (30 mg/kg/day, followed by prednisone: 2 mg/kg/day, then gradually tapered) + low-dose aspirin (3–5 mg/kg/day)	IVIG + GCS + ASA Such patients should be treated with 2 g/kg of IVIG in combination with either 2 mg/kg per day prednisolone or 30 mg/kg per day intravenous methylprednisolone pulse If the patients fail to respond to these treatments, a third-line treatment will be upgraded to a second-line treatment
GCS	Single-dose pulse methylprednisolone should not be administered with IVIG as routine primary therapy for patients with KD. Administration of a longer course of corticosteroids (e.g., tapering over 2–3 weeks), together with IVIG 2 g/kg and ASA, may be considered for treatment of high-risk patients with acute KD, when such high risk can be identified in patients before initiation of treatment. Administration of high-dose pulse steroids (usually methylprednisolone 20–30 mg/kg intravenously for 3 days, with or without a subsequent course and taper of oral prednisone) may be considered as an alternative to a second infusion of IVIG or for retreatment of patients with KD who have had recurrent or recrudescent fever after additional IVIG. Administration of a longer (e.g., 2–3 weeks) tapering course of prednisolone or prednisone, together with IVIG 2 g/kg and ASA, may be considered in the retreatment of patients with KD who have had recurrent or recrudescent fever after initial IVIG treatment	Corticosteroid treatment should be given to patients with severe KD (see high-risk patients, **Table 1**)	In high risk patients. In case of failure treatment	In patients suspected of being IVIG resistant on the basis of clinical symptoms and laboratory findings. In patients found to be IVIG resistant after first-line IVIG treatment

(Continued)

TABLE 2 | Continued

	AHA 2017	SHARE 2018	ISP 2021	JPS 2014
Treatment options for IVIG-Resistant KD patients and Refractory KD	IVIG GCS Infliximab CsA ANA CYP PE — CsA: Administration of cyclosporine may be considered in patients with refractory KD in whom a second IVIG infusion, infliximab, or a course of steroids has failed. Administration of immunomodulatory monoclonal antibody therapy (except TNF-α blockers), cytotoxic agents, or (rarely) plasma exchange may be considered in highly refractory patients who have failed to respond to a second infusion of IVIG, an extended course of steroids, or infliximab	IVIG GCS Infliximab — TNF-alpha blockade (e.g., infliximab) should be considered in KD patients with persistent inflammation despite IVIG, aspirin and corticosteroid treatment, after consultation with a specialist unit. The use of DMARDs such as cyclosporin, cyclophosphamide and methotrexate, along with anakinra and plasma exchange, cannot be recommended, except on an individual basis after consultation with a specialist unit	IVIG GCS Infliximab Anakinra Canakinumab — Current evidence supports the use of infliximab as rescue therapy in IVIG- and methylprednisolone-refractory patients with KD; IL-1 blockade with anakinra is highly promising in treating the most dramatically severe multi-refractory patients with KD, with potential benefits also on the cardiovascular complications	IVIG GCS Infliximab Ulinastatin CsA Methotrexate PE —
Prednisone/ prednisolone	Prednisolone 2 mg/kg i.v. divided every 8 h until afebrile, then prednisone orally until CRP normalized, then taper over 2–3 weeks	–	After intravenours methylprednisolone treatment. Prednisone at the initial dose of 2 mg/kg/day, then tapered up to the resolution of symptoms and normalization of CRP	During fever: 2 mg/kg/day of prednisolone, i.v. in 3 divided doses After defervescence: Once patient is no longer febrile and general status has improved, prednisolone is given orally. When CRP normalizes, the dose of prednisolone is tapered over 15 days, in 5 day steps, from 2 mg/kg/day in 3 divided doses to 1 mg/kg/day in 2 divided doses to 0.5 mg/kg/day in a single dose
Methylprednisolone	Usually 20–30 mg/kg intravenously for 3 days, with or without a subsequent course and taper of oral prednisone	Regimen 1: methylprednisolone 2 × 0.8 mg/kg for 5–7 days or until CRP normalizes; then convert to oral prednisone/prednisolone 2 mg/kg/day and wean off over next 2–3 weeks. Regimen 2: methylprednisolone 10–30 mg/kg (up to maximum of 1 g/day) once daily for 3 days followed by oral prednisone/prednisolone 2 mg/kg per day until day 7 or until CRP normalizes; then wean over next 2–3 weeks	In high-risk patients with KD initial treatment should include: IVIG + single intravenous pulse of methylprednisolone (30 mg/kg/day) + low-dose aspirin (3–5 mg/kg/day). In case of failure treatment should be implemented with a further infusion of IVIG and three pulses of intravenous methylprednisolone (30 mg/kg/day, followed by prednisone: 2 mg/kg/day, then gradually tapered) + low-dose aspirin (3–5 mg/kg/day). In low-risk KD patients resistant to two previous infusions of IVIG: pulses of methylprednisolone (30 mg/kg/day) for 3 days. followed by oral prednisone (2 mg/kg/day, then gradually tapered)	When used in combination with first-line IVIG: 1 dose of 30 mg/kg methylprednisolone. When used to treat IVIG-resistant patients: 30 mg/kg methylprednisolone once a day, for 1–3 days. Some reports suggest additional prednisolone (started at 1–2 mg/kg/day and gradually tapered over a period of 1–3 weeks) after methylprednisolone
Infliximab	Administration of infliximab (5 mg/kg) may be considered as an alternative to a second infusion of IVIG or corticosteroids for IVIG-resistant patients. Single infusion: 5 mg/kg IV given over 2 h	Infliximab should be considered in KD patients with persistent inflammation despite IVIG, aspirin and corticosteroid treatment, after consultation with a specialist unit	Current evidence supports the use of infliximab, a chimeric monoclonal antibody against TNF-α, as rescue therapy at a single intravenous dose of 5 mg/kg of body weight (given in 2 h) for IVIG- and corticosteroid resistant KD patients	i.v. drip infusion of 5 mg/kg (may only be given once)

(Continued)

TABLE 2 | Continued

	AHA 2017	SHARE 2018	ISP 2021	JPS 2014
Anakinra	2–6 mg/kg given by subcutaneous injection	The use of DMARDs such as ciclosporin, cyclophosphamide and methotrexate, along with anakinra and plasma exchange, cannot be recommended, except on an individual basis after consultation with a specialist unit	In children with a refractory KD, given subcutaneously at a daily dose of 4–8 mg/kg of body weight for an overall period of 15 days or for a longer period, depending on the specific clinical scenery	–
Cyclosporin A	i.v.: 3 mg/kg divided every 12 h p.o.: 4–8 mg/kg divided every 12 h. Adjust dose to achieve trough 50–150 ng/mL; 2-h peak level 300–600 ng/mL	The use of DMARDs such as cyclosporin, cyclophosphamide and methotrexate, along with anakinra and plasma exchange, cannot be recommended, except on an individual basis after consultation with a specialist unit	4 mg/kg/day in 2 doses p.o.; in case of persistence of fever the dosage can be increased to 5–8 mg/kg/day; administered until CRP normalization or for 10–14 days	Start on 2 divided oral doses (1 each before meal) of 4–5 mg/kg/day Target trough level: 60–200 ng/mL
Plasma Exchange	Plasma exchange should be reserved for patients in whom all reasonable medical therapies have failed	The use of DMARDs such as cyclosporin, cyclophosphamide and methotrexate, along with anakinra and plasma exchange, cannot be recommended, except on an individual basis after consultation with a specialist unit	–	Displacing solution set at 5% albumin; 1–1.5× the patient's circulating plasma volume is exchanged. Usually given for 3 continuous days (upper limit: 6 days)

ANA, anakinra; ASA, aspirin; CsA, cyclosporin A; CRP, C-reactive protein; CYP, cyclophosphamide; DMARDs, disease-modifying antirheumatic drugs; GCS, glucocorticosteroids; INF, inliximab; IVIG, intravenous immunoglobulins; KD, Kawasaki disease; PE, plasma exchange; AHA, American Heart Association; SHARE, The European Single Hub and Access point for pediatric Rheumatology in Europe; ISP, Italian Society of Pediatrics; JPS, Japan Pediatric Society.

adjunctive GCS: infants <1 year of age and children presented with coronary and/or peripheral aneurysms at diagnosis. It is unclear whether corticosteroids should be used in children with less severe KD, and the optimal corticosteroid dosing regimen to use is uncertain. Italian and Japanese guidelines indicate the use of GCS for patients suspected of being IVIG resistant on the basis of clinical symptoms and laboratory findings and for patients found to be IVIG resistant after first-line IVIG (18, 28). The problem is that there are no predictive instruments or scores for reliable identification of high-risk children outside Japan, further research is needed to test the efficacy of GCS in this population. KD-CAAP is a multi-center, randomized trial comparing the effectiveness of corticosteroids with standard treatment vs. standard treatment alone to prevent KD heart complications. The study is ongoing.

Infliximab

Monoclonal antibodies may target the presumed key-cytokines involved in KD pathogenesis, particularly tumor necrosis factor (TNF)-α and interleukin (IL)-1 (71, 72). Elevated serum TNF-alpha is elevated in patients with KD and it correlates with the development of CAL. Infliximab is a chimeric murine/human IgG1 monoclonal antibody that binds specifically to TNF-alpha with high affinity and neutralizes the biological activity of soluble TNF-α (73). Among monoclonal antibodies, infliximab is the most widely tested drug in KD. It is safe and well-tolerated drug that reduces fever duration and inflammation, but the addition of infliximab to primary treatment in acute Kawasaki disease did not reduce treatment resistance. No trials have evaluated its use as adjunctive therapy in patients with early evidence of CAL (74). Thus, current guidelines supports the use of infliximab, as a rescue therapy at a single intravenous dose (5 mg/kg of body weight given in 2 h) for IVIG- and corticosteroid resistant KD patients.

The efficacy of another tumor necrosis factor-α receptor blocker (etanercept) was also evaluated (75, 76). However, the disadvantage of etanercept is that it only binds to circulating and not cell-bound TNF-alpha which could potentially impair its efficacy (77).

Anakinra

The IL-1 signaling pathway seems to be key to the pathogenesis of KD, especially in the development of coronary artery aneurysms (78). Upregulated IL-1 pathway genes and elevated IL-1 concentrations have been demonstrated in the peripheral blood of KD patients during the acute phase of the disease (79, 80). Weng et al. showed that polymorphisms in the genes coding for IL-1 (-31 CC and−511 TT) were associated with a greater risk of resistance to IVIG treatment (81). The use of IL-1 inhibitors in patients with KD has been reported, but data are largely limited to small case series. Ferrara et al. summarized the scientific literature related to the use of anakinra, analyzing preclinical and clinical data (82). Reasons for using anakinra are as followed: Kawasaki disease shock syndrome, macrophage activation syndrome, persistent fever and laboratory abnormalities, worsening of coronary aneurysms, coronary aneurysms and increased proBNP levels. The dose ranged from 1 to 10 mg/kg/day; the duration ranged from 6 days to 6 months (83–88). According to compared recommendations only IPS mentioned about the duration of treatment for an overall period of 15 days or for a longer period, depending on the specific clinical scenery (28, 89). In the largest study concerning anakinra

(KAWAKINRA), starting doses were 2 mg/kg of anakinra (4 mg/kg in patients who were age <8 months and who weighed ≥5 kg), and the dose was increased up to 6 mg/kg every 24 h if the patient's was febrile. Treatment duration was 2 weeks. Almost all patients (sixteen patients included) received a clinical benefit (reducing fever, markers of systemic inflammation, and coronary artery dilatation), and no relevant side effects were noted. Authors concluded that anakinra may be considered as an option after the failure of the first IVIG infusion, especially in patients with coronary involvement (90). Mastrolia MV et al. have recently reported two cases of children, diagnosed with KD, non-responsive to two doses of intravenous immunoglobulins, successfully treated with ANA, without prior use of steroids (91). Further studies are planned/ongoing to reveal its clinical significance (ANACOMP, ANAKID) and to better define the place of IL-1 blockade in KD step-up treatment.

Interestingly, other anti-IL drugs could be regarded as an alternative treatment. Canakinumab is a human monoclonal antibody targeted at IL-1β, with no cross-reactivity with other members of the IL-1 family. It has been authorized for the treatment of systemic juvenile idiopathic arthritis and different hereditary autoinflammatory syndromes. According to ISP guidelines using a single subcutaneous injection of 4 mg/kg/dose of canakinumab may be also a future option for cases of IVIG-resistant and corticosteroid-resistant KD (28).

Cyclosporin A

Cyclosporin A is a calcineurin inhibitor that exerts its immunosuppressive effects through the down-regulation of NFAT (nuclear factor of activated T cells) transcription factor, and suppresses cytokine production such as IL-2 by inhibiting nuclear factor of activated T cells (17, 92). It has been studied as both a second-line therapy and as rescue therapy for KD.

The largest study (KAICA trial) was conducted on Japanese participants. Hamada et al. found that combined primary therapy with IVIG and cyclosporin was safe and effective for favorable coronary artery outcomes in Kawasaki disease patients who were predicted to be unresponsive to IVIG (93). Despite this CsA is reserved only for refractory KD according to current guidelines (including Japanese) (16, 17, 27, 28).

Other Treatment

Cyclophosphamide, methotrexate, ulinastatin have also been used in refractory-KD however according to all current guidelines these medicaments should only be considered in severe refractory cases because of potential adverse reactions and better experience with previously mentioned medicaments (77, 94–97). Plasma exchange (PE) could act via mechanical removal of inflammatory cytokines and was used in patients with refractory KD (17, 98, 99). The largest series reported to date included 125 patients who were resistant to IVIG and treated with plasma exchange (100). Authors conclude that outcomes of PE for Kawasaki disease refractory to IVIG are favorable, although not statistically significant. Because PE is a high-risk procedure and there are no controlled clinical trials it could be considered only in extreme cases of refractory KD.

TREATMENT OF OTHER CLINICAL CONDITIONS RELATED TO KD

Macrophage Activation Syndrome (MAS)

Macrophage Activation Syndrome (MAS) is a form of secondary hemophagocytic lymphohistiocytosis (HLH). It is a life-threatening systemic extreme-inflammatory syndrome caused by multifactorial immune dysregulation and pathological hyperactivation of the immune system. The most common form of HLH is MAS in the course of systemic-onset juvenile idiopathic arthritis (so-JIA) but it could also occur as the manifestation of Kawasaki disease (101, 102). Macrophage activation syndrome is characterized by fever, hepato- and/or splenomegaly, non-characteristic skin lesions, lymphadenopathy, coagulopathy, central nervous system dysfunction. Symptoms of the respiratory system and heart failure could also be present. Uncharacteristic clinical symptoms often mistakenly suggest sepsis, are accompanied by more characteristic additional diagnostic work-up. Cytopenias, hypofibrinogenemia, hypertriglyceridemia, hyperferritinemia are the most common findings. MAS may be frequently under-recognized in children with KD because there are no distinct criteria for MAS complicating KD (103). Some authors recommend that Histiocyte Society criteria may be used for the diagnosis of MAS in KD (104, 105). The MAS criteria are validated for systemic juvenile idiopathic arthritis, but they are commonly used by other physicians for other systemic autoinflammatory diseases such as Kawasaki disease (106, 107). KD patients with MAS show high intravenous immunoglobulin (IVIG) resistance and coronary complications, they usually present with hepatosplenomegaly, cytopenia, liver dysfunction, hyperferritinemia, elevated serum LDH, hypofibrinogenemia, hypertriglyceridemia (103, 104).

The main goal of the therapy of MAS is to stop "cytokine storm," the treatment should be implemented as soon as possible. The antimicrobial therapy usually is necessary because of fact that each form of HLH is triggered by an infectious agent. The chemotherapy protocol (HLH-2004) including etoposide, cyclosporine, dexamethasone, and transplantation of hematopoietic stem cells is widely used in primary HLH. For patients with acquired HLH there are no recommendations and guidelines. Glucocorticosteroids, intravenous immunoglobulins and cyclosporine A are commonly used. Anti-cytokines antibodies, cyclophosphamide, vincristine, anti-thymocyte globulin, granulocyte-colony stimulating factor, plasma exchange or hemofiltration could be used in severe and refractory HLH (102, 108–110). Some authors start with HLH-2004 protocol for secondary HLH (102, 105). Inappropriate treatment such as immunosuppression monotherapy and a delay in the start of treatment may be one of the main unfavorable prognostic factors in patients with MAS. The combined immunosuppression (high-dose GCS in combination with CsA and IVIG) is usually given as the initial therapy for patients with secondary HLH (102, 108, 109, 111). The commonly used treatment in children with MAS and KD is combination therapy with GCS, IVIG, cyclosporine, IL-1 blockers (103, 104, 112). Furthermore, in MAS there is a high risk of thrombosis because of the massive activation of the coagulation cascade. In cases of highly

elevated level of D-dimers (seen especially in MAS and other hyperinflammatory conditions like pediatric inflammatory multisystem syndrome-temporally associated with SARS-CoV-2) the use of anticoagulant drugs (e.g., enoxaparin) could be required.

Appropriate treatment of MAS requires the collaboration of pediatric, infectious disease, and intensive care unit specialists with other experts such as rheumatologists, immunologists, hematologists.

Pediatric Inflammatory Multisystem Syndrome-Temporally Associated With SARS-CoV-2 (PIMS-TS)

Since late April 2020, many articles have been published describing the increasing incidence of Kawasaki-like disease after the beginning of the SARS-CoV-2 epidemic (107, 113–117). The new entity was proposed so-called Pediatric Inflammatory Multisystem Syndrome-temporally associated with SARS-CoV-2 (PIMS-TS). Multisystem inflammatory syndrome in children (MIS-C) is an alternative name proposed in the United States of America (USA) and adopted by the World Health Organization (WHO). Whether this is a particular form of KD triggered by SARS-CoV-2 or a different entity is still a matter of debate. Some of the clinical manifestations of PIMS-TS mimic KD and MAS. Children with PIMS-TS are usually older at disease onset, classic mucocutaneous symptoms are less common, gastrointestinal and respiratory symptoms are more frequently observed. Patients are at higher risk to develop myocarditis with heart insufficiency and require longer time in the hospital and ICU admittance, for the occurrence of shock, need of vasoactive agents, and invasive ventilation. Many treatment protocols recommends the use of IVIG and aspirin with/without high-dose corticosteroids as first-line therapy. Indications for the use of GCS and dosing depends on the phenotype of the disease and differs in many medical centers. Approximately 30–80% of patients do not respond to IVIG alone and may require adjunctive immunomodulatory therapy to control inflammation. This is in contrast to classic KD where IVIG resistance has been seen in <15% of patients. Anakinra is the most common anticytokine drug used in a subgroup of children with PIMS-TS in many medical institutions, given in cases of persistent severe inflammatory state despite previous treatment (113, 116, 118–121). Treatment with tocilizumab (humanized anti-IL-6 receptor antibody, inhibiting IL-6) or infliximab was also initiated in patients with PIMS-TS with a favorable outcomes. The effect of immunomodulatory therapy needs further evaluation in both observational and trial settings to determine the influence on inflammation (116, 118, 122).

PERSPECTIVES

KD and SoJIA

Systemic-onset juvenile idiopathic arthritis (so-JIA) is a systemic inflammatory disease classified as a subtype of juvenile idiopathic arthritis. It is associated with dysregulation of the innate immune system, suggesting that it belongs to the spectrum of autoinflammatory disorders. KD and so-JIA share many common clinical and laboratory features. So-JIA can be initially diagnosed as KD and vice versa (123–125). CAL can be also found in soJIA. Most children with soJIA and coronary artery dilatations are classified initially as KD and treated with multiple doses of IVIG. Although KD and so-JIA could mimic each other at the presentation, the follow-up is quite different. Non-responsiveness to standard therapy with GCS and classical disease-modifying antirheumatic drugs is not uncommon in children with so-JIA. Recently, biologic agents that specifically inhibit the cytokines interleukin (IL)-1 and IL-6 have demonstrated remarkable clinical effectiveness and confirmed the importance of these cytokines in the process of so-JIA (126). The three IL-1 blockers that have been tested so far (anakinra, canakinumab, and rilonacept) have all been proven effective and safe, although only canakinumab is currently approved for use in so-JIA (127–130). IL-18 is another proinflammatory cytokine elevated in so-JIA and may represent a pathogenic link between so-JIA and MAS (131). Based on this, some authors suggested using exogenous IL-18BP (IL-18 binding protein) as a novel therapeutic approach for inflammatory diseases (132). A recent Phase II trial of recombinant IL-18BP (tadekinig alfa) showed promising results for adult-onset Still's disease (133). Some authors found that it could be useful in resistant systemic juvenile idiopathic arthritis and recurrent macrophage activation syndrome (134). Interestingly, IL-18 is also elevated in the acute phase of KD and may be protective for those at high-risk for treatment failure (135). Above mentioned findings warrant future research on these drugs as a promising therapeutic option also in Kawasaki disease.

Potential Therapeutic Target

Many recent studies found novel immunobiological pathways involved in KD and allowed to identify potential therapeutic targets for KD, they are listed in **Table 3** (15, 37, 136–147). Literature data indicate that researchers focused especially on JAK / STAT pathway in the context of vasculitis, thus it could be regarded as a most promising potential target.

CONCLUSIONS

IVIG and ASA are now the gold standard recommendation for the treatment of Kawasaki disease according to all guidelines. However new scientific data indicate that in the future this regimen can change. Definition of high-risk patients, as well as the indication for additional treatment in these patients, varies depending on the national recommendations. Stratification of patients and optimalization of the second-line therapy is the most urgent issue in Kawasaki disease and the effect of immunomodulatory therapy needs further evaluation in carefully designed observational and trial settings to determine the effect on inflammation. There is currently a lack of evidence for choosing optimal treatment for refractory KD.

The use of glucocorticosteroids in children with KD is still controversial. Monoclonal antibodies are currently regarded as a rescue therapy, althought some data could indicate that anakinra and infliximab may be considered as an option after the failure of the first IVIG infusion. Other medicaments should only be considered in severe refractory cases because of potential adverse

TABLE 3 | Potential therapeutic target for Kawasaki disease.

Potential target	Description	References
S100A12	One of serum protein-based biomarkers of KD (S100A12 promoted *in vitro* neutrophil infiltration which is the cause of *in vivo* CAL formation	(136)
Platelet miR-223 or VSMC PDGFRβ	Uptake of platelets and platelet-derived miRNAs influences vascular smooth muscle cell phenotype *in vivo*	(137)
ANXA1	Annexin A1 (ANXA1) is an endogenous anti-inflammatory agent and pro-resolving mediator involved in inflammation-related diseases	(138)
NLRP3	NLRP3 inflammasome is a large multiprotein complex that plays a key role in IL-1β-driven sterile inflammatory diseases	(139)
ITGAM	In KD coronary artery lesions, Integrin αM (ITGAM) might enhance subacute/chronic vasculitis, resulting in the transition of smooth muscle cells to myofibroblasts and their subsequent proliferation	(140, 141)
JAK/STAT	RPN2 inhibits autophagy via STAT3 (signal transducer and activator of transcription-3) and NF-κB pathways STAT3 is activated by interleukin 6, a pro-inflammatory cytokine that is involved in early innate immune reactivity, and present in the acute phase of KD JAK1/STAT3 signaling pathway is activated in some systemic vasculitides through the activation of Th1/Th17-type cytokines such as IL-2, interferon (IFN-γ), IL-6, IL-17, and IL-23	(15, 37, 142–146)
STING	Over-activation of the STING-pathway (Stimulator of interferon (IFN) genes), could increase the risk of delayed aneurysms in KD and COVID-19 vasculitis	(147)
KCa3.1	KCa3.1 (calcium-activated potassium channel) blockade of macrophages suppresses inflammatory reaction leading to mouse coronary artery endothelial cell injury in a cell model of KD by hampering the activation of NF-κB and STAT3 signaling pathway	(37)

reactions. Results of many ongoing studies are awaited and may provide changes in the future management of KD patients.

So-JIA overlaps clinical and immunological presentation with KD and these findings could encourage to perform further studies based on previous results on so-JIA and other autoinflammatory syndromes. Many recently described immunobiological pathways could serve as a promising potential therapeutic target.

AUTHOR CONTRIBUTIONS

PB have made a substantial contribution to the concept or design of the article, or the acquisition, analysis, or interpretation of data for the article, and drafted the article. JF-G and JK revised the article critically for important intellectual content and approved the version to be published. All authors reviewed the results and approved the final version of the manuscript.

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frontiers in Medicine

REVIEW
published: 12 January 2022
doi: 10.3389/fmed.2021.814075

Novel Therapies in Takayasu Arteritis

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Takayasu Arteritis (TAK) is a large-vessel vasculitis that preferentially involves the aorta and its primary branches. Cardiac involvement is frequent in TAK and is a major determinant of the patient's outcome. Glucocorticoids (GC) are the mainstay of therapy for TAK, with high doses of GC effective to induce remission. However, relapses are common and lead to repeated and prolonged GC treatments with high risk of related adverse events. Potential GC toxicity is a major concern, especially because patients with TAK are young and need to be treated for several years, often for the whole life. Conventional immunosuppressive drugs are used in patients with severe manifestations but present some limitations. New therapeutic approaches are needed for patients with refractory disease or contraindications to conventional therapies. Fortunately, major progress has been made in understanding TAK pathogenesis, leading to the development of targeted biotherapies. In particular, IL-6 and TNF-α pathways seems to be the most promising therapeutic targets, with emerging data on Tocilizumab and TNF inhibitors. On the other hand, new insights on JAK-Inhibitors, Rituximab, Ustekinumab and Abatacept have been explored in recent studies. This review summarizes the emerging therapies used in TAK, focusing on the most recent studies on biologics and analyzing their efficacy and safety.

Keywords: Takayasu Arteritis, novel therapies, bDMARDs, biologics, heart

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *12 November 2021*
Accepted: *16 December 2021*
Published: *12 January 2022*

Citation:
Regola F, Uzzo M, Toniati P, Trezzi B, Sinico RA and Franceschini F (2022) Novel Therapies in Takayasu Arteritis. Front. Med. 8:814075. doi: 10.3389/fmed.2021.814075

INTRODUCTION

Takayasu Arteritis (TAK) is an idiopathic large-vessel vasculitis (LVV) that preferentially involves the aorta and its primary branches. It is usually considered to be most common in Asia, while in the USA and Europe is defined as a rare disease with an incidence of 1–3 per million people (1). It is most prevalent among females between the ages of 10–40 years (2). TAK is characterized by granulomatous inflammation of the aorta and large arteries wall, leading to stenosis, occlusion, dilatation and aneurysm formation. Main symptoms are consequences of vessels occlusion and reduced blood flow, like limb claudication, angina, hypertension secondary to renal arteries stenosis, lightheadedness or other neurologic symptoms due to cerebral arteries insufficiency. However, patients can also report arterial pain, like carotidynia, and non-specific constitutional symptoms, such as weight loss, low-grade fever and fatigue. In addition to these, TAK is often complicated by cardiovascular, cerebrovascular and renal morbidity.

CARDIOVASCULAR INSIGHTS IN TAKAYASU ARTERITIS

Among all kind of vasculitis, TAK is one of the diseases with the most frequent heart involvement. In TAK the whole aorta can be affected along its entire length, and all aortic branches can be involved. Thoracic and abdominal aorta are the most common affected vessels, but heart involvement has been demonstrated in up to a third of TAK patients. Cardiac manifestations can be various with coronary, valvular and myocardial involvement. They are not always clinically evident, especially in early phases, but are related to a poorer prognosis (3).

Acute myocardial infarction (MI) is rarely reported as a clinical manifestation of TAK but coronary lesions have been detected by coronary computed tomography angiography (CTA) in up to 53% of TAK patients (4). In particular, coronary stenosis is the most typical lesion found in TAK patients, and usually affects the coronary ostia and proximal vessel segments. Coronary aneurysms may also occur but are less frequent (5). Moreover, using myocardial scintigraphy and cardiac magnetic resonance imaging (MRI) has been demonstrated that 53% of TAK patients present myocardial perfusion abnormalities (6) and 27% of them present myocardial scarring (7), indicating previously unrecognized or chronic myocardial damage. However, <10% of patients became symptomatic for angina or MI (8, 9).

Besides coronary involvement, the most frequent cardiac manifestation of TAK are valvular abnormalities, found in more than 60% of patients (10). Aortic regurgitation is the most common type of valve disease and is associated with disease activity (11). Aortic insufficiency is considered to be related to aortic valves thickening or aortic root enlargement (12). Pulmonary, mitral and tricuspid regurgitations are less common and valvular stenosis is rare (13).

In patients with TAK other rarer cardiac manifestations have been reported, like myocarditis, pericarditis and pulmonary hypertension. More specifically, myocardial involvement has been reported in 6% of patients, usually presenting with mild insidious symptoms at onset, but leading to a later heart failure with poor prognosis (13). On the other hand, only few cases of pericarditis associated with TAK has been reported, usually as an initial manifestation of the disease (14) and only in a minority of patients a mild pulmonary hypertension has been observed (15).

Finally, the association between adverse cardiovascular events and glucocorticoids (GC) is a major concern that needs to be considered in the management of TAK patients. In fact, GC treatment contributes to the exacerbation of cardiovascular risk factors. GC administration increases blood pressure in a dose dependent fashion, mediated by both an increased peripheral vascular resistance and by a direct effect on mineralocorticoid receptor. GC treatment also increases the risk of glucose intolerance and diabetes, dyslipidaemia and central obesity. For these reasons, EULAR recommendations suggest screening all patients with TAK for treatment-related and cardiovascular comorbidities and recommend prophylaxis and life-style advice to reduce cardiovascular risk and treatment-related complications (16).

TAKAYASU ARTERITIS: CONVENTIONAL DMARDS

The mainstay of therapy for the induction of remission in TAK are systemic glucocorticoids (GC), with a commonly used initial prednisone dosage of 0.5–1 mg/Kg/day. EULAR recommendations published in 2018 suggest an initial dose of 40–60 mg/day for the majority of patients and, to date, there is no evidence that a higher starting dose improves the outcome (16). A high initial dose of GC is recommended also by the very recent 2021 ACR guidelines, due to the potential organ damage and life-threatening events associated with TAK onset. However, ACR guidelines allows to consider lower doses for patients with newly active, non-severe disease (e.g., patients with constitutional symptoms and without limb ischemia) (17).

However, although most patients initially achieve disease remission, relapses or disease progression are seen in more than half of patients during GC tapering (18). In addition, chronic GC therapy is associated with adverse effects, such as diabetes, hypertension, early cardiovascular disease, infections, osteoporosis and growth restriction in children.

Given the high frequency of GC adverse effects and the high rate of relapse during tapering, the upfront use of immunosuppressives in addition to GC seems to be the most preferable management strategy in TAK patients. Based on these considerations, EULAR recommendations and ACR guidelines advise an initial treatment with high-dose GC in combination with a GC-sparing agent in all TAK patients rather than GC alone (16, 17).

However, the choice of the immunosuppressive drug remains a challenge for several reasons. First of all, most of the evidence on their efficacy comes from observational studies with limited number of patients, especially for conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs). Cohort studies have showed that methotrexate (45), azathioprine (46), mycophenolate mofetil (47), leflunomide (48) and cyclophosphamide (49) could improve clinical and radiological manifestations of TAK and have a GC-sparing effect. However, there are no randomized trials comparing the efficacy of different csDMARDs, and two recent meta-analysis on csDMARDs in TAK indicated similar efficacy rates between these drugs (25, 50). Therefore, clinical practice typically reflects the results of these low level of evidence data and expert opinion.

Secondly, TAK patients can be very different, and several individual factors need to be considered in order to choose the better treatment, including disease manifestations and severity, age, sex, comorbidities, contraindications, pregnancy plan, and also cost and availability of specific agents.

EULAR recommendations in 2018 suggested choosing as first line agent a csDMARD among methotrexate, azathioprine, mycophenolate mofetil or leflunomide. Switching from one csDMARD to another is considered a feasible option when a patient does not tolerate the first choice. On the other hand, cyclophosphamide is suggested to be used only if other treatments have failed or have not been tolerated, because of its high risk of long-term adverse events and infertility (16).

However, new therapeutic approaches are needed for patients with refractory disease or contraindications to conventional

TABLE 1 | New targeted therapies in Takayasu Arteritis.

Drug	Pathogenetic basis	Evidence in TAK	Recommendations and clinical use
TNF-α inhibitors (infliximab, etanercept, adalimumab, golimumab, certolizumab pegol)	**Inhibitors of TNF-α (bDMARDs):** • TNF-α has a major role in the development of TAK granulomatous inflammation • In active TAK higher serum levels of TNF-α and higher mRNA expression and intracellular production by T cells	• Cohort studies and open-label prospective study, showing positive results in TAK patients (clinical improvement, GC sparing effect, higher sustained remission rate compared to cDMARDs) (19–24) • No RCTs • Meta-analysis with 19 observational studies, showing more than 80% of treated patients attaining at least partial clinical response (25) • Good safety profile in cohort studies	• **2018 EULAR recommendations:** TNF-α inhibitors as second line treatment in TAK patients resistant to csDMARD • **2021 ACR guidelines:** TNF-α inhibitors as first line treatment, like methotrexate and azathioprine
Tocilizumab	**Anti-IL-6r (bDMARD)** • IL-6 is a pro-inflammatory cytokine • Higher IL-6 levels in TAK patients compared to HC and in TAK patients with active disease compared to patients with low disease activity	• Cohort studies, showing positive results in TAK patients (clinical improvement, GC sparing effect, higher sustained remission rate compared to cDMARDs) (22, 26–30) • Meta-analysis with 22 observational studies, showing more than 87% of treated patients attaining at least partial clinical response (25) • One RCT (TAKT study): relapse-free survival tended to be improved in treated patients, but no statistical significance. Longer-term open-label extension showed GC sparing effect, lower radiological disease progression, better PROs (31, 32) • Good safety profile in cohort studies and RCT	• **2018 EULAR recommendations:** tocilizumab as second line treatment in TAK patients resistant to csDMARD • **2021 ACR guidelines:** tocilizumab as second line treatment in patients with inadequate response to other immunosuppressive therapies
JAK-Inhibitors (tofacitinib, upadacitinib)	**Inhibitors of JAK-STAT signaling pathway (tsDMARDs)** • block signaling of cytokine implicated in TAK pathogenesis (type 1 and 2 interferons, IL-6, IL-12, IL-17 and IL-23) • suppress tissue-resident memory T cells and reduce inflammatory-related vascular damage	**Tofacitinib:** • Case reports and one prospective observational study, showing positive results in TAK patients (clinical improvement, lower radiological disease progression, superior to methotrexate) (33–37) • Good safety profile • One ongoing RCT (NCT04299971) **Upadacitinib:** • One ongoing RCT (NCT04161898) **No data on other JAK-Inhibitors**	• Only case reports • Not included in 2018 EULAR recommendations or 2021 ACR guidelines
Rituximab	**Anti-CD20 (bDMARD)** • In TAK patients B-cells infiltrates in the inflamed arteries adventitia and high levels of activated B-cell subsets in the peripheral blood • Rituximab blocks B cell differentiation and B-T cell stimulation	• Isolated case reports on rituximab in TAK with contradictory results (38–42) • No RCTs, no meta-analysis, no ongoing trial	• Very limited evidence with contradictory results • Only case reports • Not included in 2018 EULAR recommendations or 2021 ACR guidelines
Abatacept	**Soluble fusion protein CTLA4-Ig (bDMARD)** • In TAK patients B-cells infiltrates in the inflamed arteries adventitia and high levels of activated B-cell subsets in the peripheral blood • Abatacept blocks B-T cell co-stimulation	• One RCT with 34 TAK patients: abatacept not associated with a longer median duration of remission compared to placebo (43)	• **2021 ACR guidelines**: Abatacept is not recommended in TAK

(Continued)

TABLE 1 | Continued

Drug	Pathogenetic basis	Evidence in TAK	Recommendations and clinical use
Ustekinumab	**Anti-p40. IL-12 and IL-23 inhibitor (bDMARD)** • Th17 and Th1 pathways contribute to TAK pathogenesis • Ustekinumab target p40, a common subunit of IL-12 and IL-23 (main cytokines involved in Th17 and Th1 pathways)	• A small prospective observational study (improvement in clinical symptoms but no changes in intramural enhancement on MRA) (44) • One ongoing RCT (NCT04882072)	• Very limited evidence • Not included in 2018 EULAR recommendations or 2021 ACR guidelines

TNF, Tumor necrosis factor; GC, glucocorticoids; cDMARDs, conventional disease modifying agents; bDMARDs, biological disease modifying agents; tsDMARDs, targeted synthetic disease modifying agents; IL, interleukin; IL-6r, interleukin-6 receptor; HC, healthy controls; MRA, magnetic resonance angiography; RCT, randomized controlled trial.

therapies. Fortunately, major progress has been made in understanding the pathogenesis of TAK, leading to the development of targeted biological disease modifying agents (bDMARDs). Tumor Necrosis Factor-α (TNF-α) and Interleukin 6 (IL-6) seem to be the most promising therapeutic targets, but other pathways have been studied, and will be discussed in the next sections (**Table 1**).

TAKAYASU ARTERITIS: NEW TARGETED THERAPIES

TNF-α Inhibitors (TNFi)

Tumor necrosis factor α (TNF-α) plays a major role in the development of granulomatous inflammation that is typical of TAK. Moreover, TAK patients with an active disease showed higher serum levels of TNF-α and higher mRNA expression and intracellular production by T cells if compared to inactive TAK patients (51, 52).

Currently, five TNF-α inhibitors (TNFi) are approved for rheumatic diseases by FDA and EMA: infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol. Adalimumab and golimumab are fully human IgG1 antibodies, infliximab is a chimeric IgG1 antibody, etanercept is a fusion protein comprised of a human IgG1 Fc portion and the p75 TNF receptor, and certolizumab pegol is a PEGylated Fab fragment of a humanized anti-TNF antibody.

Several cohort studies on the successful use of different TNFi have been reported in patients with TAK but to date no RCTs have been published. A French multicenter open-label prospective study published in 2020, described the benefit-risk ratio of infliximab in TAK patients with refractory disease to conventional therapy. Between 2014 and 2017, 23 patients were treated with infliximab and a clinical improvement was observed in 64% of patients after a median treatment duration of 36.9 months. The median GC dose was reduced by 50% and no safety concerns were raised by the study, with only few reported adverse event during the 3 years of follow-up (19).

A recent meta-analysis by Misra et al. (25) analyzed 19 observational studies on TNFi in TAK, showing that more than 80% of treated patients attained at least partial clinical response, angiographic stabilization, improvement in PET-CT and normalization of inflammatory markers. Relapse rate was estimated as 32% but with considerable heterogeneity across studies. TNFi showed also a GC-sparing effect.

Similar results were reported in a previous review published in 2014 and including 120 TAK patients with active disease and treated with anti TNFi in 20 observational studies: 109 patients received infliximab, 17 etanercept and 9 adalimumab. Remission was achieved in 70–90% of cases after TNFi treatment and 40% of patients stopped glucocorticoids (20).

More specifically, a population-based cohort study from Norway included 78 TAK patients, comparing patients treated with TNFi and with csDMARDs. Patients treated with TNFi had a higher sustained remission rate and a lower risk of new lesion development if compared to patients on csDMARDs (42 vs. 20% and 10 vs. 40%, respectively) (21). Similar results were reported in 2015 by Mekinian et al. (22) on behalf of the French Takayasu Network.

All together, these data support the use of TNFi in TAK. Notably, the great majority of these patients received infliximab (19), while the experience with etanercept, adalimumab and golimumab is more limited (22, 23). Only one case series on certolizumab pegol has been published. In this report 10 female patients with TAK were treated with certolizumab pegol, achieving remission in all cases. Interesting, due to its safety during pregnancy, certolizumab pegol could present a specific advantage in TAK patients who are frequently female and young (24).

Tocilizumab

IL-6 is a pro-inflammatory cytokine, responsible for stimulating acute phase protein synthesis and for neutrophils production and B- and T- cells activation. Several studies had suggested that IL-6 plays a crucial role in TAK pathogenesis (53, 54). Higher levels of IL-6 have been demonstrated in TAK patients compared to healthy controls and in TAK patients with active disease compared to patients with low disease activity (51). Tocilizumab is a humanized monoclonal antibody blocker of IL-6 signaling and has been approved for the treatment of Giant Cells Arteritis. The clinical efficacy of tocilizumab in TAK was reported for the first time in 2008 (26), followed by several cohort studies published in the subsequent years (22, 27, 28).

In 2018 the French Takayasu network published a retrospective multicenter study on 46 TAK patients treated with tocilizumab. Under tocilizumab treatment, a significant

decrease in the median NIH scale and in the daily prednisone dose was observed. Moreover, the event-free survival was significantly better in patients treated with tocilizumab compared to cDMARDs (29).

The above-mentioned meta-analysis performed by Misra et al. (25) included 22 observational studies on tocilizumab in TAK patients. Pooling data from these studies, authors described tocilizumab effective in attaining at least a partial clinical response in 87% patients although the results were heterogenous among studies. Tocilizumab demonstrated also to induce angiographic stabilization, PET-CT improvement and median prednisolone dose reduction. In a previous review 70 cases of TAK patients with relapsing or refractory disease and treated with tocilizumab were reported. Overall, 80% of patients showed a clinical and laboratory improvement after 3 months of therapy and <20% of patients had a relapse during the treatment period (30).

Besides observational studies, a phase 3 RCT was published in 2018: the TAKT study. Thirty-six patients with relapsing TAK were randomized to receive tocilizumab 162 mg subcutaneous weekly or placebo. The primary endpoint was the time to occurrence of the first relapse, as defined by Kerr's criteria, but it was not met. In fact, after 1 year of follow-up, relapse-free survival tended to be improved in patients treated with tocilizumab, but the results did not reach statistical significance [HR 0.41 (95.41% CI 0.15 to 1.10; $p = 0.0596$)] (31). However, this study was felt to be underpowered (36 participants). Recently, the longer-term open-label extension of this trial was published, with patients in both arms treated with tocilizumab until 96 weeks. Endpoints of the extension analysis were steroid-sparing effects of tocilizumab, radiological disease progression, patient-reported outcomes and safety. The median glucocorticoid dose was significantly reduced from baseline to week 96, with 46.4% of patients reducing their prednisolone dose below 0.1 mg/kg/day. Most patients presented an improvement (17.9%) or a stabilization (67.9%) on imaging evaluations after 96 weeks compared to baseline, with only 4 patients showing a progression of vascular involvement. Mean SF-36 mental component summary scores improved rapidly by week 12 and 24 of tocilizumab treatment and improvement was maintained till week 96. The most frequently recorded adverse effects in the trial were infections but the long-term safety of tocilizumab in patients with TAK was consistent with the known safety profile of this drug in Rheumatoid Arthritis (32).

JAK-Inhibitors

JAK-Inhibitors are a more recent family of drugs, classified as targeted synthetic DMARDs (tsDMARDs). They inhibit the activity of one or more Janus kinase enzymes (JAK1, JAK2, JAK3, TYK2), interfering with the JAK-STAT signaling pathway and thereby blocking cytokine signaling. In particular, JAK inhibition suppresses the production of type 1 and 2 interferons and many cytokines including IL-6, IL-12, IL-17, and IL-23, which are implicated in TAK pathogenesis (55, 56). It has also been demonstrated that JAK1 and JAK3 signaling is important in chronic inflammation of large arteries and that JAK inhibition can suppress tissue-resident memory T cells and reduce inflammatory-related vascular damage (57).

Tofacitinib (TOF) is a JAK3 and JAK1 inhibitor and it has been studied in TAK patients in the last 2 years (33–35). A recent observational study on 5 patients reported its efficacy in TAK, with 4 patients out of 5 achieving clinical response. Moreover, three of these patients presented an improvement and a stabilizations of artery stenosis and mural thickness in vascular Doppler (36). Recently, a study comparing TOF and methotrexate in TAK patients has been published in China. TOF demonstrated to be superior to methotrexate for complete remission with a tendency to prevent relapse and tapering GC. A good safety profile for TOF was also documented in these patients (37).

More information on tofacitinib efficacy in TAK patients will be provided by an ongoing trail (ClinicalTrials.gov Identifier NCT04299971). At the same time, another JAK1 inhibitor is subject of a clinical trial: upadacitinib. In fact, a phase-3, multicenter, placebo-controlled study (SELECT-Takayasu) is now recruiting (ClinicalTrials.gov Identifier NCT04161898).

Rituximab

There is increasing evidence about the possible role of B-cells in the pathogenesis of TAK. B-cells infiltrates in the inflamed adventitia of affected arteries and high levels of activated B-cell subsets, particularly plasmablasts, in the peripheral blood of TAK patients have been described (39, 58). These findings suggest a potential role for B-cell depleting therapy in TAK. Rituximab is a chimeric anti-CD20 monoclonal antibody that induces a depletion of B-cells.

Isolated case reports on the use of rituximab reported favorable outcomes, but are limited by reporting bias (38–40). To date, only two retrospective case series of TAK patients treated with rituximab have been reported. *Pazzola et al.* described seven patients with refractory disease treated with rituximab. Despite treatment, four patients had evidence of persistent disease activity and/or radiographic disease progression during follow-up. Only three out of seven patients achieved complete remission (41). On the contrary, *Nakagomi et al.* described eight TAK patients treated with rituximab, with all but one with a clinical response after treatment (42).

In conclusion, data on the efficacy of rituximab in TAK are very heterogenous. Further studies would be necessary to understand rituximab role in TAK treatment.

Abatacept

As above mentioned, in TAK pathogenesis a possible role of B-cells has been theorized. At the same time, B-cells activation need costimulatory signals by activated T lymphocytes, macrophages, and dendritic cells. Abatacept is a soluble fusion protein comprising CTLA-4 and the Fc portion of immunoglobulin G1 (CTLA4-Ig). This drug prevents CD80/CD86 from binding to CD28 on the surface of the T-cells, resulting in failure of the costimulatory signal required for T-cell activation.

A randomized controlled trial enrolling 34 TAK patients has been conducted to test the efficacy of abatacept to prevent disease relapse (43). The primary end point of the study was not met,

with abatacept not associated with a longer median duration of remission compared to placebo (5.5 vs. 5.7 months, p: ns). Moreover, the relapse-free survival rate at 12 months was 22% for patients receiving abatacept and 40% for those receiving placebo. To date, this study does not support the use of abatacept in TAK.

Ustekinumab

The Th17 and Th1 pathways contribute to the systemic and vascular manifestations of TAK (53). IL-12 and IL-23 are two key cytokines involved in Th1 and Th17 polarizations, respectively, and IL-12B gene region has been identified as a susceptibility gene for TAK (59). These findings suggest that IL-12 and IL-23 are implicated in the pathogenesis of TAK. These two cytokines share a common subunit (p 40), which is target by ustekinumab, a humanized anti-p 40 monoclonal antibody.

In a small prospective observational study, three patients with active TAK were treated with ustekinumab in association with csDMARDs and glucocorticoids. After ustekinumab, all three patients presented an improvement in clinical symptoms and a decrease in inflammation markers, but no changes in intramural enhancement on magnetic resonance angiography (MRA) were achieved (44).

These results are very interesting but still preliminary. Further information on the efficacy of ustekinumab in TAK will be provided by a proposed phase-3, multicenter, placebo-controlled study (ClinicalTrials.gov Identifier NCT04882072).

SURGICAL MANAGEMENT

Thanks to all these new therapeutic opportunities, most patients achieve remission and do not develop irreversible vascular damage. However, it is possible that the diagnosis occurs at a stage when stenotic or occlusive lesions have already occurred. Such lesions might be not reversible by medical treatment and, if they are hemodynamically significant, may require revascularization. Most common examples are represented by cerebrovascular disease due to carotid or vertebral stenosis, coronary artery disease, severe coarctation of the aorta, renovascular hypertension or limb claudication. Such interventions need to be considered when vascular lesions are symptomatic and only if refractory to medical management, which represents the first-choice treatment (17). Not only stenotic, but also aneurismatic complication can occur in TAK patients, and surgical management can be necessary in patients with progressive aneurysm enlargement with high risk of rupture or dissection (16).

EULAR, ACR, ESC (European Society of Cardiology) and ESVS (European Society for Vascular Surgery) guidelines recommend performing elective endovascular interventions or reconstructive surgery during stable remission (16, 17, 60, 61). Surgical interventions in patients with active disease are associated with an increased risk of complications and with higher risk of requiring revision for relapse or progression of symptomatic disease (62, 63).

The method of choice for vascular interventions in patients with TAK depends on the anatomic location of the vascular damage, timing, disease activity and other factors, and should a collaborative decision between vascular surgeons and rheumatologists (16, 17). With recent advances in endovascular treatment, the use of percutaneous endoluminal angioplasty has progressively increased in TAK patients. Endovascular management is considered a feasible option especially for stenotic lesions, like in supra-aortic, iliac, and renal arteries stenosis (64–66). On the other hand, for inflammatory thoracic aortic aneurysms, open surgery with resection and replacement of the inflammatory aorta still represents the first-line standard treatment. However, successful outcomes after thoracic endovascular aortic repair (TEVAR) have been recently reported, showing that in the future TEVAR could represent a less invasive alternative in selected patients (67).

CONCLUSIONS

TAK is a chronic disease, typically affecting young patients and associated with potential organ damage and life-threatening events. It requires a prompt and aggressive treatment with immunosuppressants to avoid irreversible complications. GC have been considered the mainstay in the treatment of TAK but they are characterized by high incidence of side effects and relapse during tapering. Alternative therapies with cDMARDs showed partial efficacy, with half of the patients experiencing relapses.

As discussed above, new therapeutic approaches with bDMARDs and tsDMARDs have showed promising results, with high efficacy and acceptable safety profile.

In 2018, based on these new insights, EULAR recommendations advised the use of bDMARDs in TAK. In particular, TNFi and Tocilizumab were suggested to be used as second line agents in patients with relapsing or refractory disease despite treatment with csDMARDs or in patients with contraindications to csDMARDs (16, 68).

The most recent ACR guidelines, published in 2021, suggest a similar but different approach. Also in this case, non-glucocorticoid immunosuppressive agents plus GC are recommended over GC monotherapy in all patients with TAK to minimize GC-related toxicity. However, ACR guidelines specifically referred to methotrexate, azathioprine and TNFi as first line therapies. Notably, among bDMARDs the panel specified favoring TNFi use over tocilizumab, even if the latest is suggested to be considered, especially when TNFi are contraindicated (17).

In conclusion, biological therapies can provide additional benefits to TAK patients, and they are gradually becoming part of the clinical practice. Nevertheless, there is still a need for high-quality studies, especially RCTs, to guide the management of TAK. Hopefully, the results of the above-mentioned ongoing trials will help to better treat this challenging disease in the future.

AUTHOR CONTRIBUTIONS

FF and RS conceived the study. FR and MU conducted a review of the literature and drafted the manuscript. FR, MU, BT, PT, FF, and RS reviewed and edited the manuscript and supported the study. All authors checked the final version of the manuscript.

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Conflict of Interest: RS has received consulting fees from GSK, Roche and Otzuka.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
published: 07 March 2022
doi: 10.3389/fmed.2022.838564

Immunomodulating Therapies in Acute Myocarditis and Recurrent/Acute Pericarditis

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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *17 December 2021*
Accepted: *28 January 2022*
Published: *07 March 2022*

Citation:
Ammirati E, Bizzi E, Veronese G, Groh M, Van de Heyning CM, Lehtonen J, Pineton de Chambrun M, Cereda A, Picchi C, Trotta L, Moslehi JJ and Brucato A (2022) Immunomodulating Therapies in Acute Myocarditis and Recurrent/Acute Pericarditis. Front. Med. 9:838564. doi: 10.3389/fmed.2022.838564

The field of inflammatory disease of the heart or "cardio-immunology" is rapidly evolving due to the wider use of non-invasive diagnostic tools able to detect and monitor myocardial inflammation. In acute myocarditis, recent data on the use of immunomodulating therapies have been reported both in the setting of systemic autoimmune disorders and in the setting of isolated forms, especially in patients with specific histology (e.g., eosinophilic myocarditis) or with an arrhythmicburden. A role for immunosuppressive therapies has been also shown in severe cases of coronavirus disease 2019 (COVID-19), a condition that can be associated with cardiac injury and acute myocarditis. Furthermore, ongoing clinical trials are assessing the role of high dosage methylprednisolone in the context of acute myocarditis complicated by heart failure or fulminant presentation or the role of anakinra to treat patients with acute myocarditis excluding patients with hemodynamically unstable conditions. In addition, the explosion of immune-mediated therapies in oncology has introduced new pathophysiological entities, such as immune-checkpoint inhibitor-associated myocarditis and new basic research models to understand the interaction between the cardiac and immune systems. Here we provide a broad overview of evolving areas in cardio-immunology. We summarize the use of new imaging tools in combination with endomyocardial biopsy and laboratory parameters such as high sensitivity troponin to monitor the response to immunomodulating therapies based on recent evidence and clinical experience. Concerning pericarditis, the normal composition of pericardial fluid has been recently elucidated, allowing to assess the actual presence of inflammation; indeed, normal pericardial fluid is rich in nucleated cells, protein, albumin, LDH, at levels consistent with inflammatory exudates in other

biological fluids. Importantly, recent findings showed how innate immunity plays a pivotal role in the pathogenesis of recurrent pericarditis with raised C-reactive protein, with inflammasome and IL-1 overproduction as drivers for systemic inflammatory response. In the era of tailored medicine, anti-IL-1 agents such as anakinra and rilonacept have been demonstrated highly effective in patients with recurrent pericarditis associated with an inflammatory phenotype.

Keywords: acute myocarditis, pericarditis, immunosuppressive therapy, eosinophilic myocarditis, COVID-19, cardiac sarcoidosis, corticosteroids, anti-IL-1 therapy

INTRODUCTION

The field of inflammatory disease of the heart or "cardio-Immunology" is rapidly evolving thanks to the wider use of non-invasive diagnostic tools able to detect and monitor myocardial inflammation, such as cardiac magnetic resonance imaging (CMRI) and fluorodeoxyglucose positron emission tomography (FDG-PET) (1). In acute myocarditis (AM), recent data on the use of immunomodulating therapies have been reported both in the setting of systemic autoimmune disorders and in the setting of isolated forms, especially in patients with specific histology (i.e., eosinophilic myocarditis, giant cell myocarditis [GCM] or cardiac sarcoidosis [CS]) or characterized by an arrhythmic burden (2). We elucidate the rationale to test the use of immunomodulating therapies in patients with lymphocytic AM. In addition, AM has also emerged as a complication in the setting of coronavirus disease 2019 (COVID-19), mRNA vaccine (3–7), and immune checkpoint inhibitors (ICI) (8–10). Here, we summarize the clinical approach toward the use of immunosuppressive therapies in these specific settings. Finally, we propose the use of new imaging tools in combination with endomyocardial biopsy (EMB) and laboratory parameters such as high sensitivity troponin to monitor the response to immunomodulating therapies based on recent evidence and clinical experience.

In the second section of this review, we examine the rationale and the evidence of immunosuppression in pericarditis. We highlight recent findings defining a pivotal role for innate immunity in the pathogenesis of recurrent pericarditis with raised C-reactive protein (CRP), focusing on the emerging role of anti-IL-1 agents (i.e., anakinra and rilonacept) for this subset of patients with recurrent pericarditis.

LYMPHOCYTIC MYOCARDITIS

Lymphocytic AM is the most common histologic subset reported in AM cohorts (11). Due to the fact that in the setting of suspected AM, histologic diagnosis is more often recommended in specific scenarios (e.g., acute heart failure [HF], presence of ventricular arrhythmias (VA) or II/III-degree atrio-ventricular block [AVB]) (1, 12), the prevalence of lymphocytic AM is frequently estimated on cohorts of complicated AM. From a recent international retrospective case collection of AM presenting with left ventricular (LV) systolic dysfunction, the prevalence of lymphocytic AM has been estimated to be ∼72%, being the most frequently diagnosed form both in fulminant myocarditis [FM], a clinical entity defined by the need of circulatory support, and non-FM (11). The etiology of lymphocytic AM is broad and includes heterogeneous pathogens, drugs or autoimmune-mediated injury in the setting of systemic inflammatory diseases (10, 13, 14). The role of viruses in myocarditis etiology has been historically recognized, with Parvovirus (PV)B-19, adenoviruses, Human Herpesvirus (HHV)-6, enteroviruses being the most common agents identified in the myocardium of patients with AM (15, 16). Whether viruses have a direct or indirect causal relationship in clinical myocarditis etiology has been a matter of great debate throughout the years with expert opinions varying according to the evidence of the moment (17). The controversy matters as it has been stated that the presence of specific viruses in the heart may be a contraindication to the use of immunosuppression (18). A growing body of literature indicates that viruses, particularly PVB-19 and HHV6, may be found in a large proportion of patients who do not have myocarditis, questioning their direct causal role in the pathogenesis of myocarditis (19, 20). Of note, PVB-19 was the only virus identified in patients with lymphocytic FM in an international registry (21). Except for enteroviruses (22, 23), such as coxsackievirus, whose ability to cause direct myocardial damage has been demonstrated and seems more common in newborns/infants (24), most of the available evidence suggests that virus-triggered immune-mediated reactions are the principal cause of cardiomyocyte injury (1). Respiratory viruses, such as influenza and coronaviruses, are examples of common viruses that can trigger immune-mediated lymphocytic myocarditis with no evidence of viral genome in the myocardium (25, 26). Molecular mimicry between viral and cardiac antigens is suspected to be a key mechanism of myocardial injury in virus-triggered AM (27, 28). Furthermore, the concept that FM may resemble the presentation of a high-grade cellular rejection observed after heart transplantation (HTx) is recently emerging. These findings may suggest that the identification of viruses in the setting of AM may not represent an absolute contraindication to immunosuppression (29). At present, the role of a routine viral genome search on EMB in guiding patient management and immunosuppression therapy in patients with AM remains unknown (17). This concept holds true especially in FM where early immunosuppression may be crucial to damper the inflammatory process sustaining AM. However, most studies focusing on immunomodulation have included patients with chronic inflammatory cardiomyopathy with HF

symptoms for more than 6 months rather than those with a fulminant or complicated course (30–32). Though not supported by evidence from randomized clinical trials, recommendations for immunosuppression exist in the setting of complicated AM based on case series, expert opinions, and pathophysiological considerations (1) (**Figure 1**). The American Heart Association (AHA) suggests that, if a high suspicion for immune-mediated FM exists, pulse steroid therapy (i.e., 1 g of methylprednisolone) should be administered urgently, before biopsy-confirmed diagnosis or further diagnostic testing (33). Intravenous (IV) immunoglobulin (IG) (at a dose ranging from 0.5 g to 1 g/kg) is frequently used in pediatric lymphocytic myocarditis with evidence of some benefits in terms of functional recovery and survival, but the experience in adults has been limited (34, 35). Even though not standardized, maintenance therapy with low dose steroids often in combination with mycophenolate mofetil, cyclosporine, azathioprine (AZA) as steroid-sparing drugs may be used in those patients showing poor functional recovery associated with persistence of troponin release or any evidence of residual myocardial inflammation (30, 36). Standardized Corticosteroid therapy (IV methylprednisolone 200–400 mg or dexamethasone 20–40 mg) qd for 3–5 days and then gradually down titrated and weaned in 7–10 days, and IVIG 10–20 g qd for 3–5 days followed by 10 g for another 3–5 days has been described from a Chinese registry of 138 FM and has been associated with improved survival (37). According to several researchers, even though robust evidence is substantially lacking in the setting of AM, high viral loads may contraindicate the use of immunosuppression in favor of treatment with antiviral drugs or with agents boosting the native immune response (e.g., interferon-β) (38). Lymphocytic AM can also be associated with systemic autoimmune or inflammatory disorders (e.g., systemic lupus erythematosus [SLE], inflammatory bowel disorders, COVID-19) (39). The Lombardy registry of AM reported that 7.2% of patients had associated autoimmune or systemic disorders, being more frequent in patients presenting with complicated AM (40). The identification of the myocarditis-associated condition is essential to initiate disease-specific treatments. IV corticosteroids have been successfully used in cases of SARS-CoV-2 related FM, suggesting the relevance of the systemic inflammatory response in determining cardiac injury in COVID-19, even though more evidence is needed (41, 42).

Ongoing Trials

Anakinra is the recombinant form of the naturally occurring interleukin 1α (IL-1Rα) and blocks the activity of both IL-1α and IL-1β. The Anakinra vs. Placebo for the Treatment of Acute Myocarditis (ARAMIS) trial (ClinicalTrials.gov identifier: NCT03018834) is a double-blind randomized clinical trial testing the superiority of anakinra in addition to standard of care, defined as the maximum tolerated dosage of any beta-blockers and angiotensin receptor blockade in acute myocarditis. The ARAMIS trial has completed the randomization phase and will directly assess the role of the IL-1 immune innate pathway in the setting of AM. The rationale of blocking the (IL-1β) pathway in myocarditis relies on prior studies that suggested the central role of the Nucleotide-binding domain (NACHT) and Leucine-rich repeat (LRR) and Pyrin domain (PYD) (NLR) containing protein 3 (NLRP3) inflammasome predominately expressed in macrophages (43–45). Despite anecdotal evidence, ARAMIS will directly test this concept and the results are expected by the end of 2022 (46, 47). This double-blinded French study has assessed 120 patients with symptomatic AM defined by elevated cardiac troponin (at least 1.5-fold upper the normal reference limit) and CMRI consistent with myocarditis performed within 72 h after admission (**Figure 2**). Patients in the treatment arm received a daily subcutaneous dose of anakinra 100 mg during the hospitalization including an angiotensin-converting-enzyme inhibitor (ACE-i) and a beta-blocker. The primary endpoint of this study is the number of days alive free of any myocarditis complications including (1) VA, (2) HF, (3) recurrent chest pain requiring medication, (4) left ventricular ejection fraction (LVEF) <50%, up to 28 days after randomization. This trial has also a sub-study that has assessed ACE-i continuation or discontinuation after 1 month in patients with normal LVEF that are followed for 1 year. This trial excluded the patients with the poorest outcome, specifically those on mechanical ventilation or temporary mechanical circulatory supports (t-MCS). To address specifically patients with FM or acute HF the MYocarditis THerapy with Steroids (MYTHS) trial (ClinicalTrials.gov identifier: NCT05150704) will randomize 288 patients with FM (need for inotropes and/or t-MCS) or AM complicated by HF and severely impaired LVEF (<41%) to pulsed corticosteroid therapy (methylprednisolone 1 g IV qd for 3 days) on top of standard therapy and maximal supportive care vs. placebo (**Figure 2**). The combined primary endpoint is defined as the time from randomization to the first event occurring within 6 months including (1) all-cause death, or (2) HTx, or (3) long-term left-ventricular assistance device (LVAD) implant, or (4) need for an upgrading of the t-MCS, or (5) a ventricular tachycardia (VT)/fibrillation (VF) treated with direct current (DC) shock (excluding VT/VF in patients on t-MCS other than intra-aortic balloon pump [IABP]), or (6) first rehospitalization due to HF or VA, or advanced AVB. The trial started the enrollment in October 2021 and the estimated duration is ∼3–4 years. The rationale for the MYTHS trial is based on clinical practice. Indeed, several case series and case reports support the effectiveness of high dosage corticosteroids (48–50).

SPECIFIC SUBSET OF MYOCARDITIS

Myocarditis in Systemic Lupus Erythematosus and Antiphospholipid Antibody Syndrome

SLE is a rare disease (prevalence 48–350 per 100,000 individuals) in which the immune system attacks healthy cells and tissues. Immune system activation is characterized by exaggerated B/T cell responses and loss of tolerance against self-antigens. Production and defective elimination of antibodies, tissue deposition of immune complexes, and complement and cytokine activation contribute to clinical manifestations ranging from joint and skin inflammation to life-threatening organ damage

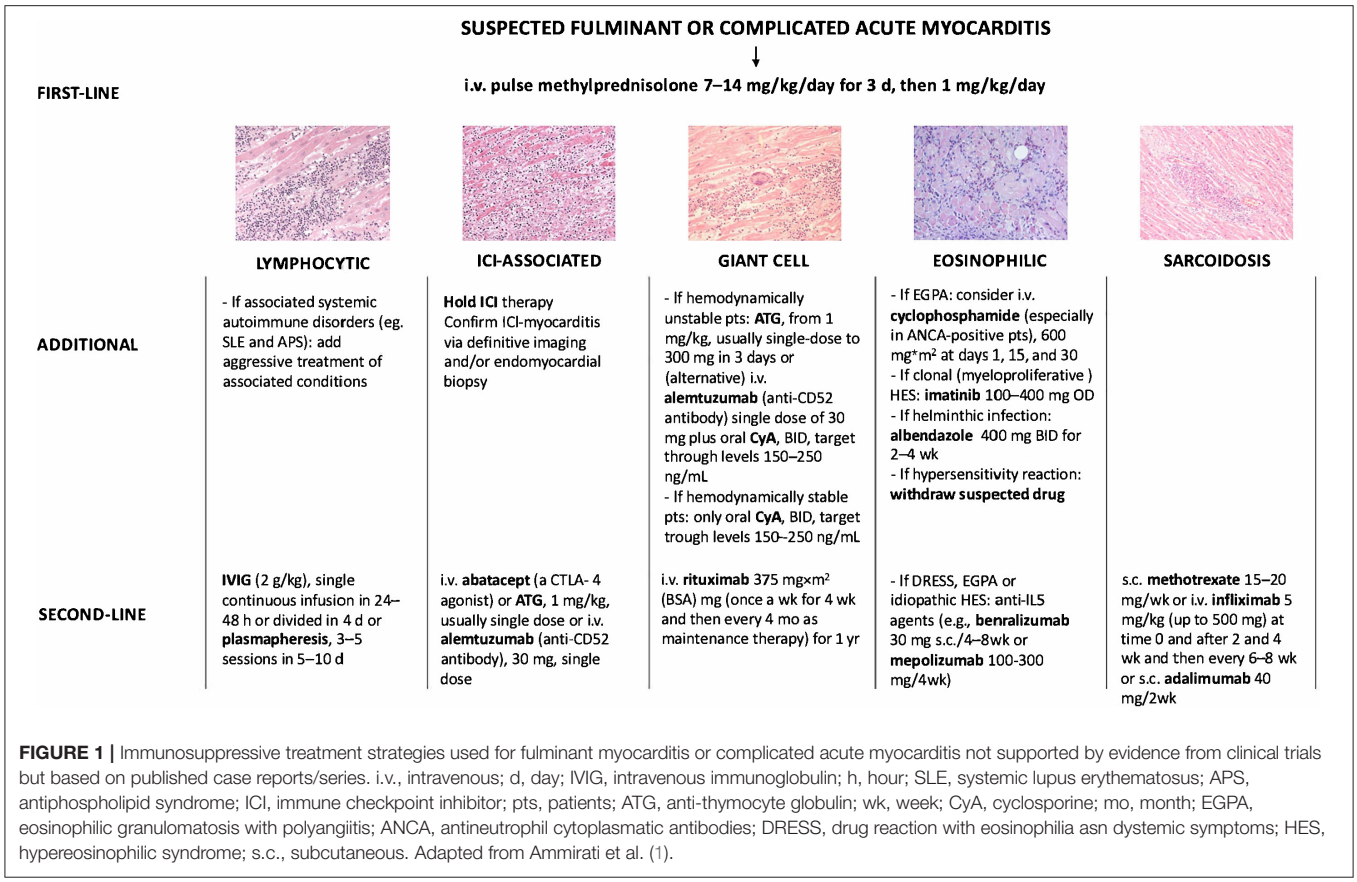

FIGURE 1 | Immunosuppressive treatment strategies used for fulminant myocarditis or complicated acute myocarditis not supported by evidence from clinical trials but based on published case reports/series. i.v., intravenous; d, day; IVIG, intravenous immunoglobulin; h, hour; SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome; ICI, immune checkpoint inhibitor; pts, patients; ATG, anti-thymocyte globulin; wk, week; CyA, cyclosporine; mo, month; EGPA, eosinophilic granulomatosis with polyangiitis; ANCA, antineutrophil cytoplasmatic antibodies; DRESS, drug reaction with eosinophilia asn dystemic symptoms; HES, hypereosinophilic syndrome; s.c., subcutaneous. Adapted from Ammirati et al. (1).

(51). Young women are disproportionately affected by SLE with a female-to-male sex ratio around 10:1 (52). Lupus myocarditis is a rare manifestation of SLE occurring in <5% of patients (53, 54) frequently at disease onset (≈60% of cases) (55). Cardiac manifestations of SLE-myocarditis are nonspecific: elevated troponin 80%, abnormal electrocardiogram 90%, altered LVEF (≤45%) 66%, pericardial effusion 69% (55), and usually associated with other SLE clinical features (e.g., fever, skin rash, joint inflammation, lupus nephritis). When isolated lupus myocarditis is suspected, SLE diagnosis relies on: positive anti-nuclear, anti-dsDNA (ELISA, Crithidia luciliae or Farr tests) or anti-extractable nuclear antigen (especially anti-SM) antibodies; low C3 complement fraction and/or elevated serum interferon-alpha (56, 57). CMRI usually reveals cardiac inflammation and the presence of late gadolinium enhancement (LGE) (69%) (55). EMB for the diagnosis of lupus myocarditis has shown disappointing results (58) and its use is debatable owing to the numerous non-invasive diagnosis methods of SLE, at least in the case of patients with stable hemodynamic conditions. Moreover, the histopathologic abnormalities of lupus myocarditis (lymphocytic myocarditis) are non-specific, even if SLE can be occasionally associated with GCM (59). The management of lupus myocarditis is not specifically addressed in the latest guidelines for the management of SLE (60). General consensus suggests the use of high-dose corticosteroids with the addition of an immunosuppressive drug (e.g., cyclophosphamide) in patients who are refractory to corticosteroids alone (**Figure 1**). Under these therapies, LVEF can recover to a normal value in most patients (>80%) (55).

Antiphospholipid antibody syndrome (APLAS) is a rare systemic autoimmune disease responsible for thrombotic events and obstetric morbidity in patients with persistent antiphospholipid autoantibodies (lupus anticoagulant, anticardiolipin and/or anti-beta2 glycoprotein [GP]-1 antibodies) (61). APLAS is the leading cause of acquired thrombophilia accounting for 10% of arterial or venous thrombosis. The disease mainly occurs in young adults (mean age at diagnosis 34–54 years) with a sex ratio slightly favoring women (55–82%) (62, 63) and can be associated with other autoimmune diseases, especially SLE. APLAS can induce chronic valvular lesions (Libman-Sacks endocarditis) responsible for mitral (more frequently) and/or aortic stenosis and/or regurgitation (64). Myocardial infarction in the setting of APLAS can be related to macrovascular thrombosis of coronary vessels or to microvascular thrombosis (myocardial infarction with non-obstructive coronary arteries [MINOCA]). The clinical features of APS-related MINOCAs are non-specific and associated with chest pain, electrocardiographic changes, a rise in cardiac necrosis markers, and evidence of myocardial LV systolic dysfunction. Macrovascular or microvascular thrombosis frequently occurs as *thrombotic storm* termed catastrophic APLAS (C-APLAS). The C-APLAS is defined as the occurrence of (1) at least the involvement of 3 organs, tissues, or systems in <7 days; (2) with biopsy-proven small vessel occlusion; (3) in patients with persistent high title of antiphospholipid antibodies

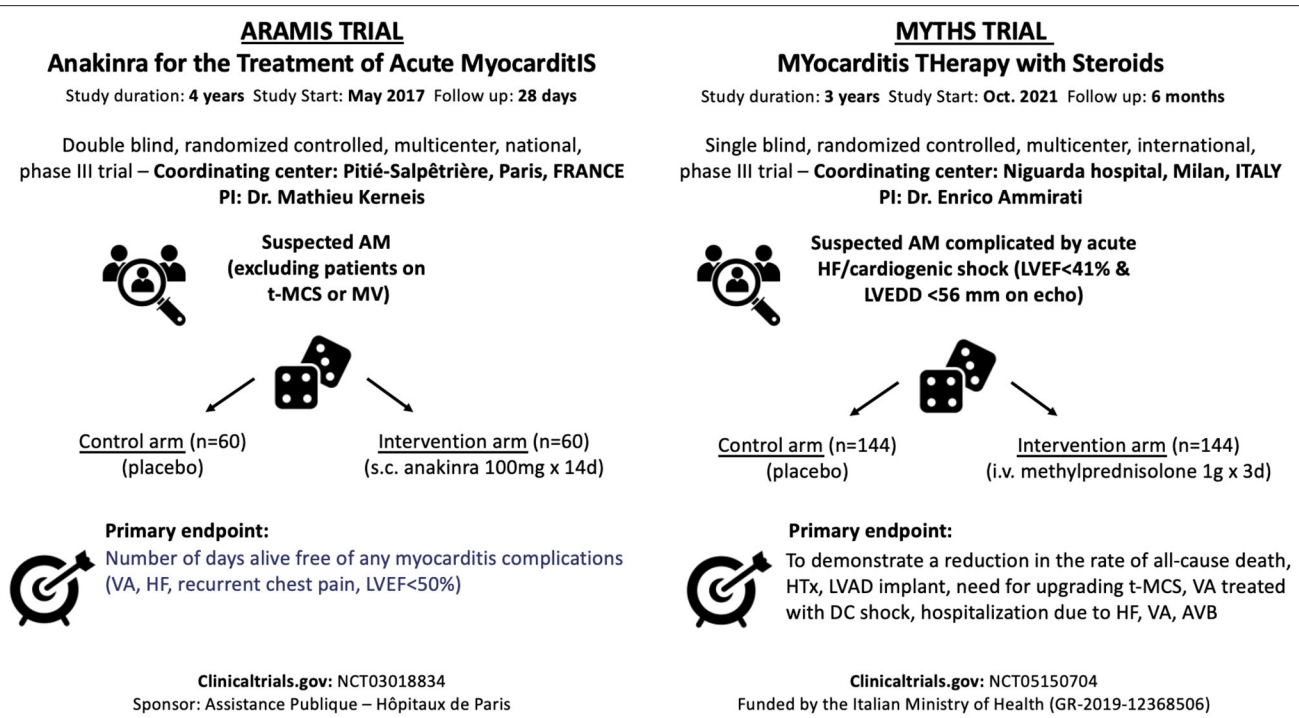

FIGURE 2 | Ongoing trials in the setting of acute myocarditis evaluating the use of immunosuppressive drugs. PI, principal investigator; AM, acute myocarditis; HF, heart failure; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; iv, intravenous; d, days; HTx, heart transplant; LVAD, left ventricular assist device; t-MCS, temporary-mechanical circulatory support; VA, ventricular arrhythmias; AVB, atrioventricular block; MV, mechanical ventilation; sc, subcutaneous.

(65). These classification criteria should be considered with great caution as they do not encompass the full spectrum of severe APLAS microvascular thrombotic episodes and some patients may require treatment escalation even though they do not fulfill the criteria for C-APLAS (66). When available, EMB can reveal myocyte necrosis with small vessels occlusions (67). However, EMB is generally not performed as it is perceived at increased risk of a bleeding complication. Small vessel occlusion can alternatively be disclosed on biopsy from other organs (i.e., skin) and CMRI can help identify microvascular occlusion (68). Nevertheless, in critically-ill patients EMB can differentiate scenario where inflammatory infiltrates prevails over the small vessels occlusions or it can reveal a GCM (1). The treatment of APS relies on anticoagulation as neither corticosteroids nor immunosuppressants nor biologics have proven their efficacy (69). Nevertheless, patients with C-APLAS should be given a triple therapy associating anticoagulation, high-dose corticosteroids, and either IVIG or plasma exchange (70). Rituximab has been also frequently used in combination with plasma exchange in C-APLAS with myocarditis (67, 71, 72). In refractory cases, the use of complement inhibitors (i.e., eculizumab) can be discussed on a case-by-case basis (73).

Immune Checkpoint Inhibitor Associated Myocarditis

ICIs have transformed cancer treatment and include monoclonal antibodies which block immune brakes such as CTLA-4 (cytotoxic T-lymphocyte antigen-4), PD-1 (programmed death receptor-1), and its ligand (PD-L1 [programmed death-ligand 1]), leading to reinvigoration of T cell responses against cancer (74). By activating the immune system, ICI can also lead to immune-related adverse events (irAE) which can affect any organ (75, 76). Myocarditis is one of the most serious irAE associated with ICI use (77). Initially described in 2016, ICI-myocarditis is now considered an infrequent but potentially lethal complication of ICI (78). ICI-myocarditis is especially arrhythmogenic and is pathologically characterized by T-cell and macrophage infiltration of the myocardium (79). Systolic HF occurs in about half of patients. On the other hand, ICI-myocarditis often occurs concomitantly with myositis, as well as a myasthenia-like syndrome (80–82). The main risk factor is combination ICI treatment, for example, when ipilimumab (anti-CTLA-4) and nivolumab (anti-PD1) are combined for more effective treatment (9). Clinical definitions have been established and advocate for the use of biomarkers, imaging, and EMB for optimal and prompt diagnosis of treatment (83, 84). Preclinical models of ICI-myocarditis have been established and suggest a critical role for immune checkpoints in the heart. For example, genetic absence of *Pdcd1* (encoding PD-1) and *Ctla4* (encoding for CTLA-4) haploinsufficiency recapitulate various features of ICI-associated myocarditis, including myocardial infiltration by T cells and severe electrocardiographic abnormalities (i.e., sinus node dysfunction, sinus arrest, and atrioventricular conduction block) (85, 86). Therapeutic intervention with abatacept (recombinant CTLA-4 immunoglobulin) rescues the fatal myocarditis in this

mouse model, providing mechanistic support for inhibition of T cell co-stimulation mediated by CTLA-4 as a treatment for ICI-associated myocarditis. Anecdotal evidence supports the use of abatacept in severe cases of ICI-myocarditis (87). ICI-induced myocarditis affects elder patients (median age of 65 years) with more comorbidities compared with non-ICI-induced myocarditis (median age between 30 and 40 years) (40, 88–90). One of the largest case series of 122 patients with ICI-associated myocarditis had early onset of symptoms (median 30 days after initial exposure to ICI), and up to 50% of deaths (9). A systematic analysis of the World Health Organization pharmacovigilance database confirmed a 32.5% of mortality in patients who had myocarditis associated with the administration of ICIs with a median time-to-onset of 33 days (10). The increased reports of cases in the last years are perhaps consistent with growing recognition of this new clinical syndrome, as well as the more widespread use of ICIs. High-dose IV corticosteroids and withdrawal of ICI are considered the first-line therapy (1, 91, 92), while alemtuzumab (anti-CD52 antibody), antithymocyte globulin (anti-CD3 antibody), and abatacept (a CTLA-4 agonist) have been proposed in corticosteroid-resistant forms (**Figure 1**) (87, 93, 94). Retrospective data suggest that earlier (within the first 24 h) and high doses (501–1,000 mg/day) of corticosteroids lead to an improved outcome (95). Prompt diagnosis and immediate treatment of ICI-myocarditis becomes a critical issue among the cardio-oncology population, as indications for ICI increase for various cancer types. In 2021, nearly 50% of cancer patients are eligible for ICI treatment. In many cases, ICIs are combined with other cancer therapies with their own inherent cardiotoxicities (96–98). In addition, long-term cardiovascular effects of ICI become an important consideration as a growing number of cancer patients respond to therapy (99–101). Finally, the emergence of ICI-myocarditis has opened new avenues for more fundamental investigation about the role of immune checkpoints (e.g., PD-1/PD-L1 signaling) in other forms of inflammatory heart disease (102, 103). These issues need to be a focus of future investigations.

Ventricular Arrhythmias and Myocarditis

AM can be complicated by VA. Specifically, ∼40% of patients presenting with life-threatening VA can experience a recurrence at a median time of 8 months based on a recent international registry including 156 patients (104). Factors associated with arrhythmic recurrence were initial presentation with sustained VT, LGE involving ≥2 myocardial segments, and absence of T2-weighted short-tau inversion recovery (STIR) signal suggestive for residual edema on CMRI (104). In this registry, 98 patients underwent EMB showing in the large majority of cases a lymphocytic myocarditis (88.8%). An immunosuppressive therapy was initiated in 21% of cases and there was no difference in the use of immunosuppressive therapy between patients who subsequently experience an arrhythmic recurrence vs. those who did not (104). A second registry of 185 patients with VA (including VF/VT, non-sustained VT, and Lown's ≥2 premature ventricular complexes [PVC]) and myocarditis confirmed a 30% of recurrence of malignant VA at 2 years (105). Another study evaluated 58 patients with histologically proven lymphocytic myocarditis and VA as above described who underwent immunosuppressive therapy vs. a matched population of 58 cases not treated with immunosuppressive agents (2). Immunosuppressive therapy in most patients was a combination of prednisone 1 mg/kg for 6 month and AZA 2 mg/kg for 1 year. Alternatively, mycophenolate mofetil at dosage of 1–3 g/day was used instead of AZA. At 24-month follow-up, no significant differences in VF/VT occurrence were observed in patients treated with immunosuppressive agents vs. those who did not (10 vs. 17%, respectively, $p = 0.42$), even if patients who were treated with immunosuppressive agents showed a significant reduction in the PVC burden (2). Another prospective registry included 107 symptomatic patients with >5,000 PVCs/24 h without ischemic etiology who underwent a combination of laboratory testing, FDG-PET scan, CMRI and EMB (106). A positive FDG-PET scan consistent with cardiac inflammation was observed in up to 51% of patients and CS was the final diagnosis in 24% of patients with positive FDG-PET scan. Patients with signs consistent with myocarditis started an immunosuppressive therapy (prednisone 40 mg for 3 months) alone or in combination with catheter ablation, showing an optimal response in 67% of cases. Optimal response was defined as a reduction in the PVC burden >80% and negative FDG-PET scan at follow up. Furthermore, patients with LV systolic dysfunction showed an improvement in 37% of cases with a mean increase in LVEF of 13% (106). Although these studies are promising, the lack of randomization vs. a control group, the absence of reports of side effects and the fact that the immunosuppression therapy did not significantly reduce VF/VT or cardiovascular death cannot routinely support the use of corticosteroids in the management of patients with myocarditis complicated by VA or frequent PVC. Specific randomized trials are required to assess whether immunosuppression can ameliorate myocardial inflammation and reduce the risk of major VA. In addition, VA is especially a hallmark of ICI-myocarditis. In an international registry of patients with ICI-myocarditis, consisting of 147 patients, a total of 22 (15.0%) patients experienced 1 or more life-threatening ventricular arrhythmia episodes, including 16/147 (10.9%) sustained ventricular tachycardia, 4/147 (2.7%) ventricular fibrillation, and 2/147 (1.4%) torsade de pointes (107).

COVID-19 Associated Acute Myocarditis

Cardiac injury with release of troponin has been observed quite often in patients who were hospitalized with COVID-19 (108), nevertheless cases of well-characterized AM are anecdotal (3). Data on clinically suspected AM complicated by acute HF among hospitalized patients with COVID-19 suggests a 0.12% incidence (109). Nevertheless, good data on the incidence of AM are still lacking. It has been recognized that asymptomatic forms of AM associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exposure range between 0.3 and 3% based on a CMRI diagnosis. This population has been largely studied among athletes who underwent systematic cardiac tests (ECG, troponin assessment, or transthoracic echocardiography) and, when clinically indicated, CMRI (110–112). It must be acknowledged that proportionally, individuals with mild

COVID-19 related symptoms have a higher likelihood of signs of myocardial inflammation compared with asymptomatic individuals. Patients with cardiac tests consistent with AM should be advised not to practice vigorous physical activities in the 3–6 months following SARS-CoV-2 exposure if they have preserved LVEF, whereas if patients have reduced LVEF, patients should initiate specific HF therapies (113), while there is no indication for immunosuppression. Patients complaining of cardiac symptoms or signs associated with COVID-19 and diagnostic findings consistent with AM can be further divided between those with COVID-19 associated AM with concurrent pneumonia and those without pneumonia (isolated COVID-19 myocarditis). Delayed-onset AM has been described after SARS-CoV-2 exposure and typically these patients can present with high titer of SARS-CoV-2-specific antibodies and recent history consistent with COVID-19 in the absence of SARS-CoV-2 by RT-PCR on a nasopharyngeal swab. Delayed-onset myocarditis is thought to be triggered by SARS-CoV-2 induced immune-mediated reactions. Immunomodulating therapies include non-steroidal anti-inflammatory drugs (NSAIDs) to relieve chest pain, low dosage of colchicine in case of associated pericardial involvement. Corticosteroids are generally used in patients with delayed onset AM that present an associated hyperinflammatory status (114, 115). In severe COVID-19 AM presenting as FM, EMB can be deemed necessary with the aim to differentiate AM from sepsis-induced acute cardiomyopathy, especially in patients with hyperinflammatory status. Identification of inflammatory infiltrates in the myocardium could support the empirical use of immunosuppressive drugs (33), even if, diffuse inflammatory infiltrates have been rarely seen (116). Hyperinflammatory status and acute HF/cardiogenic shock in which a predominant septic state has been excluded could be treated with immunosuppressive treatments, as suggested by small series where intravenous corticosteroids have been associated with a favorable prognosis (114, 115). This condition has been termed multisystem inflammatory syndrome in adults (MIS-A) and is often associated with a delayed onset of myocarditis. The condition is usually associated with high levels of inflammatory biomarkers and ferritin (117). The Multisystem inflammatory syndrome in children (MIS-C) presents overlapping characteristics with myocarditis in adults (118). It has been that although a third of patients with MIS-C can require a t-MCS, but none died in a series of 35 children who were treated with IVIG plus a third with the addition of corticosteroids (119). Finally, patients with concurrent severe myocarditis, pneumonia, and respiratory insufficiency should receive corticosteroids (120). A review article that collected data on 38 published cases of COVID-19 associated AM reported use of corticosteroids in 34% of cases and a mortality of 15% (121), even if larger series are needed to better understand optimal therapies.

mRNA COVID19 Vaccine-Related Acute Myocarditis

The association between vaccine administration and the onset of myocarditis is supported by several case reports, case series, and at the level of the national health care system (4–7, 122–124). The United States Vaccine Adverse Event Reporting System (VAERS), even if subject to bias, also revealed a clear signal for vaccine-associated myocarditis with nearly 1,300 cases reported from more than 350 million doses in the United States (8). Most cases have been reported in young men, thus, for 18–24-year-old males, the expected prevalence of vaccine-associated myocarditis is ∼3 cases per 100,000 doses (0.003%) based on VAERS data (8). Nationwide observational data confirmed a COVID-19 vaccine-associated myocarditis at ∼3 per 100,000 patients (0.003%) vs. ∼11 per 100,000 patients (0.01%) for acute COVID-19 myocarditis (125). An analysis conducted in England revealed that the increased risk of myocarditis associated with the two mRNA vaccines was present only in those younger than 40 years (6).

Historically, the vaccine that is most associated with myocarditis is the anti-smallpox (10, 126). Smallpox vaccine was associated with eosinophilic myocarditis, while almost all the present cases of mRNA COVID-19 vaccine are not associated with eosinophilia. We revised 90 cases published of mRNA COVID-19 vaccine myocarditis up to the end of August 2021 (see **Supplementary Tables 1, 2**), and we summarized major features, and anti-inflammatory and immunomodulatory drugs used. The median age at presentation was 25 years (interquartile range 17–27), in agreement with a median age observed in VAERS (8), with a marked male prevalence (93%). Even if a higher number of BNT162b2-related myocarditis is reported, disproportionality analyses using the Bayesian information component, revealed a higher likelihood of association between mRNA 1273 and myocarditis (126). In 90% of cases, myocarditis occurs after the second dose, following a median time of 3 days between the last dose and symptoms' onset, including chest pain (observed in 96% of cases) generally preceded by fever (in 85%). All these findings suggest an immune-mediated reaction related to vaccine administration. AM is generally not severe. While electrocardiographic abnormalities are present in 77% of cases, diagnostic tools revealed only a slight reduction in the LVEF (mean value of 53%) with a pericardial effusion observed in 14% of cases. Information on anti-inflammatory/immunomodulatory therapy was available for 56 of 90 patients (62.2%). In 38 out of 56 patients, the administered drugs were reported as follows: aspirin, NSAIDs, corticosteroids, IVIG, colchicine, and anakinra. Patients who received anti-inflammatory/immunomodulatory therapy did not differ in relation with age (23 ± 9 vs. 29 ± 19 years, p-value 0.10) and LVEF on the first echocardiogram (53 ± 11 vs. $53 \pm 13\%$, p-value 0.90). The use of immunosuppressive therapy was similar in the adult and pediatric populations (39.5 vs. 44.4%, p-value 0.72). Overall, NSAIDs (including aspirin) were the most used drugs (23/56 patients, 41.1%), and aspirin was used only in 3 out of 56 patients (5.4%). Corticosteroids were used in 19 of 56 patients (33.9%), IVIG in 12 patients (21.4%), colchicine in 15 patients (26.8%), and anakinra in only 2 patients. Most of the time, immunosuppressive agents were used in combination. NSAIDs were used together with the corticosteroids in 5 patients. IVIG along with corticosteroids was used in 11 patients, including 10 pediatric patients. NSAIDs along with colchicine were used in 11 of 56 patients (19.6%). Prognosis

is considered favorable, with only three (3.3%) deaths reported out of 90 patients, a figure in line with the one observed in AM patients in pre-COVID19 era (40). These data are largely consistent with a series of 139 adolescents (all with age <21 years) with suspected AM within 30 days of COVID-19 vaccination (7). In fact, the male prevalence was 91%, symptoms started a median of 2 days after vaccination, and the most common symptom was chest pain (99%) (7). Again, NSAIDs were the most used drugs in 81% of cases, followed by corticosteroids (22%) and IVIG (22%), while colchicine was administered in 8% (7). No patient died or required a t-MCS.

EOSINOPHILIC MYOCARDITIS

Eosinophils have widespread procoagulant effects, including the production of tissue factor (127), oxidation of phospholipids (128) (both of which activate the intrinsic pathway), the release of platelet-activating factor (129), reactive oxygen species, and eosinophil extracellular traps (130). Moreover, activated eosinophils are potent producers of vasospastic mediators (including histamine, leukotrienes C_4 and D_4 and prostaglandin D_2) and are able to modulate mast cell functions (131). Lastly, the shedding of both cytotoxic granules and pro-inflammatory mediators (i.e., tumor necrosis factor [TNF]-α, IL-1 and IL-6) are contributing factors of endothelial injury and procoagulant state (132). Eosinophil-mediated toxicity can lead to protean cardiovascular manifestations, including venous thromboembolism (133), eosinophilia-related coronary vasospasm (134), thromboangiitis obliterans-like disease (135), eosinophilic coronaritis, systemic eosinophilic vasculitis (136), eosinophilic myocarditis (137), and Loeffler cardiomyopathy, a chronic inflammatory cardiomyopathy (1, 138). The natural history of eosinophil-related heart involvement involves three successive (and potentially overlapping) phases: (1) AM, due to eosinophilic infiltration of the endocardium, that can be either asymptomatic or lead to acute HF or FM (137). High troponin levels, LV systolic dysfunction, and subendocardial LGE pattern on CMRI can be observed (2, 137) a thrombotic stage characterized by the occurrence of ventricular thrombi and the risk of systemic embolism; (3) a fibrotic stage, characterized by endomyocardial fibro-thrombosis that can lead to restrictive cardiomyopathy (i.e., Loeffler cardiomyopathy) and/or atrioventricular valvular disease (139). The diagnosis of eosinophilic myocarditis is usually straightforward in the presence of hypereosinophilia, increased cardiac troponin, and CMRI consistent with subendocardial inflammation (137). EMB can be considered when the initial presentation is characterized by cardiogenic shock (1, 33), or CMRI findings are atypical (i.e., subepicardial LGE) or when absolute eosinophil counts are within the normal range (which has been reported in up to 25% of patients with biopsy-proven eosinophilic myocarditis) (137, 139). Conversely, EMB is at risk of thromboembolism if ventricular thrombi are present, and can yield false-negative findings when endomyocardial fibrosis is prominent and eosinophil infiltration has partially or completely vanished (138). Eosinophil-related heart involvement can be encountered within the full spectrum of eosinophil-associated diseases (137), including drug hypersensitivity (even in the absence of skin manifestations) (10), parasitic infections (namely toxocariasis, trichinosis, filarial infections or sarcocystosis), aspirin-exacerbated respiratory disease, eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome), hypereosinophilic syndromes (HES) (mainly idiopathic and *FIP1L1-PDGFRA*-associated HES, formerly chronic eosinophilic leukemia) and high-grade hematological malignancies [e.g., Hodgkin and angioimmunoblastic T-cell lymphomas, as well as B-cell acute lymphoblastic lymphoma with t (5, 14) (q31;q32); IGH-IL3 rearrangement (140). In a review of 179 cases of biopsy-proven eosinophilic myocarditis, the main identified causes were drug hypersensitivity, EPGA, HES and parasitic infection, accounting for 34%, 13% and 8% of cases, respectively, while 36% of cases were idiopathic or eosinophilic myocarditis with undefined cause (137).

Heart involvement is the leading cause of death in patients with EGPA and is more frequent in antineutrophil cytoplasmic antibodies (ANCA)-negative patients (141). Of note, the differential diagnosis between ANCA-negative EGPA and HES is a frequent diagnostic and therapeutic dilemma. The European Respiratory Society and European Federation of Internal Medicine-endorsed Task Force suggested restriction in the use of EGPA to patients with eosinophilic asthma who test positive for ANCA and/or who exhibit genuine features of vasculitis (either biopsy-proven or clinical surrogates) (142). Likewise, in a retrospective analysis of 166 patients with blood eosinophilia $>1{,}000/mm^3$ and systemic manifestations, it was recently suggested that serum CRP levels could be a reliable biomarker able to distinguish EGPA from idiopathic HES, with low (i.e., < 36 mg/L) levels being suggestive of idiopathic HES rather than EGPA (143). A workup to identify associated systemic disorders should be performed in all patients with eosinophilic myocarditis. The workup should include testing for ANCA (positive in 10–40% of EGPA patients), serological testing for toxocariasis (which has a broad geographic distribution), ova and parasite tests (while further serologies for parasitic infections are generally guided by the patient's country of origin, travel history and dietary habits), serum vitamin B12 and tryptase levels (which are sensitive for the diagnosis of myeloid variant HES), total IgE levels (which are suggestive of reactive polyclonal eosinophilia mediated by IL-5, when elevated), lactate dehydrogenase (suggestive of lymphoma), thoraco-abdominopelvic CT scan (seeking for extra-cardiac eosinophil-related organ involvements as well as underlying solid or hematological malignancies). Furthermore, brain CT or brain MRI should be performed when embolic stroke is suspected in patients with eosinophilic myocarditis or Loeffler cardiomyopathy (144). Additionally, testing for *FIP1L1-PDGFRA* fusion gene should be performed in selected cases when clinical (e.g., male sex, splenomegaly), biologic (e.g., high B12 vitamin and/or tryptase levels) features and/or primary resistance to steroids are observed (145). Polymerase chain reaction testing for specific viruses (e.g., Herpesviridae, especially HHV 6) and the RegiSCAR scoring system can be useful in patients with suspected Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) (146). Additional imaging, endoscopic and histologic investigations are usually performed on a case-by-case basis after first-line investigations. In a retrospective

series of 19 patients with biopsy-proven myocarditis with fulminant presentation, the rate of either cardiac death or heart transplantation at 60 days was up to 26% (11).

The cornerstone of the treatment relies on systemic glucocorticoids, starting dose: 1 mg/kg qd, preceded in case of severe LV systolic dysfunction by intravenous pulses of 7.5–15 mg/kg of methylprednisolone for 1–3 days (**Figures 1**, **3**) (137, 139). In patients at risk of strongyloidiasis (owing to their past travel history), concomitant prescription of a single dose of ivermectin (200 μg/kg) is warranted to prevent *Strongyloides stercoralis* hyperinfection. When toxocariasis or trichinosis are evidenced, a 10/15-day course of albendazole (400 mg bid) is warranted (147). Likewise, in patients with evidence of intracavitary thrombus, anticoagulation should be initiated (while prophylactic anticoagulation is mandatory in all other patients until absolute eosinophil counts normalize). The diagnoses of myeloid variant HES, DRESS or EGPA should be suspected and investigated accordingly, after 2–4 days of corticosteroid-refractory eosinophilia. Specifically, the treatment of *FIP1L1-PDGFRA*-positive HES relies on the tyrosine kinase inhibitor imatinib (100 mg/d), and eosinophils generally plummet within days after imatinib initiation (145). Yet, transient worsening of HF after onset of imatinib has been reported, likely due to treatment-induced lysis of eosinophils (148). Conversely, IVIG and/or cyclosporine are the most common drugs used for the treatment of corticosteroid-refractory DRESS (149, 150), yet benralizumab (a humanized afucosylated monoclonal antibody that targets IL-5 receptor α) is on the rise in this setting (151). Historically, besides systemic corticosteroids, the treatment of EGPA-associated eosinophilic myocarditis complicated by severe HF relies on cyclophosphamide pulses (152, 153), yet it should be emphasized that there is no data proving that adding cyclophosphamide pulses to steroids improves outcomes. Whatever the underlying disorder, the aim is to quickly and persistently normalize eosinophil count ($< 500/mm^3$). Of note, both in EGPA (154–156) and in *FIP1L1-PDGFRA*-negative HES (157, 158) targeting IL-5 has emerged as clinically relevant. Anti-IL-5 agents, such as mepolizumab and benralizumab are likely to become game changers and tend to replace the use of disease-modifying anti-rheumatic drugs (i.e., AZA, methotrexate, peginterferon alpha-2a and hydroxycarbamide), even if trials are needed. In case of persistent eosinophilia and subsequent occurrence of endomyocardial fibrosis, heart surgery with resection of fibrotic endocardium (endomyocardectomy) combined with valve repair or replacement can be considered (159). Finally, in case of refractory end-stage HF, orthotopic heart transplantation has been reported to be safe and feasible in both EGPA and HES (160, 161).

GIANT CELL MYOCARDITIS

GCM is a rare but often fatal form of AM. The pathophysiology of GCM is thought to be a T-cell mediated autoimmune process leading to diffuse or multifocal inflammatory infiltrate, including lymphocytes with multinucleated giant cells, and definitive diagnosis requires EMB. An immune-mediated mechanism in the etiology of GCM is further supported by the fact that no nucleic acids from viruses implicated in myocarditis were detected in cardiac tissue samples from 9 patients with GCM (162).

However, the characteristic giant cells can take 1–2 weeks to appear, therefore, while EMB in the first few days of the illness may suggest myocarditis, it may render a false negative result for GCM; for this reason, EMB repetition can increase sensitivity in GCM diagnosis (163). It has been estimated to occur at a rate of 1 case per 200 patients with AM and constitutes about 10% of FM (11, 13). GCM affects men and women equally with a median age at onset between 43 and 53 years. Association with other autoimmune disorders has been observed in about 20% of cases, especially autoimmune thyroiditis and inflammatory bowel disease (59). Recent data where RNA-Sequencing (RNA-Seq) was applied to a small series of GCM cases reveals a distinct transcriptomic signature for GCM compared to other forms of myocarditis (164). Specifically, it has been observed downregulation of pathways involved in muscle contraction, ion homeostasis, and cardiac conduction, potentially explaining the typical patient presentation with acute heart failure and arrhythmias) (164).

Clinically, GCM generally presents with rapid hemodynamic deterioration (FM), VA, and at times bradyarrhythmia. The rate of death or HTx has been estimated at 81% at 3 years from the initial admission when GCM presents specifically as FM (11); whereas a 73% mortality rate at 5 years has been estimated more recently considering all GCM (165). It is characterized by the lack of spontaneous recovery on t-MCS which more commonly occurs in FM. Prolonged use of intravascular microaxial pump and VA-ECMO has been reported (166–168). Pharmacologic treatment includes multi-drug immunosuppression that typically involves combinations of anti-T-cell drugs (i.e., antithymocyte globulin, muromonab and cyclosporine) and high dose corticosteroids. No standardized protocols exist, though several regimens have been proposed in recent review articles (1, 169). Clinically relevant, immunosuppressive therapy should be initiated promptly. Treatment with anti–T-lymphocyte–based and calcineurin inhibitor therapy can lead to clinical remission in up to two-thirds of patients, in particular in those not requiring t-MCS (163, 168). The initial approach may vary based on the clinical presentation. In case of FM, antithymocyte globulin (dose raging from 1 mg/kg to 300 mg in the first 3 days) associated with pulsed high-dose corticosteroids (generally 1 g methylprednisolone per 3 days) is preferred; even if alternative protocols including alemtuzumab (an anti-CD52 antibody; at dose of 15 mg per 2 days) instead of antithymocyte globulin have been reported. Cyclosporine is then added and titrated to trough levels of 150 to 250 ng/L as maintenance therapy. There is a variable rate of LVEF recovery without transplant. Dosage of oral prednisone after the acute phase is generally 1 mg/kg in the 1st months with subsequent slow tapering over 1 year, while cyclosporine is generally maintained >2 years, with a target plasma through level of 80–100 ng/L. AZA at 1–2 mg/kg/day divided into 2 daily doses or mycophenolate mofetil (500–1,000 mg BID) can be

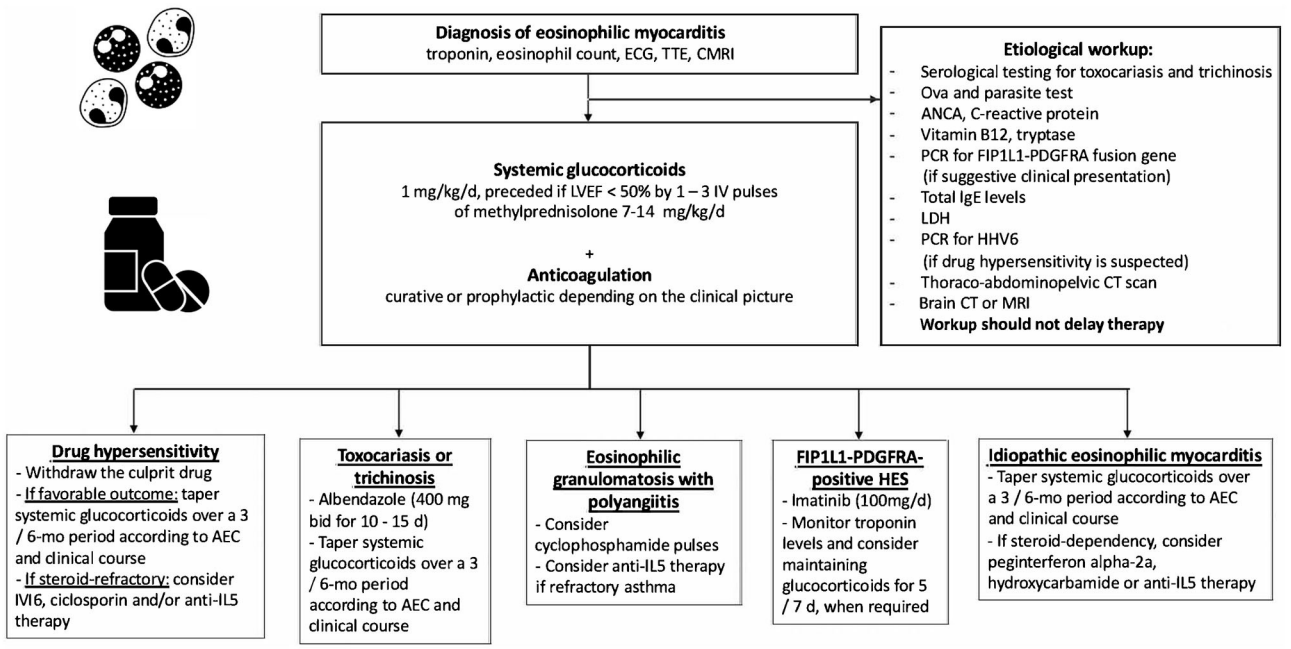

FIGURE 3 | Etiological workup and immunosuppressive treatment strategies used for eosinophilic myocarditis. TTE, transthoracic echocardiography; CMRI, cardiac magnetic resonance; LVEF, left ventricular ejection fraction; ANCA, antineutrophil cytoplasmic antibodies; PCR, polymerase chain reaction; LDH, lactate dehydrogenase; HHV6, human herpes virus 6; CT, computed tomography; d, day; mo, month; AEC, absolute eosinophil count; HES, hypereosinophilic syndrome.

added. In case of non-fulminant presentation a combination of mycophenolate mofetil and cyclosporine (or tacrolimus, trough levels in the first 6 months: 10–15 ng/mL) and corticosteroids can be added. Also, in cases with less severe presentation, pulsed high-dose corticosteroids are still suggested. If no recovery is obtained, HTx is an effective therapy, with similar post-transplant survival in patients with GCM as in those with other causes (170). Nevertheless, recurrence of GCM can happen in up to 25% of transplant patients, and again warrants aggressive immunosuppression which is typically sufficient for disease remission (169).

CARDIAC SARCOIDOSIS

CS can present as a chronic inflammatory cardiomyopathy, while infrequently can manifest as an AM (1, 165). The most reported clinical cardiac features are complete AVB, VA, LV systolic dysfunction and HF (165, 171). CS can be isolated or be part of a systemic disorder that meanly affects lungs and hilar lymph nodes. About 5% of patients with systemic sarcoidosis have clinically manifest CS (172). Myocardial histology is the gold standard of CS diagnosis but has low sensitivity (20–30%) (1). Histology is characterized by the presence of epithelioid granulomas with associated giant cells and lymphocytes, well-defined areas of inflammation and fibrosis, and absence of significant myocardial necrosis (1). Therefore, quite often the diagnosis of CS can be supported by clinical and imaging findings with contrast-enhanced CMRI and FDG-PET (1, 173). Based on this assumption, it must be accepted that if the diagnosis of CS relies on clinical and imaging criteria, we could face the risk of treating with immunosuppressive therapies patients with other inflammatory or non-inflammatory cardiomyopathies that are potentially less responsive to long-term steroid therapy or might be potentially harmed by the treatment. The immunosuppressive therapeutic approach to patients with CS is similar either presenting as a chronic inflammatory cardiomyopathy or as an AM, and it is based on corticosteroids as first line therapy (**Figures 1**, **4**) (1). Unfortunately, no randomized controlled trial supports the immunosuppressive therapy in CS, neither for corticosteroids nor for any disease-modifying therapy. Despite lack of evidence almost all patients with CS receive systemic therapy. This is distinctly different from pulmonary sarcoidosis in which only half of the patients need systemic therapy (174). We do not know at present whether these patients with a good prognosis and mild myocardial involvement benefit from immunosuppressive therapy. Patients having at the time of initial presentation, normal LV function and only 5% of LGE, have very few adverse events (175).

Corticosteroids

There is still controversy about the clinical efficacy, the optimal initial dose and duration of corticosteroid treatment for CS. It is plausible to assume that corticosteroids have similar effect in CS than in other forms of sarcoidosis. Consistent with this idea glucocorticoid treatment decreases myocardial troponin (176). By expert consensus, corticosteroids still constitute the first-line treatment at relatively high doses for 1–2 years. Although mechanistically plausible, we do not currently know if corticosteroid treatment improves prognosis in CS. Nevertheless, some patients do not respond to glucocorticoids.

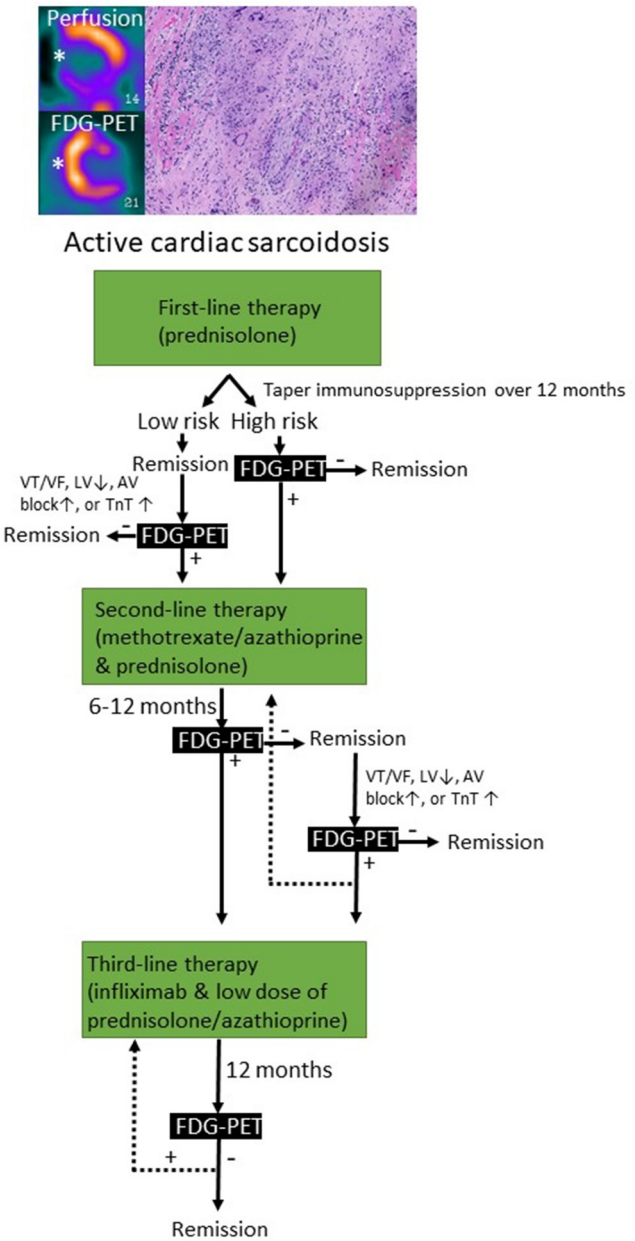

FIGURE 4 | Immunosuppressive treatment strategies used for cardiac sarcoidosis based on clinical and imaging-based monitoring. FDG-PET, fluorodeoxyglucose positron emission tomography; VT/VF, ventricular tachycardia/ventricular fibrillation; LV, left ventricular ejection fraction; TnT, troponin.

The clinical evidence for the utility of corticosteroids is based on retrospective, uncontrolled observational studies (177). Corticosteroids have been reported to improve LV systolic function at least in some patients (176, 178, 179), reverse AVB (180), and decrease VA in some studies but not in others (181, 182).

DNA Synthesis Inhibitors

DNA synthesis inhibitors (such as AZA or methotrexate) that prevent nucleotide synthesis are used as steroid-sparing agents (183). AZA acts by suppressing the activation of Rac1 target genes such as NF-κB in T-cells (184). AZA and methotrexate have been used to enable rapid reduction in the glucocorticoid doses in order to reduce the dose-dependent side effects of glucocorticoids. Methotrexate combined with glucocorticoids decreases the risk of radiologic relapse in CS (183, 185). In pulmonary sarcoidosis, steroid-sparing agents-treated patients had a higher rate of infections compared to prednisone monotherapy (186). The major weakness of glucocorticoids and DNA synthesis inhibitors are their wide-ranging effects beyond immunosuppression.

Infliximab or Other Anti-TNF-α Agents

TNF-α governs formation of granuloma thought NF-κB-mediated orchestration of cytokine expression and hence controls the hallmark tissue response in sarcoidosis (187). TNF-α antagonists are more selective and effective inhibitors of NF-κB activation than glucocorticoids and thus lack most of glucocorticoid side effects. However, not all the immunosuppressive effects of glucocorticoids may be mediated via NF-κB. Current recommendations based on expert consensus suggest anti-TNF-α agents to be used as a third-line therapy in the management of severe refractory sarcoidosis (188). Infliximab is a chimeric IgG1 monoclonal antibody that binds TNF-α with high affinity and neutralizes its effect in promoting inflammatory response. In pulmonary sarcoidosis, randomized, controlled trials with infliximab have shown that it is safe to use if proper precautions are followed (189, 190). Infliximab decreases inflammatory activity measured by FDG-PET and this correlated with improvement in forced vital capacity. In pulmonary sarcoidosis FDG-PET activity is predictive for treatment response in severe and refractory pulmonary sarcoidosis (190) and might add value in individualizing infliximab treatment. The effectiveness of adalimumab in pulmonary sarcoidosis was shown in a small open-label study (191). Adalimumab reduces the relapse rate as measured by FDG-PET (183). In CS, infliximab has been used successfully as a bailout therapy in glucocorticoid failures (192, 193). In addition to being more specific and potent inhibitor of granulomatous inflammation, a major benefit of TNF-α blockers is the lack of numerous side effects typical of corticosteroids. Despite TNF-α antagonists being very effective immunosuppressants, risk of serious infections is not higher than in corticosteroids (194). TNF-α is well-tolerated at dosage <10 mg/kg even in patients with HF (195). To reduce the production of neutralizing antibodies, infliximab and adalimumab are often combined with low-dose methotrexate or AZA (196).

Ongoing Trials

The Cardiac Sarcoidosis Multi-Center Randomized Controlled Trial (CHASM CS-RCT) is a multicenter randomized controlled trial designed to compare treatment with a higher dose prednisone vs. prednisone plus methotrexate (197). The aim is to evaluate whether a low dose prednisone/methotrexate combination have similar efficacy to standard dose prednisone

leading to an improvement in the quality of life, as a result of a reduced burden of side effects. Eligible subjects will have active clinically manifest CS with advanced conduction system disease, non-sustained or sustained VA, LV or right ventricular systolic dysfunction. The primary endpoint is a measure of myocardial fibrosis/scar, summed perfusion rest score on FDG-PET scan after 6 months from randomization.

IMAGING TO GUIDE IMMUNOSUPPRESSIVE THERAPY IN MYOCARDITIS AND CARDIAC SARCOIDOSIS

Echocardiography is routinely performed in patients with suspected AM to evaluate LV systolic and diastolic function and the presence of pericardial effusion. However, its role to guide therapy is limited, since it does not allow tissue characterization. CMRI has emerged as a powerful non-invasive diagnostic tool for the assessment of edema, inflammation and fibrosis (198). According to the Updated Lake Louise Criteria, AM can accurately be diagnosed if both edema and myocardial injury (necrosis or fibrosis) are demonstrated by, respectively, T2-weighted (STIR or T2-mapping) and T1-weighted imaging (T1 mapping or LGE) (198). In healed myocarditis, residual scar can be depicted by LGE (with or without elevated focal T1-values), while persistence of edema, as assessed by T2-weighted imaging, suggests active inflammation. Moreover, CMRI is the gold standard for quantification of ventricular volumes and function. In this respect, CMRI can be used to select patients who might benefit from immunosuppressive therapy, as well as to evaluate the impact of treatment on myocardial function, ongoing inflammation and scar formation. Furthermore, assessment of the disease stage of myocarditis is especially relevant for patients with myocarditis and drug-refractory VT, as recent data show a high recurrence rate post VT ablation if signs of active myocarditis are present on EMB or CMRI (199). Importantly, the Lake Louis Criteria are less accurate in detecting active myocarditis in the context of systemic immune-mediated diseases (200, 201), making CMRI less suitable to guide therapy in this setting. In sarcoidosis, the presence of LGE is a sensitive marker of cardiac involvement, but assessment of active inflammation by T2-weighted imaging is not well-validated. However, extensive LGE (>20% LV mass) is associated with a poor prognosis and absence of LV recovery after immunosuppressive therapy with corticosteroids (202). In this respect, CMRI is mainly used for diagnosis and prognostication in CS.

New advances in the field of CMRI include the enhancement of ultrasmall superparamagnetic particles of iron oxide (USPIO), which are nanoparticles that are taken up by monocytes and macrophages, to directly visualize cardiovascular inflammatory processes (203). A pre-clinical study in a rat model with experimental auto-immune myocarditis showed that USPIO-enhanced CMRI outperformed conventional CMRI regarding the detection of myocardial inflammatory cellular infiltrates (204), but the only study in humans failed to show a difference between patients with AM ($n = 9$) and healthy volunteers ($n = 10$) (205). Therefore, there is currently no role in clinical practice for USPIO-enhanced CMRI in the diagnosis or follow-up of patients with myocarditis.

FDG-PET can detect T cells, macrophages, or granulocytes that infiltrate the myocardium, either as non-specific response to cell injury or as primary lesion in CS by an enhanced glucose metabolism after a carbohydrate-free diet. FDG-PET is recommended by several guidelines in patients with suspected active CS (172, 206), in fact, it can reveal hypermetabolic mediastinal and hilar lymph nodes differentiating CS from other autoimmune disease with cardiac involvement (e.g., vasculitis). Since FDG uptake correlates well with the level of granulomatous inflammation, it is assumed that immunosuppression should be up titrated in patients with increased metabolic activity on FDG-PET after steroid therapy has been initiated (207), while a dose reduction can be considered in patients with reduced FDG uptake. A recent study by Ning et al. (208) showed that serial FDG-PET in patients with CS altered patient management in most cases, resulting in complete weaning or significant tapering of prednisolone in 48 and 20%, respectively (**Figure 4**), while outcome was generally favorable. FDG-PET can be also considered as an alternative non-invasive diagnostic tool in hemodynamically stable patients with contraindication to CMRI or in patients with suspected autoimmune disease to guide immunosuppression (**Figure 5**) (1).

NEW INSIGHTS ON PERICARDITIS

Pathologies of the pericardium are a heterogeneous group, spanning from minimal pericardial effusion, often asymptomatic, to incessant multidrug-resistant pericarditis (209). Acute pericarditis is diagnosed based on two of the following criteria (210): chest pain, pericardial rubbing, typical changes in the electrocardiogram, with new and widespread ST elevation or PR depression in the acute phase, and pericardial effusion, which is generally mild. Increased CRP levels can support the diagnosis. The natural history of acute pericarditis can vary. In most cases, it can be self-limiting with complete resolution of the symptoms, whereas in some cases it can relapse. The development of relapses increases by up to 50% in patients who have received corticosteroid therapy for symptomatic control of the first episode. Some patients can develop incessant pericarditis, a pericarditis whose symptoms continue without interruption even for months (210). The etiology of pericarditis changes considerably depending on the geographic regions (211). In developing countries, pericarditis is often secondary to tuberculosis (212). On the other hand, in developed countries, pericarditis is more often idiopathic, secondary to autoinflammatory or autoimmune processes or following pericardial injury such radiotherapy or cardiac surgery (211).

The Autoinflammatory Processes in Recurrent Pericarditis

Clinical and laboratory similarities between relapsing pericarditis and some autoinflammatory disorders (i.e., familial Mediterranean fever [FMF], cryopyrin-associated periodic syndromes [CAPS], TNF receptor associated periodic

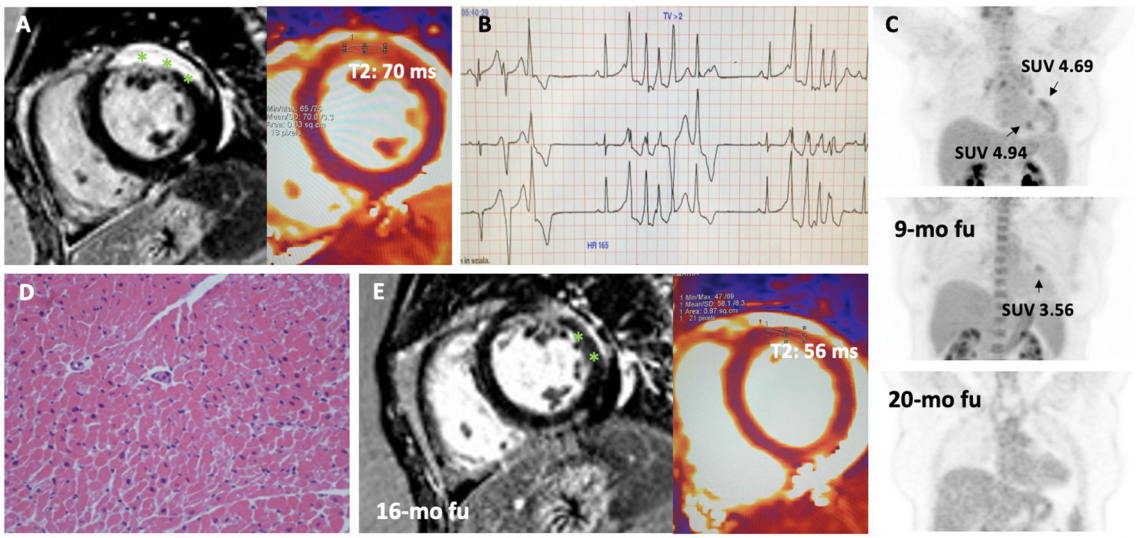

FIGURE 5 | Representative patient with acute myocarditis in whom immunosuppression was guided by FDG-PET and cardiac magnetic resonance imaging (CMRI). A 49-year-old woman with a previous history of ANA positive pericarditis presented with acute myocarditis. On CMRI she presented a transmural lesion in the anterior wall as demonstrated by late gadolinium enhancement (LGE, highlighted with asterisks) and increased T2 signal (normal value <55 ms) **(A)**. Left ventricular ejection fraction (LVEF) remain preserved, but a ventricular arrhythmic burden was observed on telemetry monitoring with frequent premature ventricular complexes (PVC) and non-sustained ventricular tachycardia (NSVT) **(B)**. An FDG-PET showed 3 focal areas of uptake in the heart with increased standardized uptake values (SUV), and an uptake in hilar nodes raising the suspect for cardiac sarcoidosis **(C)**. A septal endomyocardial biopsy (EMB) from the right ventricle was non-diagnostic for myocarditis or cardiac sarcoidosis **(D)**. Peak high sensitivity troponin T (hs-tnT) levels was 2,342 ng/L. After initial pulsed methylprednisolone, prednisone was started in combination with methotrexate (MTX) and later shift to mycophenolate mofetil (MMF) and colchicine plus bisoprolol with normalization of troponin levels, and no signs of residual inflammation on FDG-PET **(C)**, and a reduction of PVC burden. Accordingly, CMRI showed a reduction of T2 signal while LGE remains suggesting an area of residual fibrosis **(E)**.

syndromes [TRAPS] and systemic-onset juvenile idiopathic arthritis [Still's disease]) suggested a common etiological pathway (213–224). Likely, relapsing pericarditis presents family aggregation in 10% of patients (225). FMF is an autosomal-recessive disease that mainly affects patients in the Mediterranean basin (213–218). Symptoms are characterized by self-limiting and recurrent fevers associated to serositis, affecting the pleura, peritoneum, and synovium. Although not common, pericardial effusions are found in 27% of patients with FMF, while typical chest pain is found in about 50% of pediatric patients with FMF. FMF is caused by various missense mutations of the *MEFV* gene, which encodes a pyrin that composes the NLRP3 inflammasome, NOD-like receptor family pyrin domain 3, altering its functionality. Inflammasomes play a fundamental role in innate immunity and can respond to various stimuli, including damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) (226, 227). DAMPs, also known as alarmins, are released from dying cells. They consist of cytosolic or nuclear-derived proteins which, in contact with the extracellular matrix, undergo denaturation processes with consequent activation of the inflammasome through interaction with pattern recognition receptors (PRRs). In this way they give rise to an inflammatory response on a non-infectious basis (termed sterile inflammation). PAMPs, on the other hand, can be identified as phylogenetically conserved molecular patterns in some microorganisms and viruses, which are recognized by toll-like receptors (TLRs), which in turn activate the inflammasome in response to an infection (227). The inflammasome is a cytosolic macromolecule composed of procaspase, ASC adapter protein and a sensor molecule containing a nucleotide-binding oligomerization domain-like receptor (NLR), which is activated by various stimuli. In FMF, functional changes in NLRP3 inflammasome cause an increased activity of the protein complex (228–230) leading to increased caspase1 activity, higher proIL-1beta into IL-1beta cleavage, and higher circulating levels of IL-1B, a master cytokine of inflammation (231). Thus, FMF manifestations are induced by increased IL-1 levels that cause a hyperactive inflammatory state.

TRAPS are autosomal dominantly inherited syndromes characterized by periodic fevers, occurring every 5–6 days for about 1–3 weeks, associated with serositis, migrating myalgia and rash, caused by missense mutation of the TNF-α receptor gene

(219–222, 232). Previous studies reported an incidence of acute pericarditis in 7% of patients with TRAPS, while 25% of these patients reported chest pain with characteristics that resembles typical pericarditis pain (222). There are also oligosymptomatic forms of TRAPS, caused by mutations in TNFRSF1A, and characterized by delayed onset in which pericarditis can be the only manifestation (221). All these observations shed light on the inflammasome, and the hyperproduction of IL-1 in relapsing pericarditis. Similarly, to what observed in the above-mentioned autoinflammatory disorders, in patients with relapsing pericarditis physical injuries *via* DAMPs as well as infectious agents via PAMPs' pathways can elicit inflammasome hyperactivity and IL-1 overproduction.

Pericarditis as an Autoimmune Process

Pericarditis can also be a complication of various autoimmune diseases, including SLE, rheumatoid arthritis (RA), Sjogren's syndrome, Behcet's disease, chronic inflammatory bowel diseases and vasculitis, including giant cell arteritis or ANCA-associated vasculitis (233). In SLE, pericarditis is common, affecting ∼50% of patients, and generally occurs during disease flares. Pericarditis is usually associated with other serositis, malar rash, arthritis and leukopenia. The severity of pericarditis correlates with multiple serosal involvement. SLE therapies are normally effective (234–236). In RA, about 30% of patients have asymptomatic pericardial effusion on echocardiography, but <10% of cases develop symptomatic pericarditis. The incidence of pericarditis in RA patients is higher in those with more severe forms of RA, and higher levels of rheumatoid factor and anti-cyclic citrullinated peptide antibodies (237). Pericarditis can also be the initial sign of a new autoimmune disorder; thus, workup should be prompted after the first episode. Nevertheless, testing for antibodies in all patients with pericarditis is not recommended in the absence of signs or symptoms consistent with an autoimmune disorder (210).

Pericarditis of Uncertain Classification (Post-cardiac Injury)

Myocardial infarction, radiotherapy, cardiac surgery or even minor procedures such as the positioning of pacemaker leads, or radiofrequency ablations can cause pericardial layers' inflammation. Oxidative stress, cell death or tissue damage can produce the release of autoantigens and, due to altered expression or post-translational modifications, these autoantigens could trigger tolerance break after epitope spreading (238). The prevalence of anti-nuclear antibodies is 43% in patients with relapsing pericarditis, while it is 10% in healthy individuals. Similarly, anti-heart antibodies and anti-intercalated disk antibodies are found in 67.5% of patients with relapsing pericarditis (210). The presence of these autoantibodies could be explained by the release of autoantigens by physical tissue injury, then the exposure of autoantigens would trigger a T/B-cell autoimmune response. Alternatively, these autoantibodies can be just an epiphenomenon. Myocardial injury can cause the release of DAMPs and the consequent activation of the inflammasome with IL-1 overproduction. This hypothesis is corroborated by good response to anti-IL-1 drugs in patients with relapsing pericarditis secondary to myocardial or pericardial mechanical injury (239).

Pericarditis as a Systemic Disorder With Pleuro-Pulmonary Involvement

Diseases of the pericardium can be isolated or be part of a systemic condition associated a striking increase in CRP levels, erythrocyte sedimentation rate (ESR) values and neutrophilia (240–242). Approximately 53% of cases have associated pleuro-pulmonary involvement, 9% have hepatic involvement and 5% have peritoneal involvement (242). These conditions are observed more frequently in the pediatric population. Chest CT scan generally shows bilateral pleural effusion with areas of pulmonary atelectasis. Misdiagnosis with pneumonia can lead to antibiotic therapies, especially at the onset when pericardial effusion is mild. When final diagnosis of pericarditis is reached, NSAIDs (e.g., Ibuprofen 600 mg tid) and corticosteroid therapy can improve the condition. Too rapid steroid tapering can lead to pericarditis recurrence and a corticosteroid-dependent condition.

Pericardial Effusion

Pericardial effusion can be isolated or frequently associated with an underlying pericarditis (243). The symptoms span from absent or mild to severe, especially in case of rapid formation. The pericardium tends to adapt better to slowly progressing effusions, while it tends to give compression phenomena when the effusion develops abundantly and rapidly.

Pericardial effusion can result by pericarditis, edematous syndromes including HF and kidney failure, cancer, infectious diseases (i.e., tuberculosis), serositis and autoimmune diseases, and hypothyroidism (3, 212, 244, 245), even if idiopathic pericardial effusion can often occur. A pericardial effusion is defined as chronic when it lasts for more than 3 months and severe when it exceeds 20 mm in thickness. Among 100 patients with severe (>20 mm), and chronic (>3 months) idiopathic pericardial effusion, 44 patients were asymptomatic, while 56 presented with symptoms, of these 28 presented with dyspnea; 33 patients had diabetes mellitus (246). One subset of patients was symptomatic with a higher age, more likely to be diabetic, with hypertension, chronic obstructive pulmonary disease and atrial fibrillation; whereas a second subset was generally asymptomatic, younger without significant comorbidities. After a mean follow-up of 50 months, no pathology that could explain the pericardial effusion was identified and complete regression of the effusion was observed in 39%. Adverse events were observed in 38 patients, of which 8 developed cardiac tamponade (2.2%/year). Among the 100 patients, 30 underwent pericardiocentesis, 12 underwent pericardial windowing and 3 underwent pericardiotomy. Patients who underwent some invasive procedure presented worse outcomes in terms of relapse or complications than untreated patients. This study seems to emphasize that the risk of developing cardiac tamponade is quite low and therapeutic strategies should be tailored on an individual basis based on symptoms. An echocardiographic evaluation every 3–6 months is recommended for the follow-up of these patients, while invasive techniques such as pericardiocentesis or

pericardiotomy, if separated from specific symptoms, are not recommended (246). Furthermore, we recently showed that a chronic pericardial effusion is present in 37% of subjects with pectus excavatum, with the size of effusion being related to the anatomical severity of the condition, and these effusions have a good prognosis (247). Thus, in presence of chronic pericardial effusion not related to pericarditis, often with normal or near-normal serum CRP, we do not recommend any therapy, in particular, we avoid immunosuppressive therapies since there is no evidence of benefit. Low-dose corticosteroids might be considered in few selected patients on a case-by-case basis, but at present no literature deals with this topic. A study reported good efficacy and safety of intrapericardial triamcinolone in patients affected by autoreactive pericarditis with pericardial effusion (248): the use of an intrapericardial route may avoid the typical side effects of the systemic use of corticosteroids. Thus, intrapericardial use of triamcinolone remains a viable therapeutic option for patients with pericarditis and pericardial effusion. Anti-inflammatory or immunosuppressive therapies are often started because the analysis of pericardial fluid is considered suggestive of inflammation, based on the Light's criteria validated for the evaluation of pleural fluid. Data from a recent study determined the reference values of analytes and cells in pericardial fluid (249). Specifically, proteins are 1.7–4.6 g/dl, albumin 1.19–3.06 g/dl, LDH 141–2613 UI/L, total protein in pericardial fluid/serum ratio 0.29–0.83, LDH in pericardial fluid/serum ratio 0.4–42.99. According to the Light's criteria (250), pleural fluid is defined as inflammatory when at least one of the following criteria is satisfied: fluid/serum protein ratio >0.5, fluid/serum LDH ratio >0.6, and fluid LDH >2/3 of the upper limit for serum levels. The new reference values observed in this population should lead to a reappraisal concerning the classification of pericardial fluid as exudate or transudate based on Light's criteria. Efforts should be taken to stop interpreting pericardial fluid as an exudate or transudate based on evaluation tools that are not validated for this type of fluid, given the risk of misinterpreting non-inflammatory effusions into inflammatory exudates. Elevated LDH found in physiological pericardial fluid might be caused by the release of LDH by mesothelial cells, which are particularly abundant in normal pericardial fluid (249).

COVID-19 Associated and mRNA COVID-19 Vaccine-Related Acute Pericarditis

Based on a retrospective cohort study, of 718.365 patients with COVID-19, 10.706 (1.5%) developed new-onset pericarditis. Six-month all-cause mortality was 15.5% ($n = 816$) for pericarditis and 6.7% ($n = 356$) in matched controls ($p < 0.0001$), odds ratio 2.55 (95% CI: 2.24–2.91) (251). At present, only 2 published studies focused the attention toward anti-COVID-19 vaccine-related acute pericarditis. Barda et al. reported in Israel an incidence of 26 cases out of 884.828 vaccinated individuals (3/100.000) vs. 18 out of 884.828 unvaccinated controls (2/100.000); RR 1.27 (p = non-significant) (124). Diaz et al. described 37 cases in US, with an incidence of 1.8/100.000 (252). The mean monthly number of cases of pericarditis during the prevaccine period was 49.1 (95% CI, 46.4–51.9) vs. 78.8 (95% CI, 70.3–87.9) during the vaccine period ($P < 0.001$). A total of 15 cases occurred after the first dose and 22 after the second dose; 27 out of 37 subjects were males and median age was 59 years; 13 were admitted to the hospital (median stay, 1 day), none to intensive care. No patient died.

THERAPY OF PERICARDITIS

NSAIDs

NSAIDs represent the first line of therapy, exerting their action both on the pathogenesis of pericarditis and on the control of symptoms. Understanding the role of inflammasome in the pathogenesis of relapsing pericarditis explains their effectiveness (**Figure 6**). Numerous NSAIDs are used for relapsing pericarditis therapy, including ibuprofen, indomethacin and acetylsalicylic acid (ASA). All these NSAIDs are recommended in high doses as the first line of pericarditis treatment by the European Society of Cardiology (**Figure 7**). The duration of treatment is variable, but in any case, prolonged (210, 253).

Colchicine

The rationale behind the use of colchicine in pericarditis arises from the observation of good efficacy results in the control of serositis during FMF and subsequently in the control of pericarditis associated with other serositis during FMF (254). Colchicine performs its functions by inhibiting the activation of pore formation carried out by P2X2 and P2X7 receptors, that concur to the activation of inflammasome, and by inhibiting NACHT-LRRPYD-containing protein 3 inflammasome (255). The combined use of NSAIDs and colchicine has produced positive results on pericarditis in numerous clinical trials, where their use has favored both the control of symptoms and the prevention of relapses (255–261). Colchicine during relapsing pericarditis should be administered early without loading dose and its dosage might be adapted to the patient's weight: in general, we start with a dose of 0.5 mg per day and, if tolerated, the dose is then increased to 0.5 mg BID or 1 mg OD, based on compliance and tolerability. The most frequent side effects are gastrointestinal, with diarrhea that occurs mainly at the beginning of therapy in ∼10%. The dosage of colchicine can possibly be reduced in patients with this type of disorder.

Corticosteroids

The use of corticosteroids in pericarditis remains controversial. If they find their indication for the forms of pericarditis caused by autoimmune processes or in the forms resistant to the combined therapy of NSAIDs and colchicine, the probability of generating dependence for the control of symptoms is high (210). Many patients will experience a recurrence of pericarditis upon discontinuation of corticosteroid therapy, especially when corticosteroid tapering is too rapid, thus leading to a real dependence on corticosteroids and the risk of a prolonged use (253). Chronic use of corticosteroids is burdened by numerous side effects, including weight gain, osteoporosis and possible vertebral collapse, diabetes mellitus and Cushing's syndrome (262). For this reason, the use of corticosteroids should be

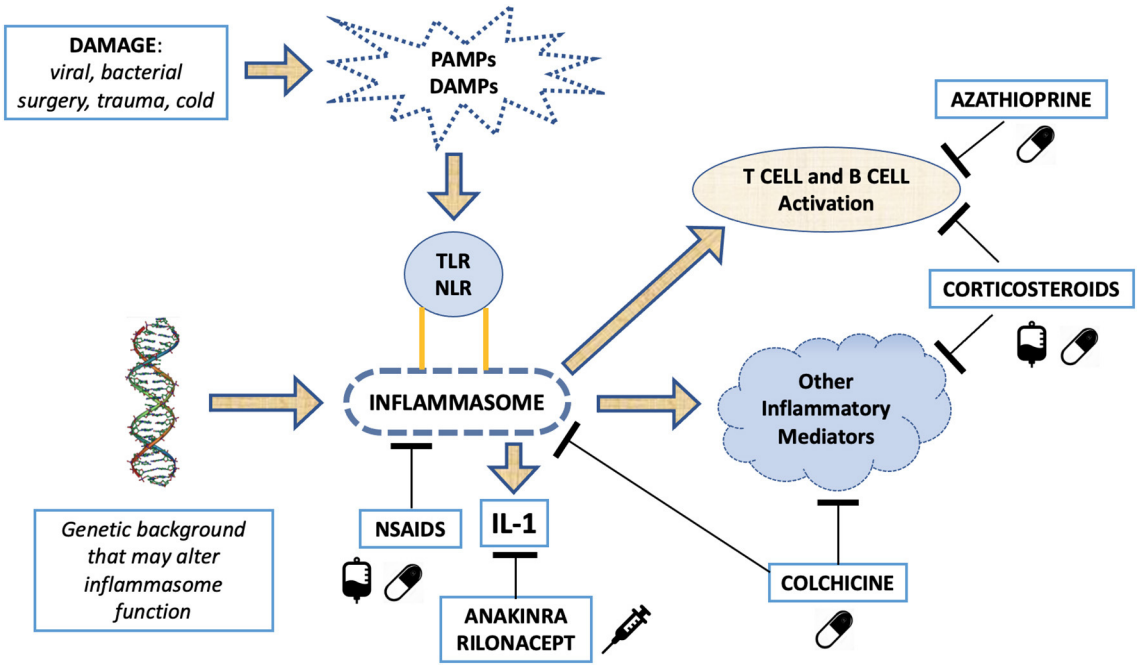

FIGURE 6 | The inflammasome-mediated inflammatory cascade and the location of the effect of currently available drugs for recurrent pericarditis. Non-specific triggers such as structural damage or microbial agents may interact with specific receptors such as NLR and TLR and thus activate the inflammasome. This naturally occurs, but a genetic background may alter the inflammasome response and consequently generate a pathologic response with a sustained inflammatory state. NSAIDs perform their effect directly on inflammasome activation. Azathioprine and corticosteroids carry out their effect mainly on B and T lymphocytes. While Anakinra and Rilonacept directly inhibit IL-1 effects, both colchicine and corticosteroids perform their action on other inflammatory mediators released after inflammasome activation. Furthermore, colchicine also exerts an inhibitory effect on inflammasome activation. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; TLR, toll-like receptor; NLR, NOD-like Receptor; NSAIDs, non-steroidal antiinflammatory drugs.

restricted to forms of pericarditis on an autoimmune basis or in forms in which NSAIDs and colchicine have been found to be ineffective and a contraindication to the use of anti-IL-1 drugs coexists. Bisphosphonates and vitamin D should be considered when corticosteroids are started, as they are often kept as long-term maintenance therapy.

Azathioprine

AZA is a prodrug that is converted into 6-mercaptopurine, and which exerts its action at intracellular level through the production of thioinosinic and thioguanilic acids, interfering with the production of adenine and guanine and therefore, consequently, with the production of deoxyribonucleic and ribonucleic acid. Its use in autoimmune diseases and chronic intestinal inflammatory diseases has produced good efficacy and safety data (263). During relapsing pericarditis, AZA can represent an effective therapeutic aid: it is well-tolerated and has shown good efficacy profiles especially as a corticosteroid-sparing agent (264). However, larger clinical trials on its use in relapsing pericarditis are lacking.

Intravenous Immunoglobulins

The use of IVIG in autoimmune diseases such as autoimmune thrombocytopenic purpura, Guillain-Barré syndrome and autoimmune demyelinating polyneuropathies or in pregnant women with SLE is now well-established (265–268). IVIG carry out their function through the blocking of the Fc-gammaRIIB receptors on macrophages and in general through the blocking of the Fc receptors. IVIG are administered at a dose of 400–500 mg per kg of body weight with one intravenous administration per day for 5 consecutive days, possibly followed by another cycles of administration at 1 month. The use of IVIG during pericarditis is limited to a few case series, and it may find a rationale in autoimmune-based forms (269).

Emerging Treatments: Anti-IL-1 Agents

The understanding of the autoinflammatory pathogenetic mechanisms, mediated by the inflammasome, in the genesis of relapsing pericarditis has shed light on IL-1 as possible therapeutic target. All drugs blocking the action of IL-1 can represent an opportunity for the control of relapsing pericarditis (270, 271). Three anti-IL-1 drugs are currently being produced, anakinra, rilonacept and canakinumab. These drugs, but especially anakinra and rilonacept, have been studied to identify their efficacy and safety profiles in patients with relapsing pericarditis.

Anakinra

Anakinra is a short-acting IL-1 receptor antagonist for daily subcutaneous administration with doses of 100 mg qd. It was approved in 2001 by the Food and Drug Administration (FDA) for the treatment of RA and juvenile idiopathic arthritis.

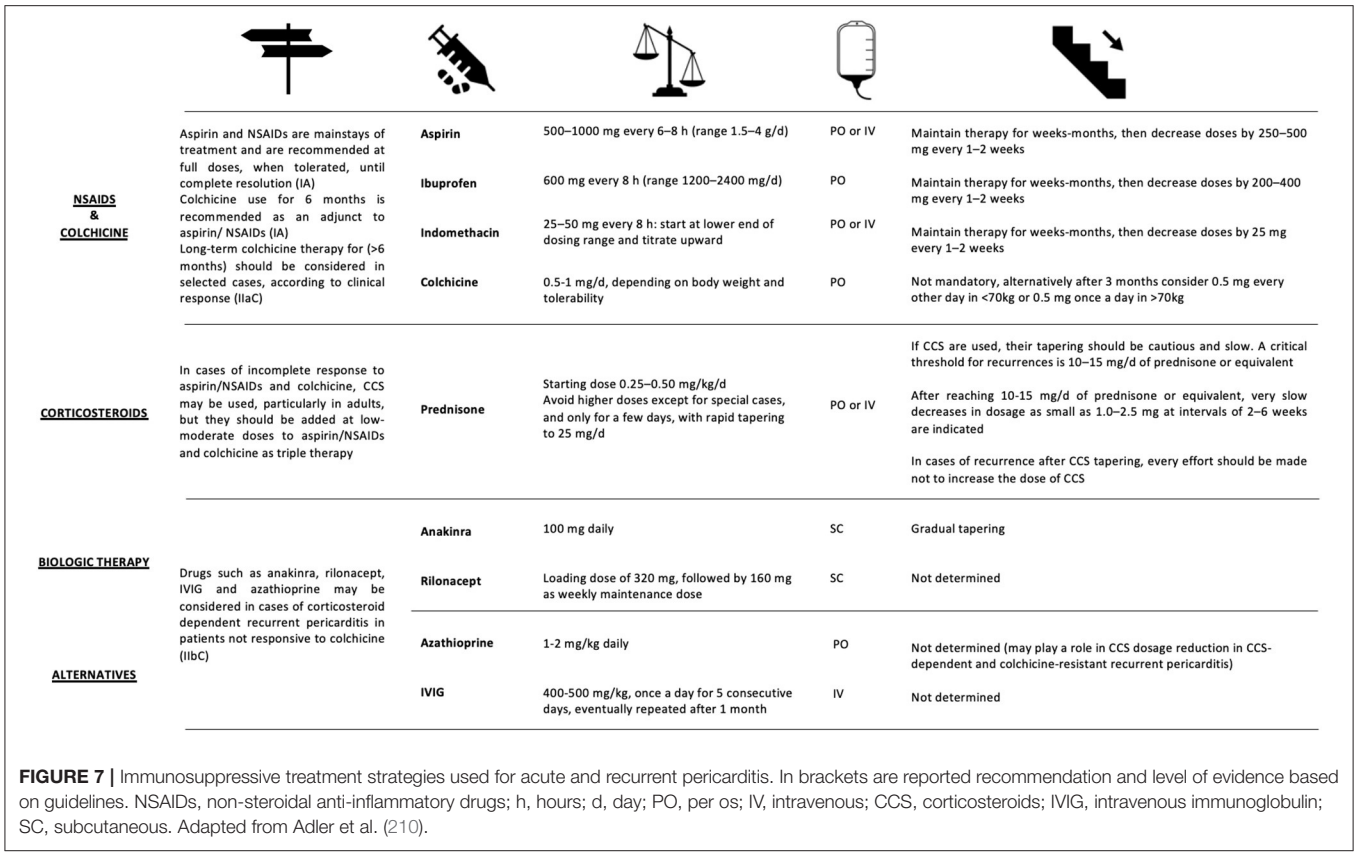

FIGURE 7 | Immunosuppressive treatment strategies used for acute and recurrent pericarditis. In brackets are reported recommendation and level of evidence based on guidelines. NSAIDs, non-steroidal anti-inflammatory drugs; h, hours; d, day; PO, per os; IV, intravenous; CCS, corticosteroids; IVIG, intravenous immunoglobulin; SC, subcutaneous. Adapted from Adler et al. (210).

Anakinra does not require dosage adjustments for the patient's age, gender or body mass index, while dosage adjustments are recommended for patients with renal impairment with a glomerular filtration rate (GFR) <50 ml /min*1.73 m^2 (272). The most common side effect is the formation of reddish, slightly burning and itchy skin plaque at the injection site (273). These cutaneous lesions tend to form mainly in the 1st months of therapy and can be reverted by the use of local ice or topical application of corticosteroids. Transient and mild increases in transaminase levels or leukopenia can also occur. Latent tuberculosis reactivation has been reported during the use of anakinra, leading to screening test for latent tuberculosis before starting anakinra (274). In addition, anakinra is contraindicated in patients with hypersensitivity to *E. coli* derived proteins. The use of anakinra in cardiovascular diseases is currently under investigation in myocardial infarction, HF, AM and pericardial disease (45). In the AIRTRIP study (271), the efficacy of anakinra was tested in a randomized, double-blind placebo-controlled trial in 21 patients with relapsing pericarditis who were colchicine-resistant and corticosteroid-dependent. In the group of patients taking anakinra, only 18% experienced a recurrence vs. 90% in patients in the placebo arm. This effect also occurred in patients with relapsing pericarditis secondary to post cardiac injury pericarditis. During the AIRTRIP study, only mild adverse events were observed. In the IRAP (International Registry of Anakinra for Pericarditis) registry, the efficacy of anakinra in reducing the dose of corticosteroids was also demonstrated in patients affected by relapsing pericarditis that was colchicine-resistant and corticosteroid-dependent for symptoms control, with a reduction of the percentage of patients needing corticosteroids for symptoms control from 80 to 27% ($p < 0.001$) (275). Tapering of anakinra in relapsing pericarditis should be very slow, as new disease flares have been reported in patients who abruptly stopped the drug and in patients who discontinued its use in <3 months (275).

Rilonacept

Rilonacept is a dimeric fusion protein formed by ligand-binding domains of IL-1R and the accessory IL-1 receptor protein linked to FC portion of human IgG1. It exerts its actions by blocking both IL-1 α and IL-1B (276). FDA approved its use in CAPS, and recently also in relapsing pericarditis (277, 278). The RHAPSODY study tested its use in patients with relapsing pericarditis associated with high CRP levels. RHAPSODY is a multicentric, double-blind, randomized trial in 86 patients with relapsing pericarditis, diagnosed based on the 2015 ESC criteria during at least a second relapse despite NSAIDs, colchicine and corticosteroids treatment or any combination of these three drugs (279). Starting dose was 320 mg, followed by weekly doses of 160 mg for 12 weeks of run-in period. All other drugs to prevent relapse were discontinued. All patients achieving a clinical response were then double-blind randomized to continue rilonacept therapy or starting placebo. Only 7% of patients experienced a new flare of disease in the rilonacept arm, while

74% of patients in the placebo arm had a pericarditis recurrence. Adverse events were reported in 74 out of 86 patients and were all categorized as mild to moderate, with mainly injection site reactions and mild upper respiratory ways infections. In four patients, adverse events led to discontinuation of therapy. Based on the results of the RHAPSODY trial (279), FDA approved rilonacept for the treatment of recurrent pericarditis in March 2021.

Canakinumab

Canakinumab is a human monoclonal antibody directed against IL-1B, which compared to anakinra has a much longer half-life, i.e., about 22–26 days, allowing an administration every 4–8 weeks (150 mg by subcutaneous injection in adults) (280, 281). Canakinumab is approved for FMF, CAPS, TRAPS, systemic-onset idiopathic juvenile arthritis and gouty arthritis (282–288). Data regarding its use in relapsing pericarditis are limited. Canakinumab was used in a case series where the use of anakinra was avoid due to adverse reactions, and data from this study were encouraging (289), but further evidences seemed contradictory (290). Canakinumab only blocks IL-1 B, while anakinra and rilonacept block both IL-1 α and IL-1B, probably explaining the better results of the latter.

Candidates for Anti-IL-1 Agents

It is important to identify the right candidate for anakinra and rilonacept (291, 292). Patients with an overt inflammatory phenotype, suffering from pericarditis with pleuropulmonary involvement, with elevated CRP levels, fever, neutrophilic leukocytosis, with repeated hospitalizations for pericarditis, are the best candidates for anti-IL-1 therapy. Prior to the administration of anti-IL-1 drugs, guideline-driven therapy should be administered, with the use of a combination of NSAIDs and colchicine. Anti-IL-1 agents could be considered before corticosteroids, and this is particularly true for pediatric patients. Also, anakinra and rilonacept may be used in patients where use of NSAIDs or corticosteroids is contraindicated, such as anticoagulated patients, patients with renal failure, gastrointestinal hemorrhages, ischemic heart disease or recent cardiac surgery. On the contrary, their use is contraindicated in pericardial effusion or in aspecific/atypical presentations of chest pain with normal serum levels of CRP.

IMAGING TO GUIDE IMMUNOSUPPRESSIVE THERAPY IN PERICARDITIS

Echocardiography is the first-line imaging tool when acute pericarditis is suspected. Although no abnormalities are seen in around 40% of cases, the presence of new or worsening pericardial effusion is considered diagnostic (293, 294). In uncomplicated cases with no or a small effusion, further imaging is usually not required. In case a moderate or large pericardial effusion is present, its hemodynamic consequences can be assessed with Doppler echocardiography. Echocardiography is also the main tool to guide pericardiocentesis in case of tamponade, and to evaluate residual effusion during follow-up.

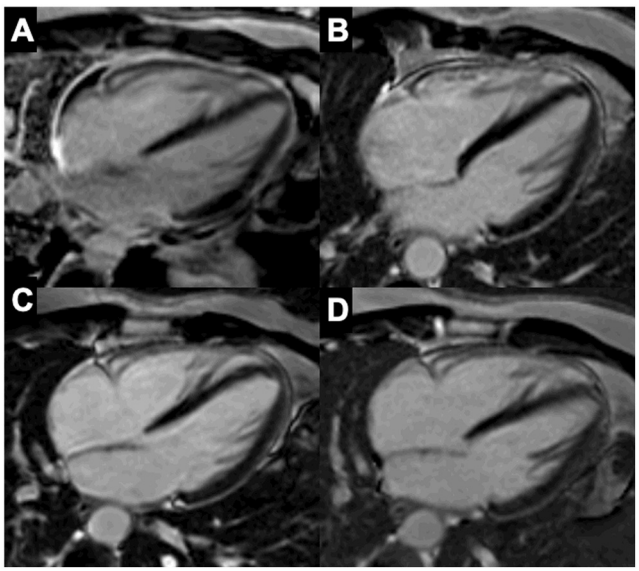

FIGURE 8 | Representative patient with pericarditis and resolution of the pericardial inflammation on sequential CMRI scans. Four-chamber late gadolinium enhancement (LGE) PSIR cardiac MRI images of a 35-year-old male patient who presented with recurrent idiopathic pericarditis under NSAIDs and colchicine. **(A)** At presentation, there was diffuse pericardial thickening and LGE, and presence of pericardial effusion. High-dose corticosteroids (prednisolone 40 mg once daily) with taper schedule and azathioprine were initiated. **(B)** Re-evaluation after 3 months showed regression of pericardial LGE and disappearance of pericardial effusion. This allowed to further decrease the dose of steroids. **(C)** Follow-up cardiac MRI 5-months later showed near resolution of pericardial LGE. A low dose of steroids (prednisolone 4 mg once daily) was maintained. **(D)** Control after 1 year showed a normal pericardium without LGE. Image courtesy of Bernard Paelinck (Antwerp University Hospital).

Other imaging modalities can be useful in patients with acute pericarditis and poor echocardiographic image quality in specific settings (e.g., complicated course, large effusions), or in dubious cases, e.g., with normal CRP. On CMRI, pericardial thickening (>3 mm) and LGE, which reflects increased vascularity, are both sensitive and specific signs of active pericarditis (295). Pericardial edema can be assessed by T2-weighted STIR imaging on CMRI but might be difficult to distinguish from pericardial effusion (296). In complicated cases with relapsing pericarditis, CMRI is not only a useful tool to assess constrictive physiology and ongoing pericardial inflammation, but also to guide treatment. In a study by Feng et al. (297), it was shown that constrictive pericarditis can be reversible after anti-inflammatory therapy (NSAIDs, colchicine and/or steroids) if pericardial LGE is present on cardiac MRI. Close follow-up to evaluate improvement of hemodynamics, and pericardial effusion if present, under medical treatment by echocardiography or CMRI is recommended (293). Moreover, CMRI using T2-weighted STIR and LGE sequences can monitor the degree of pericardial inflammation (**Figure 8**), hereby

providing important information for the clinician before anti-inflammatory or immunosuppressive medication is tapered off (296). Cardiac CT can be used to detect pericardial effusion and pericardial inflammation after contrast but implies ionizing radiation. In chronic forms of constrictive pericarditis, cardiac CT is particularly useful to assess pericardial calcifications for planning of a pericardiotomy. FDG-PET has been used to visualize pericardial inflammation and metabolic activity (298), but it is seldom performed in clinical practice and is not recommended by current guidelines (293). Nevertheless, a small study in 16 patients showed that a high FDG uptake (SUV_{max} >3.0) predicted reversibility of constrictive pericarditis with steroid treatment (299). In this respect, FDG-PET may be useful in patients with non-CMRI-conditional devices to guide immunosuppressive therapy, but further studies are needed to evaluate whether it provides incremental value to CMRI.

CONCLUSIONS

While therapies for patients with acute and recurrent pericarditis are mainly evidence-based, almost no trials are available for AM, thus immunosuppression in this setting is generally based on expert consensus. Thus, an impelling need of clinical research is to evaluate which immunosuppressive agent can be effective to improve the outcome of patients with AM and the characteristics of patients who can benefit more by immunosuppression. Thus, well-powered multicenter randomized trials are needed to test this hypothesis. In parallel, large prospective registries can better define the main determinants of outcome, even if large retrospective studies consistently demonstrated that presentation of AM complicated by reduced LVEF, HF, VA, AVB, or cardiogenic shock are associated with poor outcome (11, 40).

AUTHOR CONTRIBUTIONS

EA, EB, GV, MG, CV, JL, MP, AC, CP, LT, JM, and AB wrote the draft. EA, EB, GV, MG, JM, and AB revised the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.838564/full#supplementary-material

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Conflict of Interest: EA received a grant from the Italian Ministry of Health (GR-2019-12368506). AB: Institution received funding from Kiniksa Pharmaceuticals, Ltd., as an investigative site; unrestricted research grant from SOBI and ACARPIA; travel and accommodation for advisory committee from SOBI and Kiniksa. JM has served on advisory boards for Bristol Myers Squibb, Pfizer, Takeda, Audentes, Deciphera, Janssen, ImmunoCore, Myovant, Cytokinetics, AstraZeneca, ProteinQure, and Pharmacyclics. JM was supported by the National Institutes of Health (R01HL141466, R01HL155990, and R01HL156021). MG received consulting fees from Astrazeneca and GlaxoSmithKline.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 08 April 2022
doi: 10.3389/fmed.2022.849086

Endothelial Dysfunction in Systemic Lupus Erythematosus and Systemic Sclerosis: A Common Trigger for Different Microvascular Diseases

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This review describes the complex interplay between inflammation, vasculopathy and fibrosis that involve the heart and peripheral small vessels, leading to endothelial stiffness, vascular damage, and early aging in patients with systemic lupus erythematosus and systemic sclerosis, which represents two different models of vascular dysfunction among systemic autoimmune diseases. In fact, despite the fact that diagnostic methods and therapies have been significantly improved in the last years, affected patients show an excess of cardiovascular mortality if compared with the general population. In addition, we provide a complete overview on the new techniques which are used for the evaluation of endothelial dysfunction in a preclinical phase, which could represent a new approach in the assessment of cardiovascular risk in these patients.

Keywords: endothelial dysfunction, systemic lupus erythematosus, systemic sclerosis, microvascular disease, techniques of assessment

OPEN ACCESS

Edited by:
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University College London,
United Kingdom

Reviewed by:
George Bertsias,
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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: *05 January 2022*
Accepted: *04 March 2022*
Published: *08 April 2022*

Citation:
Moschetti L, Piantoni S, Vizzardi E, Sciatti E, Riccardi M, Franceschini F and Cavazzana I (2022) Endothelial Dysfunction in Systemic Lupus Erythematosus and Systemic Sclerosis: A Common Trigger for Different Microvascular Diseases. Front. Med. 9:849086. doi: 10.3389/fmed.2022.849086

INTRODUCTION

Systemic autoimmune diseases are disorders characterized by humoral and cell-mediated immune responses against various self-antigens. A higher cardiovascular (CV) morbidity and mortality rates were described in affected patients (1). Persistent low-grade inflammation in the vascular wall is considered the crucial trigger for CV events through endothelial dysfunction (ED) and proliferation of vascular smooth muscle cells, with subsequent vascular remodeling (2). Furthermore, the infiltration of different immune cells promotes a *milieu* of molecules that contributes to the perpetuation of inflammation itself. ED is currently considered the main mechanism explaining the microangiopathy in different clinical autoimmune conditions. An insufficient endothelium-dependent vasodilation in reply to vasoactive stimuli, principally due to the failing production of nitric oxide (NO) and/or an impaired NO function, defines ED. ED has been detected in different types of arterial vessels, and actually it is considered a systemic process (3, 4). Among systemic autoimmune diseases, ED has been extensively studied in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), which represent two different models of ED dysfunction. In SLE patients, ED is the main actor of vascular aging and pre-clinical atherosclerosis during the course of the disease, contributing to the early onset of CV disease (CVD)

and CV mortality. On the other hand, in SSc, ED and microangiopathy are key factors sustaining the development of the disease itself. The aim of this review is to analyze the factors which has a role in the pathophysiology of ED in SLE and SSc and to explore the new techniques which could be used in its evaluation in a pre-clinical phase. In fact, traditional Framingham risk factors do not fully explain the increased CV risk in rheumatic diseases (5) and, although CV risk assessment should be part of routine assessment in patients, no disease-specific models are currently available for this purpose (6, 7). Recently, the European Alliance of Associations for Rheumatology (EULAR) published some recommendations for CV risk management in these patients, suggesting the need of a precocious diagnosis without the endorsement of the use of any particular assessment tool (8).

SYSTEMIC LUPUS ERYTHEMATOSUS AND ENDOTHELIAL DYSFUNCTION

Systemic lupus erythematosus is a chronic systemic autoimmune disease characterized by a dysregulation of immune system, leading to autoantibody production, tissue inflammation, and organ damage. Since approximately 40 years, SLE is known to display a raised mortality, due to premature CVD (4). Compared to the general population, the prevalence of CVD is known to be at least double in SLE patients (9, 10), especially in young premenopausal women (11). Accelerated atherosclerosis, estimated to develop or progress in 10% of SLE patients each year (12) and that is globally sixfold more frequent in SLE compared with the general population (13), is associated to this premature CVD. Although a high cardiometabolic risk has been described in SLE (14), CVD in SLE displays atypical features, such as presentation in young women and a lack of a clear protective effect by statins (15, 16). Early CVD in SLE is known to be associated with ED and stiffness of vascular tree, that lead to atherosclerosis and clot formation, involving different pathogenetic mechanisms (17).

Pathogenesis of Endothelial Dysfunction in Systemic Lupus Erythematosus

Several mechanisms have been proposed to explain ED and atherosclerosis in SLE (18, 19), resulting in a clear predominance of injury stimuli versus protection factors on the layer of endothelial cells (ECs).

Oxidative Stress

Mitochondrial dysfunction and abnormal telomere/telomerase balance lead to a persistent oxidative stress in SLE, mainly involving circulating leukocytes and ECs (20). The oxidative process induces cell adhesion molecules (CAMs) expression (21), with consequent higher leucocyte-endothelial cell interactions and leucocytes' transmigration to sites of inflammation (22). In addition, a significant association between higher anti-double stranded-DNA (anti-dsDNA) antibodies levels and higher levels of oxidative products was reported (23, 24). The excessive production of reactive oxygen and nitrogen species (ROS and RNS) leads to modifications of different cellular molecules, such as proteins, lipids, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), generating neo-antigens with a consequent production of autoantibodies, and uncontrolled lymphocytes' activation (23, 25). In SLE, three main targets of oxidative stress have been identified: oxidized lipids, oxidized low-density lipoprotein (LDL) and proinflammatory high-density lipoprotein (HDL), all playing a crucial role in pathogenesis of SLE-related ED and atherosclerosis (26, 27).

Cytokine Cascade

Proinflammatory cytokines play a direct role in accelerating SLE atherosclerosis. In particular, all three classes of interferons (IFNs), namely IFN-I (IFN-α, IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, IFN-ω, and IFN-ζ), IFN-II (IFN-γ), IFN-III (IFN-λ1, IFN-λ2, and IFN-λ3), participated in the process of atherosclerosis (19). IFN-α and IFN-γ promote lipoproteins' oxidation (28, 29) and ED by accelerating ECs apoptosis and damaging endothelial progenitor cells (EPCs) (28, 30), one of the vascular repair mechanisms. On the other hand, IFN-γ increases vascular smooth muscle cells' (VSMC) proliferation and migration (31), VSMC and macrophages apoptosis in atherosclerotic plaques, inducing plaque instability (32). The long-term activation of IFN-I system induces the expression of different chemokine pathways that recruit leukocytes into inflammatory sites promoting the dysfunction of ECs and EPCs (19).

Neutrophil Extracellular Traps

Neutrophil Extracellular Traps (NETs), a unique type of neutrophils communication characterized by the extrusion of chromatin and other molecules, are considered a key factor in SLE atherosclerosis (33). NETs can enhance vascular leakage, endothelial-to-mesenchymal transition (34) and ECs death (35). Moreover, NETs enhance oxidation processes (36), secretion of IFN-α (37), interleukin (IL)-1β (38), and activate coagulation cascade (39).

B Cells and Autoantibodies

Many autoantibodies can affect endothelial function, by promoting pathogenic molecules and inhibiting potential protective factors (40). Antiphospholipid antibodies (aPL), that are anticardiolipin antibodies (aCL) and anti-β2-glycoprotein I antibodies (anti-β2GPI), can contribute to accelerated atherosclerosis by inducing a proinflammatory endothelial phenotype through a direct interaction with ECs (41). Different authors described ECs activation by aPL via EC-derived extracellular vesicles through a toll like receptor (TLR) 4 and 7-dependent pathway, resulting in paracrine stimulation of neighboring unstimulated ECs (42–44). In addition, aPL can upregulate the tissue factor expression on ECs and monocytes, and promote endothelial leukocyte adhesion and pro-inflammatory cytokine secretion (41). Finally, aPL are considered an independent predictor of atherosclerotic plaque progression in SLE (45). Other autoantibodies have been described as contributors of accelerated atherosclerosis in SLE: anti-HDL-IgG that induce LDL to enter the ECs; anti-apolipoprotein A1 (ApoA1)-IgG that activating the

transcriptional nuclear factor kappaB (NF-kB) favor the expression of inflammatory factors at endothelial level (46); anti-FXa-IgG can inhibit FX enzyme (47), modifying the of hemostasis/thrombosis equilibrium and promoting ED (48). Moreover, anti-C1q antibodies play a role in atherosclerosis by reducing C1q's level and lowering their protective effects on endothelium (49, 50).

T Cell Subpopulations

In general, the subset of CD28- (CD28null) T cells is characterized by pro-inflammatory properties and plays an active role in destabilization of the plaque itself, increasing endothelial oxidative markers, and arterial stiffness (51). In humans, high levels of CD4+CD28null T cells, responsible of an aberrant T-B lymphocytes' interaction, have been described during instable angina, and could be involved in the atherosclerotic plaque instability (52). The prevalence of these cells is increased in systemic autoimmune diseases because of the repeated antigenic stimulation that induces a downregulation of CD28 from the lymphocytes' membrane (53). The so-called angiogenic T cells (Tang) are characterized by the expression of CD3, the platelet-endothelial cell adhesion molecule-1 (CD31) and the receptor for stromal cell factor-1 CXCR4 (54). Due to their ability to enhance endothelial repair function (55) and promote new vessel formation (54), Tang could be used as a novel putative biological marker for CVD. A higher number of circulating Tang may be involved in ED among several autoimmune diseases, such as rheumatoid arthritis (RA), SLE and SSc, as a consequence of endothelial damage or an inefficient angiogenesis (56–58). Accordingly, in a recent study of our group, we demonstrated that the nail video-capillaroscopy (NVC) alterations in a cohort of patients with SLE and without traditional CV risk factors were associated with ED and with the increase of circulating Tang (59). A subtype of Tang called "aging" Tang (CD28null-Tang) seems to be not protective but cytotoxic, due to their ability to secrete inflammatory mediators and release cytolytic molecules from intracellular particles, inducing EC damage and accelerated atherosclerosis in most SLE patients (60). Moreover, CD28null-Tang increased in SLE patients with traditional CV risk factors and active disease (60). In our recent experience, we observed that the rate of circulating pro-angiogenic Tang decreased very early in disease course, with an increase of the rate of the "aging" CD28null subset. Our preliminary data suggest that Tang might exert their effects on the endothelium via the pro-angiogenic mediators IL-8 and metalloproteinase-9 (61). Another T lymphocyte subtype, regulatory T cells (Treg), are believed to play a protective role in autoimmune diseases. Anyway, atherosclerosis's severity does not seem to be strictly related to their numbers, but rather to their dysfunction (62, 63). In SLE, Treg cells are significantly reduced in both, number and function (64). In human studies and mouse models, Treg have been associated with a protective role in atherosclerosis (65) and their decrease is significantly associated with acute coronary events (18). Recently, the invariant natural killer T cells showed an anti-atherosclerotic phenotype in SLE patients and can induces macrophages to polarize into anti-inflammatory and anti-atherosclerotic M2 phenotype (66).

Endothelial Progenitor Cells

Endothelial progenitor cells are a group of bone marrow-derived cells, acting in vascular homeostasis control and endothelial repair (67). Some authors reported a reduced number of EPCs in patients with CV risk factors (68) and CVD (69). Therefore, EPCs could be considered a new marker of CV risk, especially in SLE patients in which traditional CV prediction models fail to estimate the risk of clinical CVD. Physiologically, after endothelial injury, vascular repair occurs by accelerating the replacement of ECs: a process that involves proliferation and migration of adjacent ECs and resident EPCs and recruitment of new EPCs. Although data in SLE are controversial, EPCs are reduced in number and are functionally impaired (19). This impairment seems to be the result of the balance between risk factors (including IFN-I) and protective factors (including Tang cells). In particular, IFN-I accelerates SLE atherosclerosis, by interfering with EPCs (19), as suggested by studies in adult- or childhood-onset SLE (67, 70). The results among studies are difficult to be compared because EPCs could be identified using different and not yet standardized methods, such as flow cytometry or through different cell isolation techniques (67). Type I IFN, overexpressed during a SLE flare and involved in SLE pathogenesis, was described as a contributor of EPCs dysfunction in the disease (67). Furthermore, some data demonstrated that recombinant IFN-α displays a toxic effect on CD133/CD34 + cells (e.g., putative EPCs) in culture. The use of monoclonal antibody blocking IFN pathways in SLE leads to a normalization of EPCs function (71).

Cardiovascular Disease Risk Assessment in Systemic Lupus Erythematosus

Systemic lupus erythematosus represents a good example of autoimmune disease associated to an inflammatory-related early atherosclerosis. It is widely known that SLE patients have a significant risk of CVD, presenting a higher rate of atherosclerotic large arterial vessels, as well as in RA and diabetes mellitus (72). Furthermore, as compared to the general population, SLE patients have a twofold increased rate of ischemic myocardial infarction (73, 74). The presence of lupus nephritis and aPL represents further risk factors for CVD in SLE (75). According with guidelines (8), the assessment of traditional but also the disease-related risk factors is recommended in SLE patients. A modified version of the Framingham risk score that used 2 as multiplicative factor was showed to increase the sensitivity in identifying patients with an increased risk of coronary artery disease (76). It became necessary to develop a SLE-specific CV risk score that combines traditional CV risk factors and SLE-specific variables: only disease activity score, C3 level, and lupus anticoagulant titer were predictive of CV outcomes (77). Petri et al., determined that patients with higher SLE disease activity index (SLEDAI) score had their 10-year risk underestimated by as much as a factor of 10 (78). Inaccurate CVD risk assessment is evident especially in young SLE patients, that are not likely to experience adverse CV events within 10 years: for these patients a more complex and

multidisciplinary risk assessment appears of utmost relevance (78). In SLE patients, levels of blood pressure lower than 130/80 mmHg are recommended because are associated with lower incidence of CV manifestations (8). For the other risk factors, treatment suggestions should follow recommendations that are used among general population. The impact of most used immunosuppressant agents in SLE on accelerated atherosclerosis has been understudied and, actually, any drugs could be recommended with the purpose of lowering CV risk (77). The maintenance of a low disease activity was demonstrated to be a good strategy to reduce CV risk among these patients, such as the limitation of the use of glucocorticoids to the lowest effective dose considering their well-known deleterious cardiometabolic effects (8, 79). Selective B cell activating factor (BAFF) inhibition, belimumab, seems to display a double effect in animal models: in low-lipid conditions, BAFF inhibition is predictably athero-protective, but in high lipid environments it is atherogenic, due to a counter function in macrophages (80). Hydroxychloroquine shows multiple protective effects (77), reducing IFN-α production, aortic stiffness, correcting lipoprotein profile, improving glycemic control, as well as reducing the risk of all thrombo-vascular events in SLE patients. Finally, mycophenolate treatment seems to improve HDL function in SLE patients, and reduces atherosclerosis mouse models, limiting the recruitment of CD4 + T cells to atherosclerotic lesions (81). Preventive strategies, such as the introduction of low-dose aspirin, is based on individual CV risk profile which should include the assessment of aPL which are more frequent in SLE than in general population (8).

SYSTEMIC SCLEROSIS AND ENDOTHELIAL DYSFUNCTION

Systemic sclerosis is a rare, acquired, systemic disease of unknown origin and uncertain pathophysiology characterized by multi organ involvement. Vascular alterations, extensive fibrosis and specific autoantibodies are the principal actors of its pathogenesis (82). While in SLE ED and accelerated atherosclerosis are a consequence of the chronic and sustained inflammation (83), in SSc microvascular dysfunction is one of the hallmarks of the disease along with immune dysregulation and widespread fibrosis, and represents a primary pathogenetic process (84). Indeed, vasculopathy is of fundamental importance in SSc, from the very early onset of the disease, manifesting with Raynaud's phenomenon that usually precede the other disease manifestations, through the late clinical complications whose prototype is the pulmonary arterial hypertension (PAH). These widespread vascular abnormalities can also present as ischemic digital ulcers (DU), mucocutaneous telangiectasias, gastric antral vascular ectasia and scleroderma renal crisis (85).

Pathogenesis of Microangiopathy in Systemic Sclerosis

Oxidative Stress

Repetitive ischemia and reperfusion processes causes oxidative stress with subsequent tissue damage in SSc, mediated by proinflammatory cytokines and activated leukocytes. These activated leukocytes also show increased expression of inducible nitric oxide synthase (iNOS), leading to the production of a huge amount of NO that reacts with oxygen in the re-perfused blood to form ROS. This causes a direct endothelial injury that leads to vasoconstriction and conversion to a procoagulant phenotype (86).

Endotheliitis

The dysregulation of EC within the vascular wall has a major role in the above-mentioned fibroproliferative vasculopathy (87). This contribute to the unbalanced production of vasoactive mediators resulting in vasoconstriction (88, 89). The alterations of mediators involved in this process were described as both quantitative and qualitative. A particular mention has to be done with regards to the alterations of the vascular endothelial growth factor (VEGF). In fact, despite the fact that higher circulating levels of this vasodilator agent were described in SSc patients in comparison with healthy controls, anti-angiogenic VEGF isoform was strongly expressed in the skin of SSc patients (90). In addition, the increased expression of adhesion molecules by damaged endothelial surface promotes leukocyte trans-endothelial migration, activation, and accumulation (91, 92). ECs transdifferentiate into myofibroblasts gaining mesenchymal cell markers (93, 94). These events culminate in the intima-media proliferation and vessel occlusion leading to tissue hypoxia, which further promotes cell injury and fibroblasts activation (87). Viral infections, coagulation cascade activation, complement system impairment and antibodies against ECs have been proposed as the initial trigger in SSc pathogenesis (95, 96). Some viral infections have been linked to activation/injury of ECs through a mechanism of molecular mimicry. For instance, human cytomegalovirus infection induces antibodies that recognize an amino acid sequence on a viral protein, which is homologous to a surface molecule highly expressed on ECs, inducing apoptosis of ECs (97). Some studies have found a correlation between the parvovirus B19 DNA expression levels and the severity of ED in SSc (98, 99). Recently, new evidence focuses on whether SARS-CoV-2 infection triggers autoimmunity and may have a role in SSc pathogenesis. Indeed, exploration of the SARS-CoV-2-related endotheliitis might provide further important information in the understanding of the early SSc pathogenesis (100).

Complement System

The complement system role in the pathogenesis of SSc vasculopathy has not been exhaustively studied. Its classical functions such as opsonization, recruitment of inflammatory cells, influence of coagulation cascade and angiogenesis are primary for ECs integrity. In normal conditions, complement attack is tightly regulated by regulatory proteins, ensuring protection of EC layer. A reduced expression of these regulators has been shown in SSc skin, potentially leading to endothelium-bound membrane attack complex of complement deposition that could cause EC apoptosis (101).

Autoantibodies

The anti-endothelial cell antibodies (AECAs) can be found in almost 50% of SSc patients and can react with various cell surface antigens on ECs leading them to apoptosis (102) through the antibody-dependent cell-mediated cytotoxicity mechanism (103–105). An association between circulating antibodies and vascular manifestations has been described for antibodies against cell surface receptors such as angiotensin II type 1 receptor and endothelin-1 type A receptor (106). Among other antibodies possibly associated with vasculopathy in SSc, aPL should be considered. Their frequency in SSc is highly heterogeneous and ranges from 0 to 57% (107). Sobanski et al., carried out a meta-analysis, revealing an overall pooled prevalence of 14% (108). ACL and anti-β2-GPI antibodies can contribute to accelerated atherosclerosis by interacting with ECs and inducing a proinflammatory endothelial phenotype (41). Some studies reported an association between aPL positivity and PAH and DU (109–113), while others did not (108, 114, 115). Lastly, considering the strong clinical associations of SSc specific antibodies (anticentromere, anti-topoisomerase 1, anti-RNA polymerase III and anti-Th/To antibodies) and their role as prognostic biomarkers, a potential pathogenicity of these antibodies was suggested. Raschi et al., demonstrated that SSc specific antibodies bound to their antigens to form immune complexes (ICs) elicit pro-inflammatory and pro-fibrotic effects on healthy ECs (95). They stated that immune complexes composed with SSc specific antibodies might contribute to scleroderma pathogenesis through a direct interaction with TLRs. Endothelial incubation with SSc-ICs modulates several molecules (endothelin-1, IL-8, inter-CAM-1, IL-6, and transforming growth factor β1) involved in the three cardinal scleroderma pathophysiologic processes (95).

T Cell Subpopulations

As previously outlined, Tang are required for endothelial progenitor colony formation, promote new vessel formation by secreting angiogenic factors such as VEGF and adhere to ECs. Tang can interact directly with the CD31 expressed by ECs via endothelial-T-cell CD31-CD31 homophilic interactions. In addition, given that these cells also express the cytotoxins granzyme B and perforin, they also have cytotoxic potential. Zhang et al., reported that these cells secrete large amounts of proinflammatory cytokines, such as tumor necrosis factor alpha, IL-6 and IFN-γ, confirming their proinflammatory features (116). Interactions these Tang related cytokines may contribute to ED by exacerbating oxidative stress and reducing phosphorylation of endothelial NOS (117). Their frequency is increased in individuals with traditional CV risk factors further supporting their role in regulating ED (60). It was found that circulating Tang were significantly increased in SSc patients with DU compared either with SSc patients without DU or with healthy controls. In addition, in SSc patients, Tang levels correlate with NVC patterns: higher levels were observed in patients presenting late NVC pattern more frequently than in those with early/active NVC patterns (58). In another study, the absolute number of Tang was higher in SSc patients compared to healthy controls, especially in SSc patients with PAH (118). Taken together, these findings demonstrated that Tang are expanded in SSc patients displaying severe peripheral vascular complications suggesting that circulating Tang increase as a reaction to ischemia and might represent a novel biomarker closely reflecting the severity of SSc-related peripheral vasculopathy.

Endothelial Progenitor Cells

The scleroderma impairment of neovascularization could be associated to both angiogenesis and vasculogenesis failure. Besides insufficient angiogenesis, the contribution of defective vasculogenesis to SSc vasculopathy has been extensively studied (119). As mentioned above, EPCs are defined as circulating primitive cells that contribute to postnatal vasculogenesis (120) and, in SSc patients, circulating EPCs were shown to be reduced in comparison with healthy controls (121). In addition to quantitative alterations, an impaired potential of SSc-derived EPCs to differentiate into mature ECs was reported in terms of functional properties of EPCs (122). It was suggested that EPC precursors were functionally altered before their release into the bloodstream because of a dysregulated microenvironment within the bone marrow (reduced microvascular density and increased fibrosis) (123, 124). In addition, the hypoxic condition of the affected tissues of SSc patients are known to stimulate the differentiation of monocytic EPCs, one EPCs subset (125), through activation of hypoxia-inducible factor (HIF)-1α (126). These local stimuli promote the accumulation of functionally altered monocytic EPCs into the affected lesions of SSc and, since monocytic EPCs are capable of differentiating into cells that produce extracellular matrix proteins (127, 128), they might participate in the fibrotic process in the affected organs (128, 129).

Cardiovascular Disease Risk Assessment in Systemic Sclerosis

SSc patients are at a higher risk of atherosclerosis, albeit, its pattern appears to be less aggressive compared with other rheumatic diseases (130). The alteration of microvasculature is a main feature of SSc and a central cause of complications, but also a macrovascular dysfunction was described (131). In fact, a high incidence of coronary artery disease among SSc patients was reported (132). Among all the connective tissue diseases, SSc is currently associated with the highest mortality rate, with an estimated 10-year survival of 66–82% (133). Due to the recent improvements in the treatment, SSc patients are dying less from SSc-related complications and more from non-SSc related causes, which now account for about 50% of all SSc deaths (133). CVD contributes significantly to SSc mortality burden, accounting for 20–30% of all SSc deaths. For this reason, an accurate understanding of CV risk is crucial in order to improve the overall outcomes of SSc patients (86). However, recommendations for cardiac assessment, CVD risk stratification and prevention strategies in this particular population are currently lacking (134). All patients with SSc should undergo a full evaluation for conventional CV risk factors, even if, compared to general population, the prevalence of traditional CV risk factors in SSc do not seem to differ significantly (135). Standard therapies have to be considered in this context. Early treatment with calcium channel

blockers (CCBs), angiotensin-converting enzyme inhibitors, and endothelin receptor antagonists (ERAs), were demonstrated to be efficacious on myocardial perfusion and contractility, as they improve cardiac microcirculation (136). Vasodilator agents such as phosphodiesterase-5 inhibitors, reducing circulating cytokines and chemokines and suppressing oxidative stress, can improve endothelial function in the patients (137). According with the last published recommendations (8), the management of blood pressure and of hyperlipidemia in these patients should follow the rules used in general population, without specific indications about the use of low-dose aspirin for the prophylaxis.

ENDOTHELIAL DYSFUNCTION ASSESSMENT

The first demonstration of ED in atherosclerotic patients was done using intracoronary infusion of acetylcholine by Ludmer and colleagues in the nineteenth century, heralding an important shift in the paradigm of human atherosclerosis regarded as a purely structural disease (138). Later, several and less invasive techniques to detect changes in the morphology and function of the microcirculation at subclinical level have been developed. The forearm circulation but also the retinal capillary bed was considered as a surrogate for coronary arteries (138). These techniques were mostly applied to primary CVD, except for NVC which is applied in the routinely SSc evaluation In this review we focused the attention on techniques evaluating peripheral circulation.

Nailfold Video-Capillaroscopy

Nailfold video-capillaroscopy is a non-invasive and reproducible imaging study of capillary circulation which is easily accessible in daily routine. It is a well-documented and established tool for the evaluation of peripheral microcirculation in SSc and it has been incorporated in the last international SSc classification criteria (139, 140). The specific alterations which are recognized in SSc form a characteristic morphological pattern known as "scleroderma pattern" (141). The "early" pattern is characterized by few enlarged/giant capillaries, few hemorrhages and relatively well-preserved capillary distribution with no evident loss; the "active" pattern is defined by frequent giant capillaries and hemorrhages and by mild disorganization of the architecture with moderate loss of capillaries; the "late" pattern is characterized by the disorganization of the normal capillary array and the presence of scarce capillaries which show irregular enlargement with ramified/bushy structure (139). Over the last years, the implications of NVC have expanded beyond the diagnostic evaluation of Raynaud's phenomenon to the point that NVC patterns are considered as potential surrogate markers of disease severity and of disease progression (142). Morphological vascular patterns are correlated to the severity of SSc as they seem to reflect the different phases of the disease. The early pattern characterizes the incipient vascular changes and the active/late patterns represents the extensive capillary damage characterizing the fibrotic phase of SSc (143). Indeed, several studies have investigated the association between NVC and SSc manifestations finding some associations of NVC alterations to PAH (144–146) and to telangiectasias (147, 148). However, these data were not confirmed throughout the studies on the topic (149–151). In view of ED and CVD risk in SSc patients, NVC patterns have been associated with arterial stiffness and CVD risk scores supporting a link between micro and macrovascular damage in this disease (152, 153). Limited data exist on the use of NVC in SLE. Many different capillary forms and patterns and a variable prevalence of capillary abnormalities has been reported. In morphometric studies longer capillaries have been described as characteristics of SLE, while in the presence of an associated antiphospholipid syndrome the typical NVC findings are called "comb-like" hemorrhages and consists in multiple hemorrhages from normal shaped capillaries (154, 155). Non-specific morphological alterations, can be found in approximately 75% of SLE patients and relevant capillaroscopic changes correlate with disease activity and with the presence of anti-U1RNP antibodies and aCL (154). However, reported data on association between these findings and disease-related organ involvement are conflicting (156). In addition to morphological and structural evaluation of capillary bed, a dynamic method for studying skin capillaries has been applied to NVC, based on the principle of reactive hyperemia after arterial occlusion. It allows to investigate whether capillary rarefaction is related to a structural anatomic absence of capillaries or to a non-perfusion, reflecting both functional and structural status of the microcirculation (157). However, NVC is routinely used to evaluate structural microvascular changes without the complete estimation of the functional endothelial reserve (158). Few experiences are available on NVC in primary CVD. At present, no convincing evidence of a prognostic value of a decreased capillary density in hypertension was demonstrated (159).

Other Techniques

In the last years, methodologies that allow functional microcirculation assessment have been used, including established methods based on medium vessels, such as flow-mediated vasodilatation (FMD) of brachial artery (160), or small digital vessels, namely peripheral arterial tonometry (PAT) (161), as well as laser doppler techniques, such as laser doppler flowmetry (LDF), laser doppler imaging (LDI) (162), laser speckle contrast imaging (LSCI), laser speckle contrast analysis (LASCA), and near-infrared spectroscopy (NIRS) (163). All of these techniques found a common basic principle: a vasodilatation in healthy arteries in response to mechanical (e.g., post-occlusive reactive hyperemia), physical (e.g., thermal challenges) and chemical stimuli (e.g., pharmacological with vasoactive substances, administered through intra-arterial infusion or iontophoresis) (138, 156). However, vascular responses are not only determined by the functional condition of the vasculature, but also by the structural status of the microvasculature. Endothelium-dependent and endothelium-independent responses can be differentiated applying exogenous NO donors (e.g., glycerol-trinitrate) or direct non-NO donors (e.g., adenosine): impaired endothelial-independent function is associated with structural vascular alterations with changes in smooth muscle cells, rather than endothelium alterations

TABLE 1 | Applications of endothelial function assessment techniques in systemic lupus erythematosus and systemic sclerosis.

Technique	Method of vasodilatation detection after stimuli*	Finding in	
		Systemic lupus erythematosus	Systemic sclerosis
Flow-mediated vasodilatation (FMD)	Ultrasound measurement of diameter changes of the artery	– Lower FMD in patients compared to healthy subjects (176) – Lower FMD in patients carrying aPL and in patients with lupus nephritis history compared to the others (177, 178)	– Lower FMD in patients compared to healthy subjects (179) – Lower FMD in DU-patients compared to non-DU patients and FMD correlation with NVC patterns (179)
Peripheral arterial tonometry (PAT)	Measurement of digital pulse volume through specific plethysmographic finger probes	– Lower RHI in patients compared to healthy controls without correlation with SLEDAI (180)	– Lower RHI in patients compared to healthy subjects (181) – Decreased RHI values in DU-patients compared to non-DU patients and inverse correlation between RHI values and mean PAP at RHC in patients (182)
Laser doppler flowmetry/imaging (LDF/LDI)	Laser doppler assessment of the skin capillary perfusion by measuring the light scatter	– Higher microvascular dilatation in patients treated with antimalarial drugs compared to patients not in treatment (183)	– Impaired endothelium dependent vasodilatation in PAH- compared to non-PAH-patients (169)
Laser speckle contrast imaging/analysis (LCSI/LASCA)	Laser speckle contrast analysis of tissue microvascular blood perfusion	– Lower peripheral blood perfusion and impaired microvascular reactivity in patients compared to healthy subjects (184, 185) – Positive correlation of peripheral blood perfusion and number of capillaries evidenced at NVC in patients (184)	– Lower peripheral blood perfusion in patients compared to healthy subjects (181) – Lower peripheral blood perfusion in in DU- compared to non-DU patients with association of decreased skin perfusion to progression of NVC damage (186)
Near-infrared spectroscopy (NIRS)	Assessment of the regional tissue oxygenation through the near-infrared light	NA	– Lower StO_2 values (both at baseline and at recovery time after the ischemic stimuli) in patients compared to healthy subjects (163) – Higher StO_2 values in patients treated with sildenafil compared to patients not in treatment (163)
Microvascular imaging (MVI)	Ultrasound evaluation for flow quantification of small fingertip vessels	NA	– Peak systolic and end-diastolic flow velocities differ between patients and healthy subjects (187)

**Stimuli can be mechanical (post-occlusive reactive hyperemia), physical (thermal challenges), chemical (vasoactive drugs administered through intra-arterial infusion or iontophoresis). aPL, antiphospholipid antibodies; DU, digital ulcers; na, not applicable; NVC, nailfold video capillaroscopy; PAH, pulmonary arterial hypertension; PAP, pulmonary arterial pressure; RHC, right heart catheterization; RHI, reactive hyperemia index; SLEDAI, systemic lupus erythematosus disease activity index; StO_2, oxygen saturation.*

(138). All the aforementioned stimuli can be used substantially in the same way: the most frequently used are the brachial artery occlusion with a blood pressure cuff and the administration of sublingual nitroglycerin. The difference among the various techniques is the way to assess the vasodilatation. In the brachial artery FMD the respective diameter changes from the resting state of the artery are measured by ultrasound (160). PAT is a plethysmography technique that measures digital pulse volume through specific probes placed on the fingers. The average PAT amplitude (post-to-pre occlusion) of the tested arm, divided by that of the contralateral arm, is automatically calculated as the Reactive Hyperemia Index (RHI). An RHI < 1.67 is the cut off to define ED (161). The laser techniques are: LDF/LDI and LSCI/LASCA. LDF assesses the skin capillary perfusion by measuring the doppler shift induced by the scatter of the light induced by the flow of circulating red blood cells. LDI works as LDF but enables the evaluation of blood flow over a larger area of the skin compared to LDF. LSCI measures the fluctuating granular pattern produced by the reflection of the moving red blood cells illuminated by laser lights (162). LASCA is similar to LSCI where the contrast is calculated on a single pixel over a number of time frames, but has a greater temporal resolution and smaller spatial resolution than that of LSCI (164). NIRS-2D imaging provides indirect information about the microcirculation state by assessing the regional tissue oxygenation: a light in the near-infrared band penetrates the tissue and exploiting the difference between the oxygenated and deoxygenated hemoglobin in absorption spectra, estimates the balance between local arterial supply and tissue oxygen consumption. Consequently, NIRS-2D imaging provides an average value of tissue oxygen saturation (stO_2) that is a marker of regional tissue oxygenation (163). All these techniques, especially FMD and PAT, were firstly used in the setting of atherosclerosis (55) and essential hypertension (165). Furthermore, ED, analyzed by brachial artery FMD, predicted long-term adverse CV in healthy subjects without heart disease and low clinical risk (166–168). PAT was useful in predicting non-obstructive coronary artery disease, not well predicted

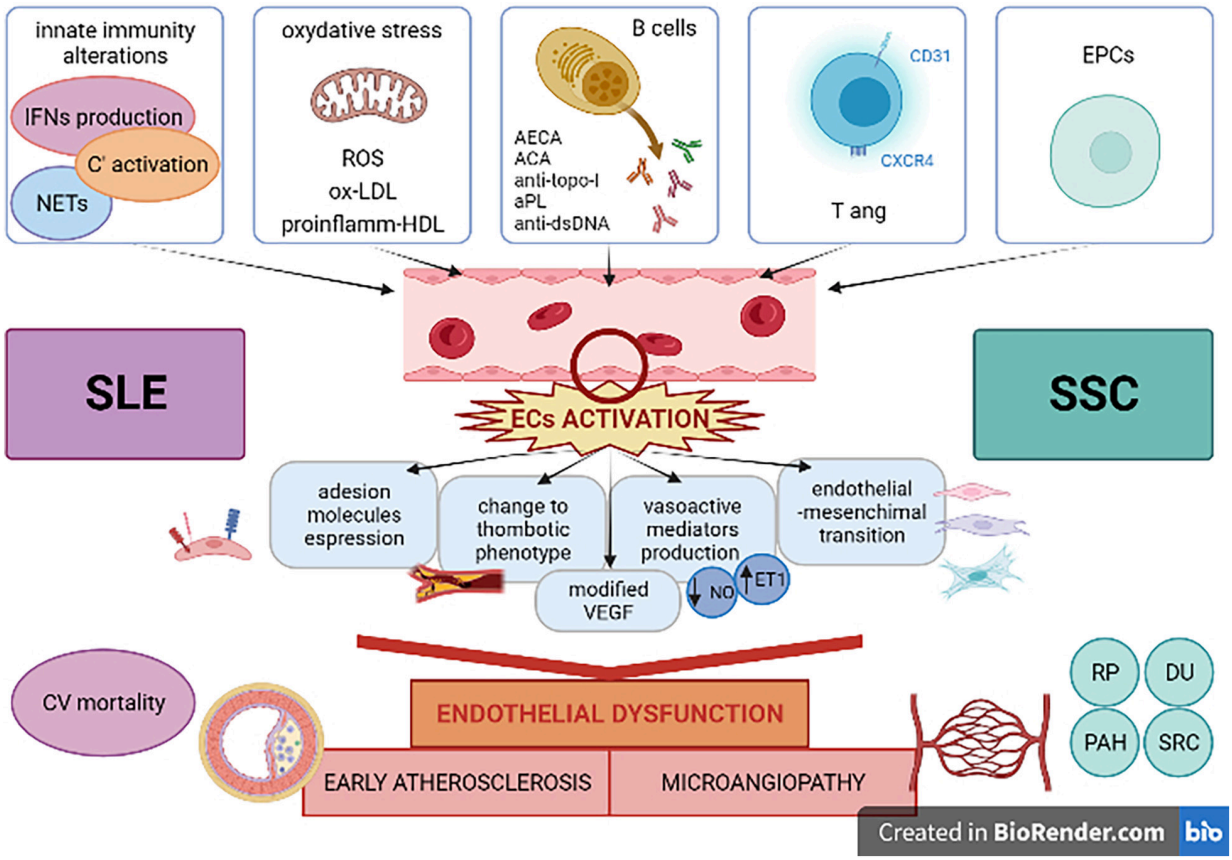

FIGURE 1 | Systemic lupus erythematosus and systemic sclerosis as paradigmatic diseases in showing multiple factors involved in cardiovascular complications related to inflammatory and autoimmune processes. ACA, anti-centromere antibodies; anti-dsDNA, anti-double stranded-DNA antibodies; AECA, anti-endothelial cell antibodies; aPL, anti-phospholipid antibodies; anti-topo-I, anti-topoisomerase-I antibodies; C', complement; CV, cardiovascular; DU, digital ulcers; ECs, endothelial cells; EPCs, endothelial progenitor cells; ET1, endothelin1; IFN, interferon; NETs, neutrophil extracellular traps; NO, nitric oxygen; ox-LDL, oxidized low-density lipoprotein; PAH, pulmonary arterial hypertension; proinflamm-HDL, proinflammatory high-density lipoprotein; ROS, reactive oxygen species; RP, Raynaud's phenomenon; SLE, systemic lupus erythematosus; SRC, scleroderma renal crisis; SSc, systemic sclerosis; Tang, angiogenic T cells; VEGF, vascular endothelial growth factor. Created with BioRender (academic license).

by the Framingham score, and late CV events in large case-series (169). FMD and PAT were confirmed to be independent predictors of CV events, with a relative risk of 0.90 per every 1% increase of FMD and 0.85 per every 0.1 increase in RHI (170). The data on the predictive values of these techniques have suggested that microvascular endothelial function assessment, which is as an earlier indicator of CV risk, could play a significant role in younger subjects or in subjects without a full-blown CVD, such as patients with autoimmune diseases. Another new technique which was recently applied in the context of autoimmune diseases is the microvascular imaging (MVI) which is a novel ultrasound modality for flow imaging, more sensitive than the conventional power doppler modality (171). It generates a high-resolution flow mapping of small vessels using adaptive image analysis to achieve an increased low-velocity blood flow stability (172). The evidence of the application of all these tools in SLE and SSc patients is reported in **Table 1**. In addition to the evaluation of the peripheral microcirculation of the skin, also the retinal district can be evaluated. In fact, retinal arterioles constitute another microvascular area directly and easily observed with relatively simple approaches and which share several common characteristics, including anatomic, physiological, and embryological features with heart and brain microcirculation. Recently, LDF of retinal arterioles and adaptive optics (AO), have been introduced in order to analyze small vessels morphology at the retinal field (173). Wall to lumen ratio (WLR) of retinal arterioles is the parameter which can be calculated for the evaluation of small resistance artery structure. Supporting the concept that changes in macrovasculature and microvasculature are strongly interrelated, a significant correlation among WLR values of retinal arterioles with other microvascular indexes, such as media to lumen ratio (MLR) of subcutaneous small resistance arteries, and macrovascular parameters, such as aortic and carotid stiffness, clinic and 24-h ambulatory blood pressure has been previously found in patients with hypertension (173) and initially evaluated in patients with autoimmune diseases (174).

CONCLUSION

Patients with systemic autoimmune diseases show an excess of CV mortality, and they represent a model for the study of pathogenetic mechanisms which have been recently evaluated as determinants in atherosclerosis and in its complications (175). In fact, the evaluation of the risk factor profile should take into account additive aspects, defined as "non-traditional drivers" which are commonly found in patients with rheumatic diseases (175). Systemic lupus erythematosus and systemic sclerosis were presented in this review as paradigmatic diseases in describing the principal factors which are involved in the determination of the excess of risk, such as ED, microangiopathy and accelerated atherosclerosis. Chronic inflammation and autoimmunity are presented as the main actors in this process and both aspects are well described in SLE and SSc (**Figure 1**). Despite the fact that they have many points in common, SLE represents an example of a disease in which immune system plays a central role in the organ manifestations, CV complications included, as a consequence of the state of inflammation, such a secondary condition. On the other hand, SSc is a disease in which ED is a primary dysfunction, responsible of many typical clinical features of the disease. The Framingham risk score underestimates the CV risk in patient with autoimmune diseases. Clinical tools that assess the microvasculature could represent a new approach in the CV risk evaluation, helping in the development of new models of risk prediction of our patients and changing the management of these diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

REVIEW
published: 30 June 2022
doi: 10.3389/fmed.2022.820263

Novel Surrogate Markers of Cardiovascular Risk in the Setting of Autoimmune Rheumatic Diseases: Current Data and Implications for the Future

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Specialty section:
This article was submitted to Rheumatology, a section of the journal Frontiers in Medicine

Received: 22 November 2021
Accepted: 30 May 2022
Published: 30 June 2022

Citation:
Mandel A, Schwarting A, Cavagna L and Triantafyllias K (2022) Novel Surrogate Markers of Cardiovascular Risk in the Setting of Autoimmune Rheumatic Diseases: Current Data and Implications for the Future. Front. Med. 9:820263. doi: 10.3389/fmed.2022.820263

Patients suffering from rheumatologic diseases are known to have an increased risk for cardiovascular disease (CVD). Although the pathological mechanisms behind this excess risk have been increasingly better understood, there still seems to be a general lack of consensus in early detection and treatment of endothelial dysfunction and CVD risk in patients suffering from rheumatologic diseases and in particular in those who haven't yet shown symptoms of CVD. Traditional CVD prediction scores, such as Systematic Coronary Risk Evaluation (SCORE), Framingham, or PROCAM Score have been proposed as valid assessment tools of CVD risk in the general population. However, these risk calculators developed for the general population do not factor in the effect of the inflammatory burden, as well as other factors that can increase CVD risk in patients with rheumatic diseases, such as glucocorticoid therapy, abnormal lipoprotein function, endothelial dysfunction or accelerated atherosclerosis. Thus, their sole use could lead to underestimation of CVD risk in patients with rheumatic diseases. Therefore, there is a need for new biomarkers which will allow a valid and early assessment of CVD risk. In recent years, different research groups, including ours, have examined the value of different CVD risk factors such as carotid sonography, carotid-femoral pulse wave velocity, flow-mediated arterial dilation and others in the assessment of CVD risk. Moreover, various novel CVD laboratory markers have been examined in the setting of autoimmune diseases, such as Paraoxonase activity, Endocan and Osteoprotegerin. Dyslipidemia in rheumatoid arthritis (RA) is for instance better quantified by lipoproteins and apolipoproteins than by cholesterol levels; screening as well as pre-emptive carotid sonography hold promise to identify patients earlier, when prophylaxis is more likely to be effective. The early detection of subtle changes indicating CVD in asymptomatic patients has been facilitated through improved imaging methods; the inclusion of artificial intelligence (AI) shows promising results in more recent studies. Even though the pathophysiology of coronary artery disease in patients with autoimmune rheumatic diseases has been examined in multiple studies, as we continuously gain

an increased understanding of this comorbidity, particularly in subclinical cases we still seem to fail in the stratification of who really is at risk—and who is not. A the time being, a multipronged and personalized approach of screening patients for traditional CVD risk factors, integrating modern imaging and further CV diagnostic tools and optimizing treatment seems to be a solid approach. There is promising research on novel biomarkers, likewise, methods using artificial intelligence in imaging provide encouraging data indicating possibilities of risk stratification that might become gold standard in the near future. The present review concentrates on showcasing the newest findings concerning CVD risk in patients with rheumatologic diseases and aims to evaluate screening methods in order to optimize CVD risk evaluation and thus avoiding underdiagnosis and undertreatment, as well as highlighting which patient groups are most at risk.

Keywords: surrogate marker, cardiovascular risk, autoimmune, rheumatic disease, prevention

INTRODUCTION

Cardiovascular disease (CVD) is one of the predominant causes of death and reduced quality of life worldwide (1). Half a century ago, traditional risk factors, such as systemic hypertension, physical inactivity, obesity, diabetes, smoking, and hypercholesterolemia have been described and then complemented by non-traditional risk factors, such as inflammation and consecutive atherosclerosis associated with RA and other autoimmune processes. Additional mechanisms linking RA and CVD include shared post translational modification of both peptides and proteins, and a multitude of subsequent immune responses, alterations in the composition and function of lipoproteins, increased oxidative stress, and endothelial dysfunction (2, 3). While the first mentioned are already broadly used for screening and diagnostics in cases with symptomatic vasculitis or corresponding genetic predisposition, and complex polygenetic risk, there seems to be a general lack of consensus in early detection and treatment of endothelial dysfunction and cardiovascular (CV) risk in patients suffering from rheumatologic diseases who haven't yet shown CVD symptoms. However, it is clearly established that CVD is between the leading comorbidities and the most common death causes in patients with autoimmune rheumatologic disorders. In fact, these patients had an increased 10-years risk of major adverse CV events like sudden cardiac death or ischemic stroke, regardless of the prior presence of a coronary artery disease. Additionally, the risk rises significantly for patients with RA and already persisting coronary artery disease (CAD) (4). Atherosclerosis might be directly mediated also by underlying autoimmune processes in patients with rheumatoid arthritis (5). Furthermore, it is expected that a part of the two-fold higher risk of heart failure and total mortality in RA may be due to myocardial disease associated with inflammation including elevated acute phase proteins, T-Cell subsets, proinflammatory cytokines and the presence of circulating auto-antibodies (5). Autoimmune rheumatic diseases are known to affect the valves, myocardium, pericardium as well as the cardiac vasculature and conduction system, leading to multiple cardiovascular manifestations that in some cases can remain clinically silent or lead to a considerable cardiovascular mortality and morbidity (6–10). Atherosclerosis plays a substantial role in CVD morbidity and mortality; the degree of coronary atherosclerosis observed in patients with rheumatic diseases can be as accelerated, diffuse, and extensive as in patients with diabetes mellitus (11). Although this high risk of CVD has been known for decades, patients with rheumatologic diseases generally receive poorer primary and secondary CVD preventive care than other high-risk patients.

In 2009, the European League Against Rheumatism (EULAR) recommended screening, identification of CVD risk factors and CVD risk management based on expert opinion and since has published an update based on a growing body of evidence. One of the overarching principles that have been defined is that the rheumatologist is responsible for risk management in patients with inflammatory joint diseases. For patients with RA, ankylosing spondylitis and psoriatic arthritis, CVD risk assessment is recommended at least once every 5 years and should be reconsidered following major changes in antirheumatic therapy. Other recommendations include optimizing disease activity control, lifestyle recommendations and screening for asymptomatic atherosclerotic plaques by use of carotid ultrasound among others (12, 13) (**Figure 1**). More recently, Drosos et al. (14) published EULAR recommendations for patients affected by gout, vasculitis, systemic sclerosis, myositis, mixed connective tissue disease, Sjögren's syndrome, systemic lupus erythematosus, and antiphospholipid syndrome. The authors put an emphasis on the importance of regular screening and management of modifiable CVR factors. Several recommendations relied on expert opinion because high-quality evidence is scarce. Due to lack of validated rheumatic diseases-specific tools, they recommend the use of generic CVR prediction tools.

The consensus, however, is that in order to evaluate CVD risk in patients with rheumatic diseases, there is an inherent need for screening methods tailored to this specific patient group. Thus, as there is a lack of high-quality evidence, more studies are needed addressing this matter.

This review's aim is to give an overview over new advancements in the field of CVD risk assessment in patients with rheumatic diseases. In particular, we want to showcase novel approaches in the field of imaging technology and biomarkers, as well as highlighting the role of established methods. This could help to facilitate earlier diagnosis and treatment, thus preventing CV events and lead to a better outcome for these patients.

CVD SURROGATE MARKERS

Arterial Stiffness: Measurements by Pulse Wave Velocity and Augmentation Index

Pulse wave velocity (PWV) (**Figure 2**) has long been established as the gold standard for the assessment of aortic stiffness (AS) and is widely used for CV risk stratification; recent studies have shown that aortic stiffness measured through PWV has an independent predictive value for CV events in multiple populations, thus heightening its diagnostic value (15, 16). Increased arterial stiffness leads to diastolic dysfunction, which is the main responsible mechanism of heart failure in chronic inflammatory rheumatic diseases. Rheumatoid arthritis has long been characterized as a systemic disease with a well-defined high atherosclerotic burden. It has been shown that PWV is increased in these patients and that there is an association with age, disease duration, and erythrocyte sedimentation rate (ESR) (17). Our research group could examine PWV during the last few years in patients with various autoimmune rheumatic diseases, such as rheumatoid arthritis (8), mixed connective tissue disease (MCTD) (7), systemic lupus erythematosus (SLE) (10), and antisynthetase syndrome (ASyS) (11). In the case of MCTD and ASyS, aortic PWV was statistically significantly higher in comparison to respective control groups even after adjustment for possible confounding factors. Thus, a higher CVD risk could be postulated. Moreover, we could find that PWV and carotid sonography could improve screening of CV and cerebrovascular risk in patients with ASyS by identifying high risk patients who could have been missed by taking into account only traditional CVD risk factors. Interestingly, there was no difference in cfPWV of patients with SLE and healthy controls in our study. However, we found an independent statistically significant inverse association between estimated glomerular filtration rate (eGFR) and cfPWV in an SLE population with a widely normally ranged eGFR (10). Patients with fibromyalgia, a disease which belongs to the so called rheumatologic chronic pain syndromes and does not have a proven autoimmune background (18), showed higher AS than healthy controls (9). Another widely used indicator for CVD risk is the augmentation index (AIx), which is a measurement of peripheral arterial wave reflections. Although both PWV and AIx deliver information on aortic stiffness, they cannot be used interchangeably: Sakura et al. (19) investigated the relationship between aortic AIx and PWV by measuring them directly using a catheter and found no significant relationship between AI and PWV. The data of the Anglo-Cardiff Collaborative Trial suggested that the AIx might be more sensitive as a marker of arterial stiffening and risk in younger individuals, whereas PWV might be better suited for older individuals (20). PWV is still considered the gold standard method to measure arterial stiffness (21) and is widely used in the scientific community.

Still, there is a large number of studies that provided evidence that PWV, as well as AIx, are predictive for CV events and all-cause mortality in asymptomatic populations (22–24). Nowadays, devices are reasonably portable, relatively easy to use, time-efficient and non-invasive. Elliot et al. (25) reported acceptable to excellent PWV measurement accuracy by a novice operator following as little as 14 practice participants. However, both methods are still not routinely used in daily clinical practice.

CARDIOVASCULAR IMAGING: DETECTING SUBTLE CHANGES

Other non-invasive cardiovascular imaging modalities such as magnetic resonance imaging (26) (**Figure 3**), positron emission tomography (27), computed tomography (28), optical coherence tomography (29), and ultrasound (30) can be used for risk assessment and early detection of CVD in asymptomatic patients. These methods offer a variety of unique information concerning the morphological variations of atherosclerosis and differ in availability, practicability, and cost. MRI can assess plaque composition, such as calcification, lipid-rich necrotic core, and the thickness of the fibrotic cap (31). Cardiac Magnetic Resonance Imaging (cMRI) has been shown to detect myocardial abnormalities in RA patients without known cardiac disease (32). CMRI was also used in a comparison of myocardial structure and function in a cohort study of patients with RA with matched controls. Interestingly, mean left-ventricular mass was 26 g lower for the RA group compared to controls ($p < 0.001$), suggesting that the progression to heart failure in RA patients might be due to reduced myocardial mass rather than hypertrophy (33). Mavrogeni et al. (34) could show that cMRI was able to detect cardiac lesions in symptomatic patients with connective tissue disease and a normal echocardiography.

CT is mostly used to evaluate the degree of carotid artery stenosis, while F-fludeoxyglucose–positron emission tomography (FDG-PET) delivers vital information on the inflammation present in carotid atherosclerotic plaque. Ultrasound, specifically of the carotid arteries, has emerged as a widely available, relatively low-cost imaging method that has been established in preventative care in clinical settings. Carotid intima-media thickness (cIMT) and the presence of plaque have been used as surrogate markers for CVD risk in multiple studies. RA patients display a high prevalence of increased cIMT and carotid plaque (18); similarly, associations have been found with systemic sclerosis (35), ASyS (11), SLE, and many other inflammatory diseases. Interestingly, Ajeganova et al. showed in a 10-year case-control-study, that SLE patients had a three- to four-fold higher risk of CV events and death, compared with persons who do not have SLE but with a similar pattern of traditional CVD risk factors and subclinical atherosclerosis measured with carotid intima-media thickness and presence of carotid plaque (36). A significantly improved prediction of the adverse outcome could be accomplished with the combination of cIMT measures with the Systemic Lupus International Collaborating Clinics (SLICC) Damage Index and coexistence

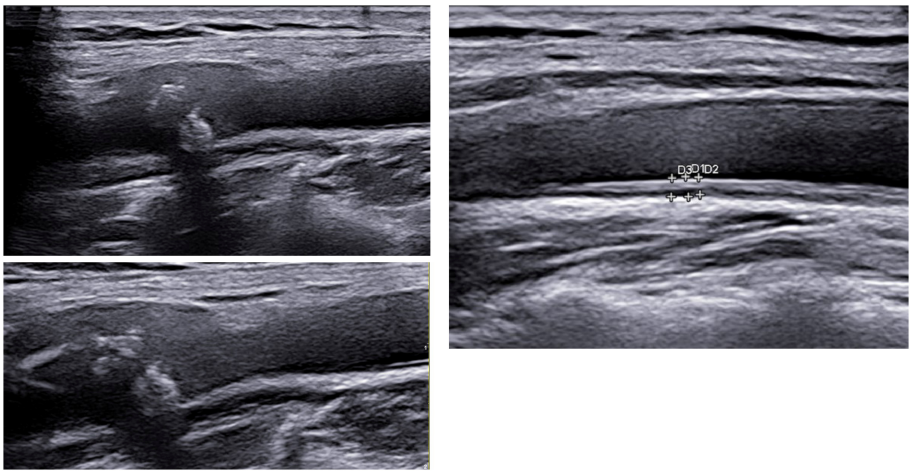

FIGURE 1 | Carotid ultrasound imaging with plaque (left) and increased carotid intima media thickness (above) in two different patients, courtesy by Dr. Konstantinos Triantafyllias, Rheumatology center in Bad Kreuznach, Germany.

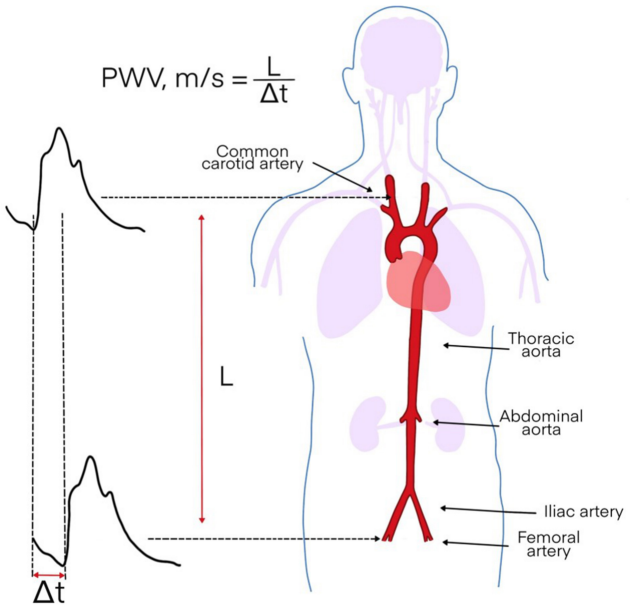

FIGURE 2 | Principles of assessment of pulse wave velocity (PWV).

of SLE-antiphospholipid syndrome (SLE-APS) (37). These findings highlight the necessity of a comprehensive approach to risk stratification and management. Echocardiography has found its way into routine preventative care in patients with autoimmune and rheumatological diseases, as the presence of cardiac abnormalities, such as heart muscle damage, pericardial involvement and valvular heart disease are relatively common. However, early structural, and functional changes are often subtle and not easy to detect (37). Therefore, conventional echocardiographic parameters may often be not sufficient to find these early abnormalities of cardiac dysfunction especially if the global ventricular function is normal. Hence, the use of echocardiographic techniques such as tissue velocity imaging (TVI), ventricular strain imaging (SI), and strain rate imaging (SRI) can be useful for analysis of regional and longitudinal myocardial function (38).

Cardiovascular magnetic resonance imaging holds a promising role in the early detection of numerous pathophysiologic phenomena in subclinical patient populations (39). It allows for the evaluation of cardiac function, the identification of various disease entities such as myocardial oedema and inflammation, ischemia, subendocardial vasculitis, and myocardial fibrosis, which are often missed by other imaging modalities, especially at an early stage of development. Plus, the presence of late gadolinium enhancement (LGE) has been linked to a worse cardiovascular prognosis in various patient populations. CMR is an important tool in the diagnosis and risk prediction for patients with sarcoidosis and could help narrow the gap between clinical and autopsy diagnosis of myocardial involvement in patients with SLE (40). CMR is surely helpful in the early detection of CVD risk, however, the considerable cost and limited availability have to be taken into account, as well as the need to perform clinical prospective trials in order to assess the specific parameters that affect CVD prognosis. Furthermore, CMR could be extremely valuable in some cases and can be considered for patients with new-onset heart failure, arrhythmia, for treatment evaluation, or if there is any mismatch between patient symptoms and routine non-invasive evaluation (41).

NUCLEAR IMAGING: FOCUS ON INFLAMMATION

Methods using nuclear imaging and CT, although promising, have the considerable disadvantage of ionizing radiation and thus, are difficult to justify as a preventative diagnostic method. In recent years, F-fluorodeoxyglucose-positron emission tomography/computed tomography (18 F-FDG-PET/CT) has shown its value in cardiac imaging for the diagnosis and

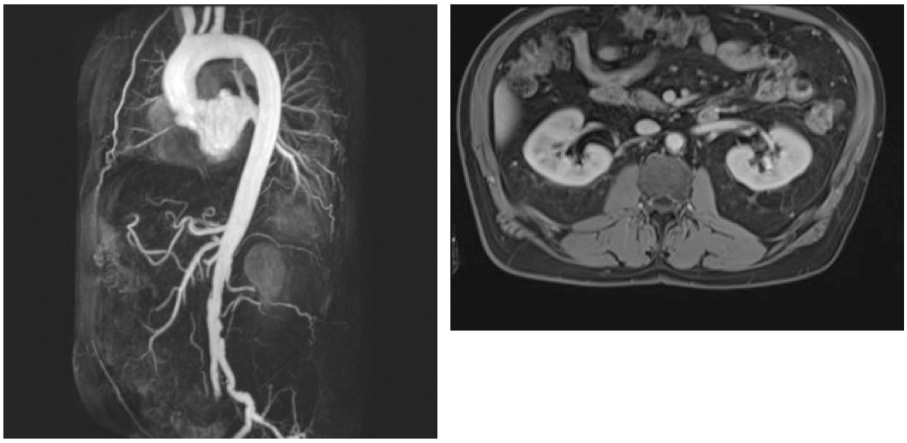

FIGURE 3 | MIP reconstruction of a CE-MRA of a 64 years old patient's aorta and transversal T1 vibe dixon with excentric plaque of the aorta. Pictures by courtesy of Dr. med. Corinna Schorn, Rheumatology center in Bad Kreuznach, Germany.

follow-up of patients with inflammatory conditions of the heart like sarcoidosis, pacemaker infections, and endocarditis. It allows to assess vascular inflammation directly; it shows the quantification of 18F-2-deoxy-D-glucose uptake, and thus vascular inflammation, as well within the atheroma as overall in the arterial wall. An increased arterial FDG uptake has been shown to predict plaque expansion and rupture and thus leading to CV events (42, 43). As arterial inflammation is believed to represent one of the earlier and possibly reversible steps of atherosclerosis, and has been known to precede subsequent calcification, FDG-PET/CT has been increasingly used as a primary outcome in randomized controlled trials of anti-atherogenic drugs (44). PET/CT has been able to detect aortic vessel wall inflammation in RA patients without CVD symptoms (45) and has been proven to predict CVD better than the traditionally used Framingham risk stratification score (46). It has been demonstrated that patients with RA have significantly higher arterial FDG uptake compared with matched controls even after adjusting for atherosclerosis risk factors and statin therapy (47). Seraj et al. suggested that NaF-PET/CT might be even more effective at identifying increased molecular calcification in the wall of the abdominal aorta among patients with RA compared to FDG-PET/CT (48). Although there is a variety of compelling reasons that highlight the value of PET/CT imaging, it is considerably high in cost, not widely available, and will most likely not be included in routine preventative risk assessment in the clinical setting, but will still provide valuable information in the further research of CVD.

Biomarkers of CVD: the role of endothelial dysfunction Endothelial dysfunction is an early event in atherogenesis and has been known to precede the formation of plaques. There are several parameters that have been implicated as markers of endothelial dysfunction; among others, PWV and flow-mediated dilation have been evaluated thoroughly in atherosclerotic diseases. Additionally, biochemical parameters have emerged, such as compounds of the arginine metabolism asymmetric dimethylarginine (ADMA) or symmetric dimethylarginine (SDMA), and endothelial microparticles (EMP). These compounds mediate endothelial dysfunction through interaction with nitric oxide (NO) metabolism, vascular inflammation, and platelet function (49). In patients with systemic sclerosis (SSc) for example, ADMA and EMP might be involved in the development of microangiopathic changes and pulmonary arterial hypertension (50, 51). ADMA has been associated with a wide array of morphological and functional parameters of subclinical vascular disease in patients with autoimmune diseases (52). Significant correlations that have been established include between ADMA and carotid intima media thickness (53) as well as coronary flow reserve in patients with early RA (54) and psoriatic arthritis (55) or between ADMA and the detection of coronary calcium in patients with lupus erythematosus (56). Similarly, ADMA has been associated with arterial stiffness (57) and CVD events (58). In patients with systemic sclerosis and pulmonary hypertension, increased ADMA serum levels are negatively associated with exercise capacity (50, 59). This suggests that the NO pathway might play a significant role in the development of pulmonary vascular disease. Similarly, Thakkar et al. (60) could demonstrate that ADMA in combination with N-terminal pro hormone BNP (NT-proBNP) show an excellent sensitivity and specifity in the non-invasive detection of pulmonary arterial hypertension in patients with systemic sclerosis. Impaired endothelial function has been found for many other rheumatologic and autoimmune diseases; patients with SLE for example show impaired flow-mediated dilation, which in itself is considered as independent predictor of CV events (61). A growing body of evidence supports the hypothesis that chronic inflammation and immune dysregulation are pivotal in the development of atherosclerosis, which can in itself be considered as autoimmune disease (62). Activated T-lymphocytes expressing major histocompatibility complex

(MHC) class II molecules with a pro-inflammatory T-helper (Th)-1 phenotype have been found in human atherosclerotic plaques; the activation of Th-1 responses contributes to a more aggressive progression of atherosclerosis (63). The adaptive immune system is targeted against self-antigens modified by a variety of biochemical factors such as oxidative stress and hypercholesterolemia. Atherosclerotic plaques have been found to express autoantigens, which are targeted by both IgM and IgG antibodies. Autoantigens, such as low-density lipoprotein (LDL), high density lipoprotein (HDL), and lesser-known autoantigens like stress-induced heat shock proteins (HSPs), beta-2-Glycoprotein 1 and oxidized hemoglobin have been associated with CVD, although their individual roles are still not entirely clear (64).

Considering autoimmune diseases, it has been found that synovium and atherosclerotic plaque show similarities in patients with RA, and thus, it has been proposed that common mechanisms might be at play in the accelerated atherosclerosis in RA patients (65). Likely as a consequence of chronic inflammation, RA patients show elevated LDL and HDL plasma levels. Similarly, patients with SLE typically have elevated levels of atherogenic lipoproteins and low levels of atheroprotective factors like paraoxonase 1 (66).

Still, there are limited studies examining the predictive value of vascular assessments on adverse cardiovascular outcomes in patients with rheumatologic diseases. Moreover, associations between disease-related inflammation and the vasculature are far from consistent (67). Even though inflammation seems to play a pivotal role in the mediation of CVD risk, the association between endothelial dysfunction and inflammation particularly in systemic inflammatory disorders stays controversial. In a prospective study with 201 RA patients and a follow-up of 6 years, classical CVD risk factors, such as hypertension, dyslipidemia and insulin resistance predicted vascular function and morphology better than disease-related inflammation (68). Another hallmark of autoimmune disorders is immune dysregulation, which in itself might increase the risk of CVD. Rheumatoid factor and antinuclear antibodies positive subjects have shown a higher risk of CVD events even after adjustment for the presence of rheumatic disease (63). Similarly, anti-CCP antibodies are associated with impaired endothelial function and myocardial involvement in patients with RA. Aforementioned ADMA could provide a promising link between endothelial dysfunction and autoimmune dysregulation as it has been shown to be associated with ds-DNA anti-SM, anti-RNP and anti-CCP among others (57).

High-density lipoproteins (HDL) are long known to have a pivotal role in the prevention of atherosclerosis. Altered levels of blood lipids and HDL have been described in a variety of autoimmune diseases, and the "lipid paradox," where low lipid levels paradoxically correlate with increased CVD risk has been widely accepted, but the mechanisms are still not understood (69). One of the mechanisms might be reduced HDL functionality due to decreased enzymatic activity of the calcium-dependent esterase paraoxonase 1 (PON 1), which has been reported in these conditions. In RA, decreased serum PON 1 levels are associated with increased cIMT and plaques; thus, could be used as atherosclerosis prediction marker (70, 71). Although it has been discovered over 50 years ago, lipoprotein a (Lpa) has not gained importance up until the past 10 years, where it has shown to be an independent, genetic, and likely causal risk factor for CVD (72). Plus, it can be used for a broad spectrum of patients, including those with an LDL level of below 70 mg/dl. Its predictive value is considered higher than traditionally used markers, such as LDL, HDL and cholesterol (73).

In recent years, the role of autoantibodies in CVD has been explored; but although there are studies that suggest a link between humoral immune response and development of CVD, specific autoantibodies and their possible targets are yet to be elucidated. There seems to be a detrimental interplay between autoantibodies and lipid profiles. Autoantibodies targeting HDL have been shown to be associated with altered lipid profiles, and lipoprotein functionality (74–76). Interestingly, there seems to be a difference in anti-HDL levels among immune-driven diseases; Rodriguez-Carrio et al. (77) found the highest levels in systemic autoimmune rheumatic conditions and inflammatory bowel disease, whereas increased levels were not observed in organ-specific autoimmune diseases. Mixed connective tissue disease (MCTD) seems to exhibit an exceptionally high prevalence of anti-HDL positivity, and an association between anti-HDL antibodies and impaired PON1 activity in MCTD has been postulated (78). Hence, anti-HDL antibody levels might be a promising novel biomarker addressing the need for the identification of patients with lipoprotein dysfunction; anti-HDL levels can be measured through conventional, operator-independent and automatized laboratory techniques, thus making it a relatively cost-effective option.

Another potentially useful biomarker is Osteoprotegerin (OPG), which, as the name suggests, is traditionally implicated in bone remodeling but has been linked to CVD. OPG is produced by a variety of tissues and is a member of the tumor-necrosis factor (TNF) receptor family; it is known to be steadily released from vascular endothelial cells in response to inflammatory stimuli and thus might play a modulatory role in vascular injury and atherosclerosis (79, 80). Increased OPG levels have been related to a multitude of cardiometabolic alterations such as diabetes, obesity, hypertension, and metabolic syndrome. In patients with SLE, increased serum OPG has been associated with subclinical atherosclerosis (81), in RA elevated OPG levels correlated with cIMT and higher PWV (82). Hence, there is evidence that circulating OPG levels could be helpful in the identification of patients with subclinical atherosclerosis.

Another novel biomarker that has recently gained attention, endocan is a soluble dermatan sulfate proteoglycan released by the endothelium; it is known to be upregulated by multiple proinflammatory cytokines and proangiogenic factors and may be pro-inflammatory itself. In addition of being used as a surrogate marker of inflammation and endothelial dysfunction, it seems to be involved in the regulation of proliferative and neovascularization processes. Therefore, endocan has been proposed as a biomarker of endothelial dysfunction and pathological angiogenesis (78), thus suggesting its usefulness as a potential predictor of CV events and its utility as a biomarker has been increasingly explored for a variety of patient populations

TABLE 1 | Recently identified potential biomarkers of inflammation and endothelial dysfunction in rheumatic diseases.

Biomarker	Implications or considerations	References
Paraoxonase 1	Decreased serum PON 1 levels are associated with increased cIMT and plaques in RA	(55)
Lipoprotein a	Independent, genetic risk factor for CVD even in populations with low to normal LDL	(57)
Anti-HDL	Highest levels in systemic autoimmune diseases	(61)
Osteoprotegerin	Increased OPG levels have been related to cardiometabolic alterations such as diabetes, obesity, hypertension, and metabolic syndrome. In patients with SLE, increased serum OPG has been associated with subclinical atherosclerosis, in RA elevated OPG levels correlated with cIMT and higher PWV	(65, 66)
Endocan	Has been proposed as a biomarker of endothelial dysfunction and pathological angiogenesis. High endocan levels were detected in autoimmune diseases as psoriasis, Behçet's disease, SLE, and SSc	(63, 67–71)

(83). High endocan levels were detected in autoimmune diseases as psoriasis (84), Behçet's disease (85), SLE (86), and SSc (87).

As liquid biopsies and new molecular biology techniques are used more frequently, a wide array of novel potential biomarkers has emerged on the horizon. A selection of the markers presented in this review can be found in **Table 1**. However, there are new and exciting markers emerging constantly. Adiponectin, for example, has been proposed as an early marker of atherosclerosis in asymptomatic type 1 diabetes mellitus patients (88). EMPs, microRNAs, ANGPTL8, CTRP9, and Galectin-3 among others have been studied in CAD patients (78), it is unclear, to which extent these findings could be applicable to patients with immune-driven conditions. Still, our knowledge of the complex interplay of the pathophysiology of endothelial dysfunction and atherosclerosis is better understood; the future will show, which of these novel markers will prove their value.

ARTIFICIAL INTELLIGENCE: TRANSFORMING THE POSSIBILITIES OF MEDICAL IMAGING

On the quest for improving and optimizing preventative diagnostics, Artificial Intelligence (AI) has recently emerged as a novel tool with the potential to radically change the way we interpret data and make clinical decisions. With increasing data volume and complexity, AI techniques such as machine learning and deep learning can be an invaluable tool to extract relevant information (89). Machine learning is a subfield of AI used to "teach" computers to analyze vast datasets quickly and efficiently; making it possible to identify patterns on new data that match with existing data. Deep learning is a machine learning technique characterized by its use of neural networks, which learn through experience, read data, can build hierarchical architectures, and provide more advanced input-output levels. Deep learning can work with more complex nonlinear patterns and is gaining popularity in the medical research field, as data is steadily increasing in volume and complexity. Deep learning techniques are already playing a pivotal role in tech companies, for example in the field of speech recognition in Apple's Siri and Amazon's Alexa, and Facebook image recognition programs (90).

Machine learning based predictive models might provide more accurate information on CVD risk: In a prospective cohort study using routine clinical data of 378,256 UK primary care patients, Weng et al. (91) were able to show that machine learning algorithms outperform established risk prediction approaches at predicting the absolute number of CVD cases correctly. Likewise, Jamthikar et al. (92) found machine learning based CVD/stroke risk calculators to be superior in terms of 10-year CVD/stroke risk prediction, compared to t13 different types of statistically derived risk calculators.

The advantage of AI techniques in the medical field are numerous; in echocardiography, inter- and intraoperator variability has been shown to be reduced and it is possible to detect additional predictive information which is too subtle for the human eye to see (93). This makes the application of AI especially compelling in the early detection of CV changes. A new up-and-coming option for AI could be its use in cardiac CT: the association between cardiac CT and machine learning algorithms has shown a promising chance in clinical practice to detect functional information beyond atherosclerotic plaque characterization (94).

It has been shown over and over again that risk stratification methods aimed at the general population fall short in the assessment of patients with immune-mediated autinflammatory diseases. AI techniques might help bridge this gap and help clinicians to tailor predictive medicine to the individual patient (95).

Although the research in this field is promising, data focusing specifically on patients with immune-mediated inflammatory diseases is scarce. Additional studies are needed to evaluate the potential of AI as a tool for more personalized and thus effective decision-making.

DISCUSSION

CVD has long been recognized as a major cause of premature morbidity and mortality among patients with immune-driven conditions. Although we gain a growing understanding of the mechanisms that fuel the vicious circle of inflammation and atherosclerosis, there is still a lack of comprehensive approaches of risk stratification, preventative care, and treatment options. A multitude of surrogate parameters have emerged to help pinpoint patients most at risk at an early stage. PWV and AIx

have been widely used in the scientific community to assess CVD risk in subclinical populations, but are not yet routinely used in a clinical setting. There are numerous studies that show that PWV, as well as AIx, are predictive for CVD events and all-cause mortality in asymptomatic populations. Given the fact that modern devices are reasonably portable, relatively easy to use, time-efficient and non-invasive, their integration in every day routine rheumatology practice could improve CVD screening of patients with systemic inflammatory diseases. Carotid ultrasound, on the other hand, has been a valuable tool in the detection of asymptomatic patients with CVD, and can be supplemented by additional imaging methods such as CMR; PET/CT scan can be considered in unclear cases. The advent of AI techniques in modern medicine is an up-and-coming tool which can be useful in the interpretation of vast data volumes and complexity. Novel biomarkers like PON1, Osteoprotegerin, or Endocan have emerged with promising data, but still need to be examined further in relation to diagnostic value and if they can be applied to different population groups. However, it is difficult to draw specific conclusions from the current evidence regarding the mechanisms through which those parameters could be interpreted about their possible prognostic value. Although there is evidence that combining several methods leads to a higher accuracy, the optimal combinations for diagnosis or prognosis still need to be defined. A holistic, comprehensive approach seems to be the most optimal way to pinpoint patients most at risk for CVD. More longitudinal studies with a variety of populations are needed to further describe and assess their prognostic value as well as the best way to employ them in daily clinical practice. Still, we already see promising evidence which might change the way we can identify patients at risk for CVD which would have otherwise been stratified incorrectly with traditional methods. Early atherosclerotic lesions are reversible, and the incorporation of diagnostic methods like PWV, AIx, newer imaging techniques and novel biomarkers could help establish an early diagnosis and prevent the occurrence of CV events early on and thus, facilitate a better outcome and quality of life.

AUTHOR CONTRIBUTIONS

AM acquired and analyzed the data and wrote the first draft of the manuscript. AS, LC and AM drafted the manuscript. AS and LC revised the work critically for important intellectual content. KT conceived of the work, drafted the manuscript, and revised it critically for important intellectual content. All authors have read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank dearly all colleagues working in the Diagnostic Department and in the Clinical Research Department of the Rheumatology Center Rhineland Palatinate and particularly Nicole Dirvonskis, Susanne Dietz, Melanie Opp, and Ursula Sengutta for their help in current and past research projects.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 14 February 2024
DOI 10.3389/fmed.2024.1247024

OPEN ACCESS

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RECEIVED 25 June 2023
ACCEPTED 05 January 2024
PUBLISHED 14 February 2024

CITATION
Piantoni S, Regola F, Angeli F, Caproli A, Trovati A, Tomasi C, Chiarini G, Rossini C, Rosei CA, De Ciuceis C, Franceschini F, Muiesan ML, Rizzoni D and Airò P (2024) Retinal microvascular alterations in patients with active rheumatoid arthritis without cardiovascular risk factors: the potential effects of T cell co-stimulation blockade. *Front. Med.* 11:1247024.
doi: 10.3389/fmed.2024.1247024

Retinal microvascular alterations in patients with active rheumatoid arthritis without cardiovascular risk factors: the potential effects of T cell co-stimulation blockade

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Background: The evaluation of microvascular alterations might provide clinically useful information for patients with an increased cardiovascular (CV) risk, such as those with rheumatoid arthritis (RA), being the small artery remodeling the earliest form of target organ damage in primary CV diseases, such as arterial hypertension. The evaluation of retinal arterioles is a non-invasive technique aimed to identify an early microvascular damage, represented by the increase of the wall-to-lumen ratio (WLR) index. Abatacept (ABA), a T-cell co-stimulator blocker, is used to treat RA. A CV protective action was hypothesized for its peculiar mechanism of action in the modulation of T-cells, potentially involved in the pathogenesis of CV comorbidity. The study aimed to non-invasively investigate morphological characteristics of retinal arterioles in a cohort of RA patients treated with ABA.

Materials and methods: Seventeen RA patients [median (25th-75thpercentile) age = 58 (48–64) years, baseline 28-joint Disease Activity Score DAS28-C-reactive protein (DAS28-CRP) = 4.4 (3.9–4.6), body mass index (BMI) = 24.2 (23.4–26) kg/m^2, rheumatoid factor positive:52.9%, anti-citrullinated peptide autoantibodies positive:76.5%] without known CV risk factors (arterial hypertension, diabetes, hypercholesterolemia, previous CV events, smoking) were evaluated by the adaptive optics imaging system of retinal arterioles before and every 6 months of therapy with ABA (T0, T6 and T12). Office blood pressure evaluation, 24-h ambulatory blood pressure monitoring and tissue-doppler echocardiography were also performed.

Results: A progressive significant reduction of the WLR of retinal arterioles was observed [T0 = 0.28 (0.25–0.30), T6 = 0.27 (0.24–0.31), T12 = 0.23 (0.23–0.26); p T0 vs. T6 = 0.414; p T6 vs. T12 = 0.02; p T0 vs. T12 = 0.009], without significant variations in other parameters. The T0-T12 reduction of WLR was correlated with that of DAS28-CRP (r:0.789; p = 0.005). Moreover, a significant reduction of diastolic office blood pressure and a trend for reduction of daily pressure measured by ambulatory monitoring were observed.

Conclusion: In a cohort of RA patients without known CV risk factors, a reduction of retinal microvascular alterations was demonstrated after treatment for 12 months with ABA, in parallel with the reduction of disease activity. These results might suggest the possibility of microvascular abnormalities regression induced by the immune system modulation.

KEYWORDS

rheumatoid arthritis, abatacept, microcirculation, cardiovascular risk, inflammation

Introduction

Rheumatoid arthritis (RA) patients have increased cardiovascular (CV) risk as compared with general population (1). Their excess risk for myocardial infarction and ischemic stroke is comparable to that observed in patients with diabetes mellitus (2). RA patients, especially if seropositive, are also at higher risk to develop heart failure (3). This increased risk is not fully explained by traditional risk factors, concomitant therapies, or genetic features, but most likely it can be traced to RA-related systemic inflammatory processes (4). Among other factors, T lymphocytes have been described to have a pivotal role in the pathogenesis of CV comorbidity in RA and in other diseases. CD4 + CD28-negative T cells were first identified in the plaques of patients with unstable angina and expansions of these cells have been reported in a range of CV conditions (5). Moreover, these cells are expanded in RA patients, especially in those with preclinical atherosclerotic changes (6, 7).

Clinicians are forced to use scores which are validated for general population for the screening of CV risk in RA, due to the lack of RA-specific CV event prediction models (8, 9). According to the last updated guidelines, CV risk assessment should be performed every 5 years in patients with low-to-moderate risk, and patients with high risk should receive appropriate treatments (1). Screening for carotid plaques may be useful, if possible, in routine clinical practice (1).

In the evaluation of CV risk in high-risk categories of patients, new techniques can be used to detect early CV alterations in clinical studies (10). Considering that small artery remodeling (i.e., thickened arterial wall) is the earliest form of target organ damage in arterial hypertension, and it has a role in increasing vascular resistance, the evaluation of microvascular alterations might provide clinically useful information extending the determination of traditional Framingham risk factors (11). Assuming that microvascular damage is present with similar characteristics in all vascular districts, retinal vessels might be considered as a window to the heart (12) and even to the brain (13) and can be evaluated by new techniques such as the adaptive optics imaging system (14). A permanent decrease in arteriolar vessel lumen and an increase in arteriolar vessel wall due to, for example, smooth muscle cells proliferation, resulting in a higher wall-to-lumen ratio (WLR), play a key role for microvascular remodeling in chronic disease states (15, 16). Previous studies have shown strong correlations of WLR with age and blood pressure (BP) (17, 18), and higher retinal WLR has been described in older people or in patients with hypertension (17–19).

Recent studies were published on vascular remodeling of the retinal microcirculation in RA, detected with methods which were different from adaptive optics. The reduction of the vessel density was demonstrated in early RA using the optical coherence tomography angiography (20) and an altered retinal microvascular morphology was showed in active RA patients by processing retinal images through a computerized software that allow to calculate the retinal vascular caliber (21, 22).

The role of antirheumatic drugs on CV risk in patient with RA is not yet fully evaluated. Little is known on the effect of abatacept (ABA), a T cell co-stimulation blocker, even if this agent was demonstrated to reduce the number of circulating CD28-negative T cells (23), therefore specifically targeting relevant players in the pathophysiology of CV comorbidity in RA. The aim of our study was therefore to perform an in-depth CV assessment in a cohort of active RA patients before and after therapy with ABA. To better evaluate the role of antirheumatic therapies we focused on a cohort of patients without known traditional CV factors.

Patients and methods

Patients

Seventeen consecutive patients with RA without known traditional CV risk factors (arterial hypertension, diabetes mellitus, hypercholesterolemia, previous CV events, smoking), treated with ABA for at least 6 months (T6), were enrolled in the study between June 2016 and April 2019, but only 11 patients have concluded 12 months follow-up (T12). Ocular diseases, that may interfere with the experimental study with adaptive optics, were excluded due to a preliminary ophthalmologic visit in all the participants. Among 6 patients who did not complete the scheduled follow-up, two were excluded because arterial hypertension was detected at baseline at the 24-h ambulatory BP monitoring (ABPM) evaluation, and anti-hypertensive treatment with calcium channel-blockers was introduced. One patient was switched to another biological disease modifying anti-rheumatic drug (bDMARD) because of a primary inefficacy of ABA. The other three patients did not complete the study because of the SARS/COV-2 pandemic that limited the frequency of face-to-face visits.

The main clinical and demographic characteristics of these patients are shown in Table 1. The study was approved by the Institution Ethics Committee (NP 2276), and patients' written consent, according to the Declaration of Helsinki, was obtained.

Clinical disease activity and the response to the treatment were evaluated, respectively, with the 28-joints disease activity score based on C-reactive protein (CRP) (DAS28-CRP), and the European Alliance of Association for Rheumatology (EULAR) criteria of Response to the treatment (24).

TABLE 1 Baseline demographic, serological and clinical features of 17 total RA patients and the 11 RA patients with a 12-month follow-up.

Features at T0	All the cohort (n = 17)	Patients who completed the study (n = 11)	Value of p
Gender: M/F, n (%)	4/13 (23.5/76.5)	3/8 (27.3/72.7)	0.823
Age, years	58 (48–64)	60 (49–64)	0.880
BMI, kg/m^2	24.2 (23.4–26)	23.4 (21.6–25.6)	0.615
Arterial hypertension, n (%)	0	0	n.a.
Diabetes, n (%)	0	0	n.a.
Hypercholesterolemia, n (%)	0	0	n.a.
Previous CV events, n (%)	0	0	n.a.
Smoking, n (%)	0	0	n.a.
RF positivity, n (%)	9 (52.9)	5 (45.5)	0.698
Anti-CCP positivity, n (%)	13 (76.5)	8 (72.7)	0.823
CRP, mg/L	7.1 (2.4–12)	7.5 (4.1–11.5)	0.755
ESR, mm/h	31 (23–34)	29.5 (25.8–33.5)	0.990
DAS28-CRP	4.4 (3.9–4.6)	4.4 (3.8–4.6)	0.999
HAQ	0.5 (0.2–1)	0.2 (0.1–0.7)	0.435
Disease duration, months	72 (18–144)	80 (15–121.5)	0.954
Currently treated with corticosteroid, n (%) Daily dose [Prednisone equivalent mg]	14 (82.4) 4.5 (2.8–5)	9 (81.8) 3.6 (2.4–4.7)	0.971 0.518
N of previous csDMARDs	2 (1–2)	1 (1–2)	0.602
Currently treated with csDMARDs, n (%)	13 (76.5)	10 (90.9)	0.329
Currently treated with MTX, n (%) Weekly dose [mg]	10 (58.8) 15 (10.6–15)	8 (72.7) 13.8 (10–15)	0.562 0.813
Naive to b/tsDMARD, n (%)	14 (82.3)	9 (81.8)	0.971
Currently treated with NSAIDs, n (%)	8 (47)	6 (54)	0.698

Data are shown as median (25th-75thpercentile). In bold, significant value of ps.
BMI, body mass index; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptides antibodies; CRP: c-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, 28-joints disease activity score; HAQ, health assessment questionnaire; DMARD, disease modifying anti-rheumatic drugs, conventional synthetic (cs), target synthetic (ts), and biologics (b); MTX, methotrexate; n, number of subjects; n.a., not applicable; NSAIDs, not steroidal anti-inflammatory drugs.

During the visit at our hospital, each patients did a rheumatologic evaluation at the Rheumatology and Clinical Immunology Unit, and a CV assessment at the Internal Medicine Unit (ASST Spedali Civili University Hospital of Brescia, Italy). Adaptive optics examination, office BP evaluation, 24-h ABPM and tissue doppler echocardiography were part of the CV assessment.

Methods

Microcirculation

Adaptive optics imaging technique

Adaptive optics apparatus is an improved version of a traditional fundus camera, allowing the investigation of vessels with 20–150 µm of diameter (16). A beam of light enters the eye, and a small amount is reflected out of the eye and into the optical system. Wavefront aberrations in the reflected image are detected by an image sensor and corrected by a deformable mirror. The achieved image resolution is of the order of 1 µm (16). Other details on the technique are described elsewhere (16). The WLR of retinal arterioles is the crucial parameter which was calculated using the formula (arteriole diameter − lumen diameter)/lumen diameter (16). Moreover, the wall thickness and the wall cross-sectional area (WCSA) were also measured.

Macrocirculation

Blood pressure measurements

Office BP evaluation and 24-h ambulatory BP monitoring (ABPM)

BP was measured three times by the same physician in all subjects in a sitting position after 10 min at rest, using a sphygmomanometer and taking the disappearance of phase V Korotkoff sounds as diastolic pressure. Hypertension was defined as a sustained increase in BP (systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) according to the World Health Organization/ International Society of Hypertension guidelines (25, 26).

Twenty-four-hour BP and heart rate were evaluated by non-invasive automatic monitoring (model 90,207; SpaceLabs, Redmond, WA, USA) (23). The procedure was described elsewhere

(27). The 24-h BP profiles were used to calculate mean 24-h systolic and diastolic values, mean daytime systolic and diastolic values, mean night-time systolic and diastolic values (27).

Tissue Doppler echocardiography

The left ventricular (LV) internal dimensions, interventricular septum and posterior wall thickness were measured according to the American Society of Echocardiography's recommendations (25). Relative wall thickness was calculated, and values ≥0.43 was considered to indicate left ventricular (LV) concentric geometry. The formula of American Society of Echocardiography was used to calculate LV mass and it was indexed by body height to the 2.7 power (LVMI). LV hypertrophy was defined if LVMI was greater than 47 g/m$^{2.7}$ in women or 50 g/m$^{2.7}$ in men (28). Trans mitral flow velocity profile was evaluated by the Doppler technique, with the sample volume placed at the tips of mitral leaflets from the apical four-chamber view, and the peak early (E wave) flow velocity, peak late (A wave) flow velocity, and the E wave deceleration time was measured. LV isovolumic relaxation time (IVRT) was also measured, as previously described (28).

Statistics

The lack of Gaussian distribution of all the variables were verified by the Kolmogorov–Smirnov test. Data were expressed as the median (25th-75thpercentile). Mann Whitney U test and Wilcoxon-signed rank test were applied to assess variations for quantitative variables, when appropriated. General linear model for repeated measurements was used as a verification test. The correlations between variables were evaluated by Spearman rank correlation test. A p value ≤0.05 was considered statistically significant. All analyses will be carried out using the software package GraphPad Prism (version 6) and IBM SPSS.

Results

Longitudinal clinical evaluation of the cohort

Patients who completed the 12 month follow up with CV evaluation (n = 11) had a progressive improvement of their symptoms during ABA therapy and were progressively treated with lower doses of prednisone (Table 2). Ten of 11 (91%) patients had a moderate response, and one subject had a good response. The results of the improvement of CRP and DAS28-CRP are shown in Table 2.

Longitudinal evaluation of microvascular parameters

As shown in Table 3, five parameters were evaluated through adaptive optics technique on retinal arterioles. Significant reduction of the WLR was observed progressively during time (general linear model, p = 0.008). Considering the variation with time of the retinal parameters in correlation with the DAS28-CRP, a significant direct correlation was found between the RA activity index and WLR variations after 12 months of treatment with ABA (r:0.789; p = 0.005) (Figure 1).

TABLE 2 Clinical disease activity features of 11 RA patients with 12-month follow-up.

Features (n = 11)	T0	T6	T12	Value of p T0 vs. T6	Value of p T0 vs. T12	Value of p T6 vs. T12
CRP, mg/L	7.5 (4.1–11.5)	4.0 (1–7)	5 (2.6–7.4)	0.167	0.320	0.476
DAS28-CRP	4.4 (3.8–4.6)	2.3 (2.1–3.2)	1.8 (1.3–2.3)	**0.002**	**0.001**	**0.025**
Currently treated with corticosteroid, n (%)	9 (81)	4 (36)	3 (27)	**0.030**	**0.010**	0.647
Daily dose [Prednisone equivalent mg]	4.5 (2.8–5)	2.3 (2–3.4)	2.5 (2.3–4.4)	**0.003**	**0.004**	>0.999

Data are shown as median (25th-75thpercentile). In bold, significant value of ps.
CRP, high sensitivity C-reactive protein; DAS28: 28-joints disease activity score.

TABLE 3 Retinal arterioles parameters of 11 RA patients with a 12-month follow-up.

Parameters (n = 11)	T0	T6	T12	Value of p T0 vs. T6	Value of p T0 vs. T12	Value of p T6 vs. T12
Lumen µm	94.4 (84.1–103.9)	94.8 (84.6–107.7)	99.2 (89.1–109.1)	0.765	0.278	0.278
External diameter µm	125.8 (111.1–131)	122.4 (109.1–134.5)	125.6 (113.9–134.4)	0.898	0.563	0.577
Wall thickness µm	13.2 (12.2–14.4)	13.4 (11.7–14.4)	12.5 (11.6–13)	0.365	0.070	0.175
Wall cross-sectional area µm^2	4581 (3789–5264)	4563 (3789–5295)	4100 (3899–5146)	0.638	0.365	0.765
Wall to lumen ratio	0.28 (0.25–0.30)	0.27 (0.24–0.31)	0.23 (0.23–0.26)	0.414	**0.009**	**0.002**

Data are shown as median (25th-75thpercentile). In bold, significant value of ps.

Longitudinal evaluation of macrovascular parameters

Blood pressure evaluation

Office arterial BP and the 24-h ambulatory BP monitoring were detected in enrolled patients. Longitudinal data are shown in Table 4. Data are presented as the mean values of all the measurements in office, 24-h, day and nighttime. During 12 months of observation, slight variations were observed. A significant decrease of the systolic [T6 vs. T12: 125 (116–133.5) mmHg vs. 116 (110–120) mmHg; $p = 0.047$] and diastolic [T6 vs. T12: 76 (70–76) mmHg vs. 69 (64–71.5) mmHg; $p = 0.005$] office BP was observed (general linear model, $p = 0.073$ and $p = 0.007$, respectively). In the intensive monitoring of the 24-h, the day and night ABPM systolic blood pressure decreased between T0 and T12. value of ps were approaching the level of significancy ($p = 0.055$ and $p = 0.063$, respectively). No correlation was found between blood pressure parameters' reduction and the decrease of WLR.

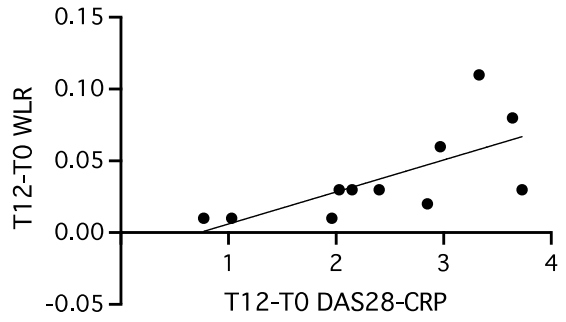

FIGURE 1
Correlation between T12-T0 variations of WLR and DAS28-CRP in 11 RA patients with a 12-month follow-up (r:0.789; $p = 0.005$). CRP, C-Reactive Protein; DAS28, 28-joints disease activity score; WLR, wall-to-lumen ratio; WLR, wall-to-lumen ratio.

Tissue Doppler echocardiography

As shown in Table 5, selected parameters of the echocardiography were evaluated. No significant variations were registered with time. Notably, however, a slight decrease of the left ventricular mass index with time [T0 vs. T6 vs. T12: 31.5 (28.2–35.9) g/m$^{2.7}$ vs. 30.4 (26.8–33.8) g/m$^{2.7}$ vs. 30 (26.5–33.2) g/m$^{2.7}$] was observed.

Discussion

Our study is the first demonstration of the reduction of microvascular alterations detected by adaptive optics technique in a cohort of patients with active RA treated with ABA. The observed alterations at retinal level in our RA patients may be a consequence of inflammation that affects blood vessels, enhancing precocious mechanisms of endothelial dysfunction which are, at least in part, responsible of the excess of CV diseases in these patients if compared with general population (29). Even though rheumatologists are aware about the presence of a higher CV risk in their patients, one of the objectives of the next years may be to propose a new model of evaluation of this risk in RA, and the methods of detection of early micro and macrovascular modifications could have a place. Retinal vessels' inspection is a standard procedure for assessing microvascular changes in hypertension or in diabetes and it represents an emerging tool to be used also in other field, like that of autoimmune diseases (20–22).

In our study we enrolled RA patients with an active disease and without any modifiable CV risk factors because we wanted to study

TABLE 4 Blood pressure parameters (mmHg) of 11 RA patients with a 12-month follow-up.

Parameter ($n = 11$)	T0	T6	T12	Value of p T0 vs. T6	Value of p T0 vs. T12	Value of p T6 vs. T12
Systolic OBP	120 (115.5–132)	125 (116–133.5)	116 (110–120)	0.449	0.125	**0.047**
Diastolic OBP	75 (72–80)	76 (70–76)	69 (64–71.5)	0.847	**0.014**	**0.005**
24-h ABPM SBP	116 (114–132)	119 (115–122)	113 (105–123)	0.813	0.063	0.672
24-h ABPM DPB	74 (71–79)	72 (71–74)	69.5 (66.3–73.5)	0.500	0.098	0.656
Day ABPM SBP	121 (118–135)	124 (120–125)	112 (110–122)	0.625	0.055	0.313
Day ABPM DBP	78 (76–82)	75 (73–78)	72 (69.5–73)	0.500	0.098	0.156
Night ABPM SBP	109 (105–127)	109 (97–113)	99.5 (95–116)	0.625	0.063	0.313
Night ABPM DBP	65 (59–74)	61 (60–63)	61.5 (58–65.5)	0.375	0.063	0.203

Data are shown as median (25th-75thpercentile). In bold, significant value of *ps*.
OBP, office blood pressure. ABPM, ambulatory blood pressure monitoring; SBP, systolic blood pressure; DBP, diastolic blood pressure.

TABLE 5 Tissue Doppler Echocardiography parameters of 11 RA patients with a 12-month follow-up.

Parameter (n = 11)	T0	T6	T12	Value of p T0 vs. T6	value of p T0 vs. T12	p-Value T6 vs. T12
LAD cm	3.3 (3.1–3.6)	3.2 (3.1–3.4)	3.1 (3.0–3.3)	0.512	0.278	0.718
LVM h ($g/m^{2.7}$)	31.5 (28.2–35.9)	30.4 (26.8–33.8)	30.0 (26.5–33.2)	0.275	0.083	0.083
RWT	0.33 (0.29–0.36)	0.30 (0.29–0.35)	0.32 (0.30–0.35)	0.563	1	0.747
Dec E (m/s)	203 (160–235)	199 (185–221)	204 (171–234)	0.447	0.320	0.848
IVRT (m/s)	81 (73.5–100.5)	88 (81–92.5)	80 (72.5–86.5)	0.711	0.449	0.206

Data are shown as median (25th-75thpercentile). In bold, significant value of *ps*.
LAD, left atrial diameter; LVM h, left ventricular mass indexed by body height to the 2.7 power; RWT: relative wall thickness; Dec E, E wave deceleration time; IVRT, left ventricular isovolumic relaxation time.

the inflammation-related CV risk eliminating potential confounding factors. A new technique of evaluation of the retinal arteriolar morphology was used for the first time in RA patients in our study (14). During the period of observation (one year), we showed a significant reduction of the WLR parameter which is a marker of arteriolar resistance. Interestingly, the reduction of the DAS28-CRP index was directly correlated with that of WLR. This let us to hypothesize a possible effect of the reduction of systemic inflammation due to the treatment in the decrease of arteriolar resistance and vascular swelling. Another hypothesis might be that ABA could have an effect in improving endothelial function thanks to its peculiar mechanism of action on the endothelium.

Notably, some parameters related to the health of the macrovascular system also varied in our patients, in particular a significant reduction of diastolic office BP and a trend for reduction of daily pressure measured by ABPM, decreases that were independent from that of WLR. It should be noted that glucocorticoid frequency of use and dosage were also reduced. In two patients, which were excluded by further analysis, anti-hypertensive therapies were introduced after the first visit because of the detection of high level of arterial pressure at the ABPM evaluation. This suggests the potential utility of the use of ABPM to detect arterial hypertension in an early phase in patients with high CV risk. Furthermore, the involvement in this study might have improved the sense of responsibility of the patients in taking care about their lifestyle. Taken together, these elements might be the main determinants of the improvement of some parameters in our cohort even if they may also represent a major limitation of the study, together with the involvement of middle-aged patients with a moderate disease activity and the lack of a control group treated with other biological treatments. Furthermore, also the progressive reduction of the prednisone daily dose might have had a positive effect on the reduction of the microvascular parameters. All those elements may interfere with the outcome of the study. As expected, considering the relatively short period of observation, no variation was instead shown in echocardiography indexes. The stability of macrovascular parameters during 12 months of ABA therapy was previously demonstrated in a similar cohort of RA patients (30), in contrast with a previous study where a worsening of aortic stiffness was found after 6 months of ABA, probably related to an insufficient decrease of systemic inflammation (31).

Currently, the control of disease activity is the most effective strategy to lower CV risk in RA patients thanks to the reduction of the inflammatory burden (32). According to EULAR recommendations, RA patients should be monitored every 5 years or after major changes in antirheumatic therapy, and lipids monitoring, smoking cessation, regular physical activity, and Mediterranean diet should be advised (1). Furthermore, the lipid increasing effects of certain bDMARDs and some adverse effects of not steroidal anti-inflammatory drugs and corticosteroids should be considered in the management of the disease (1).

Among all the bDMARDs, ABA, a lymphocyte co-stimulation blocker, has a rationale to be efficacious in inducing an improvement of endothelial function (33, 34). This might be an additional effect, strictly related to its mechanism of action that can lead to the reduction of CD28-negative T cells in the bloodstream (5). In fact, several studies support a role of circulating T cells lacking CD28 surface molecule in inducing functional impairment of arterial endothelium, that is currently considered to be the earliest stage of atheroma development, but also in enhancing plaque instability promoting CV disease progression (6). This T cell subpopulation may cause an increase in endothelial oxidative markers and in arterial stiffness, with relevant consequences on left ventricular mass (5). Unlike the common helper T cells, CD4 + CD28-negative subpopulation produces a great amount of TNF-alpha, IFN-gamma, perforin and granzyme B which have cytotoxic activity on endothelial cells (35). In a rat model, chronic administration of a potassium channels blocker prevented the development of unstable atherosclerotic plaques by blocking the release of inflammatory and cytotoxic molecules from CD4 + CD28-negative T cells (36).

A clinical study found that ABA was associated with a 20% reduced risk of CV disease in comparison with TNF-alpha inhibitors, among patients with CV disease history (37). So far, there are data from preclinical studies on atherosclerosis (34) and from large population studies confirming its potential CV benefits (37, 38).

As additional evidence of the importance of T cells co-stimulation blocking in the prevention of CV events, some authors demonstrated that ABA administration in animal models of heart failure reduced the severity of cardiac dysfunction and fibrosis, when compared to non-treated animals, even if it was administered late in the disease course (39). In these experiments, the authors showed that the ABA

effect was exerted as a combination of the T cells inhibition, but also of macrophage functions with the induction of signals in B cells, triggering a compensating anti-inflammatory IL-10 expression (39).

Considering this last evidence and the results of our study, a fascinating hypothesis that can be postulated is that, in the future, new possibilities for the treatment of CV risk in our patients could be a reality, in addition to prevention strategies. Immunosuppressants, with ABA as a possible preferred candidate among the others thanks to its peculiar mechanism of action, may be used for the cure of CV complications, at least, in inflammatory diseases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Brescia ethical committee, P.le Spedali Civili 1, Brescia. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SP, PA, DR, and MLM contributed to conception and design of the study. SP, FR, FA, AT, GC, CR, CAR, and CDC conducted the study and organized the database. SP, AC, CAR, and CC performed the statistical analysis. SP, FR, PA, FA, CAR, CC, and FF wrote sections of the manuscript. CT in performing the statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by an unrestricted research grant from Bristol- Myers Squibb to the Rheumatology and Clinical Immunology Unit, ASST Spedali Civili of Brescia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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