DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN RARE AND COMPLEX AUTOIMMUNE DISEASES

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DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN RARE AND COMPLEX AUTOIMMUNE DISEASES

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ORIGINAL RESEARCH
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Pro-inflammatory Stimulation of Monocytes by ANCA Is Linked to Changes in Cellular Metabolism

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Clinical and experimental data suggest that pathogenesis in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis is driven by ANCA-mediated activation of neutrophils and monocytes. While the role of neutrophils has been extensively investigated, the function of monocytes remains relatively understudied. We have previously demonstrated that stimulation of monocytes with anti-myeloperoxidase (MPO), but not anti-proteinase-3 (PR3), antibodies results in production of the pro-inflammatory cytokine IL-1β. Changes in cellular metabolism, particularly a switch to glycolysis, have recently been linked to activation of immune cells and production of IL-1β. Therefore, we investigated the metabolic profile of monocytes following ANCA stimulation. We found a significant increase in glucose uptake in anti-MPO stimulated monocytes. Interestingly, both anti-MPO and anti-PR3 stimulation resulted in an immediate increase in glycolysis, measured by Seahorse extracellular flux analysis. However, this increase in glycolysis was sustained (for up to 4 h) in anti-MPO- but not anti-PR3-treated cells. In addition, only anti-MPO-treated cells exhibited increased oxidative phosphorylation, a metabolic response that correlated with IL-1β production. These data indicate that monocyte metabolism is altered by ANCA, with divergent responses to anti-MPO and anti-PR3 antibodies. These metabolic changes may underlie pathologic immune activation in ANCA associated vasculitis, as well as potentially contributing to the differing clinical phenotype between PR3- and MPO-ANCA positive patients. These metabolic pathways may therefore be potential targets for therapeutic intervention.

Keywords: ANCA vasculitis, monocyte, immunometabolism, autoimmune, metabolism

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INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides (AAV) are a group of autoimmune conditions affecting the microvasculature (1). Most patients harbor autoantibodies directed against myeloperoxidase (MPO) or proteinase 3 (PR3). These antigens are found in the primary granules of neutrophils and lysosomes of monocytes and can be externalized when these cells are primed, thereby allowing engagement with circulating ANCA. The clinical phenotype of patients with anti-PR3 and anti-MPO antibodies is different, with the former often exhibiting granulomatous inflammation and the latter frequently having a sclerosing phenotype. Additionally, genomic analyses have demonstrated that AAV with PR3-ANCA is genetically distinct from that

with MPO-ANCA (2). To date, our understanding of disease pathogenesis has been dominated by experimental work in neutrophils (3). However, recent data from our laboratory and others suggests a role for inflammatory monocytes in driving disease (4–6).

IL-1β is a key pro-inflammatory cytokine that induces both local and systemic inflammation through the production of cyclooxygenase type 2 (COX-2), nitric oxide, adhesion molecules, fever, and polarization of T cells [reviewed in (7)]. We previously described increased IL-1β production from human monocytes in response to stimulation with anti-MPO antibodies (4). This result has also been shown by other groups with IL-1β acting as a mediator of proinflammatory monocyte activation in a murine system (8).

Recent studies have shown that changes in intracellular metabolism play a major role in immune cell function and polarization (9). Rather than being secondary events, these changes have been shown to instruct cell function (10) and have been implicated in several autoimmune conditions (11, 12). The most frequent metabolic event in this setting involves a programmed switch from oxidative phosphorylation (oxphos) to aerobic glycolysis (13). Most work in this emerging "immunometabolism" field has focused upon macrophages and T cells. The production of IL-1β from murine bone marrow derived macrophages (BMDMs) is linked to a switch to aerobic glycolysis in response to pro-inflammatory signals including LPS (14, 15). Some studies have shown that, like macrophages, monocytes undergo a metabolic shift from oxphos to glycolysis in response to an acid environment induced by lactate accumulation (16). However, in experiments using HIV as the stimulus, the opposite effect was observed, with glycolysis being decreased in activated monocytes (17). Monocytes activated with HIV also display increased levels of the glucose transporter Glut-1 and have increased glucose uptake (18). These data show that the metabolic response of monocytes varies depending on stimulus and, therefore, focused analysis in monocytes using condition-specific stimuli is necessary.

Here, we have investigated ANCA-mediated metabolic changes in monocytes. We report divergent metabolic responses to anti-MPO and anti-PR3 stimulation. These differences correlate with the distinct inflammatory cytokine profiles which we have previously reported (4).

METHODS

Isolation of CD14+ Monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using lymphoprep and monocytes were purified by positive selection. PBMCs were incubated with anti-CD14 magnetic beads (Miltenyi Biotec) as per the manufacturer's instructions and CD14+ cells were then isolated using an LS column.

Stimulation of Monocytes for Measurement of 2-NBDG Uptake Analysis

CD14+ monocytes were plated at a density of 2×10^6 cells/ml in Roswell Parks Memorial Institute (RPMI) Media supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 1 mg/ml streptomycin and 2 mM L-glutamine (cRPMI). Cells were treated with 5 μg/ml anti-MPO (clone 2C7), anti-PR3 (clone CLB-12.8), or isotype mAb for 60 min followed by incubation with 86.5 μg/ml 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) for 60 min @ 37°C with 5% CO_2. Conjugated anti-CD14 (clone RM02) antibody was added for the last 10 min of the incubation. Cells were then analyzed immediately on a Cyan ADP analyser (Beckman Coulter).

Treatment of Monocytes With Inhibitors and Cell Death Assay

Monocytes were plated at a density of 2×10^6 cells/ml. Cells were treated with 10 mM 2-deoxyglucose (2-DG), 8 μM oligomycin, 20 mM dichloroacetate (DCA), or MitoTempo and incubated for 20 min before the addition of 5 μg/ml anti-MPO mAb, isotype control or vehicle for 4 h @ 37°C with 5% CO_2. IL-1β levels were assessed in supernatants by ELISA. Propidium iodide (PI) staining was performed in the appropriate experiments by addition of 10 μg/ml PI immediately prior to analysis on a Cyan ADP analyser (Beckman Coulter).

Mitochondrial Stress Test and Glycolysis Stress Test

Monocytes were adhered to CellTak-coated 24-well plates at a concentration of 1×10^6 per well as described above. Cells were stimulated with 5 μg/ml anti-MPO, anti-PR3, isotype or vehicle for 4 h at 37°C with 5% CO_2. Mitochondrial stress test or glycolysis stress test were performed as per the manufacturer's instructions using a Seahorse XF24 analyser (Agilent). Sequential addition of oligomycin (2 μM), D-glucose (5.5 mM), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (4 μM), rotenone (4 μM), 2 deoxy-glucose (2-DG) (10 mM) allows for accurate calculation of mitochondrial respiratory capacity and glycolytic acidification. Each condition was evaluated in duplicate and a minimum of three measurements was performed following addition of each compound.

Measurement of Real Time Metabolic Changes in Response to ANCA

Monocytes were adhered to Seahorse plates as described above. Anti-MPO, anti-PR3 or isotype antibodies were added to port A of an XFe24 FluxPak. For ECAR measurements, D-glucose was added to port B. For OCR measurements rotenone or vehicle was added to port B. The Cell plate was added to the Seahorse XFe24 analyser. Six initial basal measurements were performed followed by injection of port A. For ECAR experiments three more measurements were performed before injection of glucose followed by nine further measurements. For OCR experiments one measurement was performed before rotenone injection followed by nine further measurements.

Measurement of Cellular and Mitochondrial ROS in Monocytes

Monocytes were isolated and stimulated with 5 μg/ml anti-MPO, anti-PR3 or isotype mAb or 5 ng/ml LPS for 1 h at 37°C with 5% CO_2. For the final 30 min of the incubation cells were treated with either 5 μM CM-H_2DCFDA (Thermo Fisher Scientific, Loughborough, UK), 2.5 μM MitoSOX red (Thermo Fisher Scientific, Loughborough, UK), or 10 μg/ml JC-1 (Thermo Fisher Scientific, Loughborough, UK). Anti-CD14 antibody was added for the final 10 min of the incubation. Cells were analyzed immediately on a Cyan ADP analyser.

Study Approval

Buffy coat samples were obtained from the Irish Blood Transfusion Service, St. James's Hospital, Dublin with ethical approval from the School of Medicine Research Ethics Committee, Trinity College Dublin.

Statistics

All statistics and correlations were performed using GraphPad Prism 6 software and nonparametric analyses were used for non-normal data. When comparing three or more groups, a one-wat ANOVA was performed for unpaired samples and a *post hoc* Friedman test was used for paired samples. Multiple comparisons were corrected using Dunn's multiple comparisons test. For comparisons between two groups, a students *T*-test was used. To assess the difference in across multiple parameters 2-way ANOVA with Tukey's *post hoc* multiple comparison test was used. Differences were only statistically significant ($p < 0.05$) when specified.

RESULTS

Anti-MPO Stimulated Monocytes Produce IL-1β in a Glycolysis Dependent Manner

We have previously demonstrated differential cytokine production in monocytes in response to anti-MPO and -PR3, with anti-MPO, but not anti-PR3 leading to increased secretion of IL-6, IL-8, and IL-1β (4). As IL-1β production is linked to changes in intracellular metabolism, we sought to investigate the metabolic correlates of IL-1β production by anti-MPO and anti-PR3 stimulated monocytes. Firstly, we investigated the overall glucose uptake by monocytes in response to these stimuli by measuring uptake of 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG), a non-metabolisable fluorescent analog of glucose. This molecule is taken up in the same way as glucose but is not broken down by the cell and it's fluorescence can therefore be used as a surrogate marker for glucose uptake (19). As expected, based on its ability to induce IL-1β, anti-MPO stimulation resulted in significantly enhanced glucose uptake (**Figures 1A,B**). We therefore studied the role of glucose metabolism in anti-MPO and -PR3 antibody induced production of this cytokine. We used 2-deoxyglucose (2-DG) to block hexokinase, and therefore inhibit glycolysis, and oligomycin to block the electron transport chain, and thus oxphos. 2-DG treatment of monocytes abolished anti-MPO induced IL-1β production (without causing cellular injury as measured by PI exclusion, (**Supplementary Figure 1**), indicating that glycolysis is required for IL-1β production in these cells (**Figure 1C**). Conversely, oligomycin treatment had no effect on anti-MPO-induced IL-1β production (**Figure 1D**).

Anti-MPO Stimulation of Monocytes Results in Both Increased Oxygen Consumption and Glycolysis

To further define the changes in monocyte metabolism in response to anti-MPO and -PR3 antibody stimulation we performed Seahorse Extracellular Flux analysis, which permits simultaneous measurement of both extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), markers of glycolysis and oxphos, respectively. We hypothesized that the increase in glucose uptake in response to anti-MPO would be associated with a switch to aerobic glycolysis, with a corresponding decrease in OCR. However, we found that anti-MPO treatment markedly increased monocyte basal OCR (**Figures 2A,B**), as well as increasing maximum and spare respiratory capacity (**Figures 2C,D**). This increase was not found in anti-PR3 stimulated cells (**Figures 2A–D**). The increase in maximum respiratory capacity in response to anti-MPO stimulation is particularly interesting due to the relatively short stimulation time (4 h). The primary mechanism by which respiratory capacity is increased is via mitochondrial biogenesis. However, an increase in mitochondrial mass over our 4 h stimulation is unlikely. Using mitotracker green we confirmed that our anti-MPO stimulation had no effect on mitochondrial mass (**Figure 2E**), suggesting that the observed upregulated oxygen consumption is not due to increased mitochondrial biogenesis.

In contrast to OCR, which was increased only by anti-MPO antibody, stimulation with both anti-MPO and -PR3 increased basal glycolysis and non-glycolytic acidification (as determined by ECAR) (**Figures 3A–C**). Contrary to the expected switch to aerobic glycolysis, we instead observed parallel increases in glucose uptake, OCR and ECAR in response to anti-MPO, suggesting a broad up-regulation of monocyte bioenergetics.

Changes in Monocyte Cellular Metabolism in Response to ANCA Occur Immediately After Stimulation

The changes in monocyte metabolism in response to ANCA described above occurred within 4 h of stimulation. The relatively short timeframe of the glycolytic and oxphos responses led us to investigate the real-time kinetics, using the Seahorse analyser, of these responses by adding anti-MPO and anti-PR3 directly to the cells. Changes in OCR (**Figure 4A**) were measured in real-time immediately following addition of antibody. Both anti-MPO and -PR3 stimulation resulted in immediate increases in OCR of similar magnitude. Approximately 45 min after antibody stimulation, OCR levels in anti-PR3 stimulated monocytes reduced rapidly to basal levels, whereas the OCR in anti-MPO stimulated cells continued to rise and was maintained for the duration of the experiment (**Figure 4A**). Such an immediate increase in oxygen consumption raised the possibility that it was

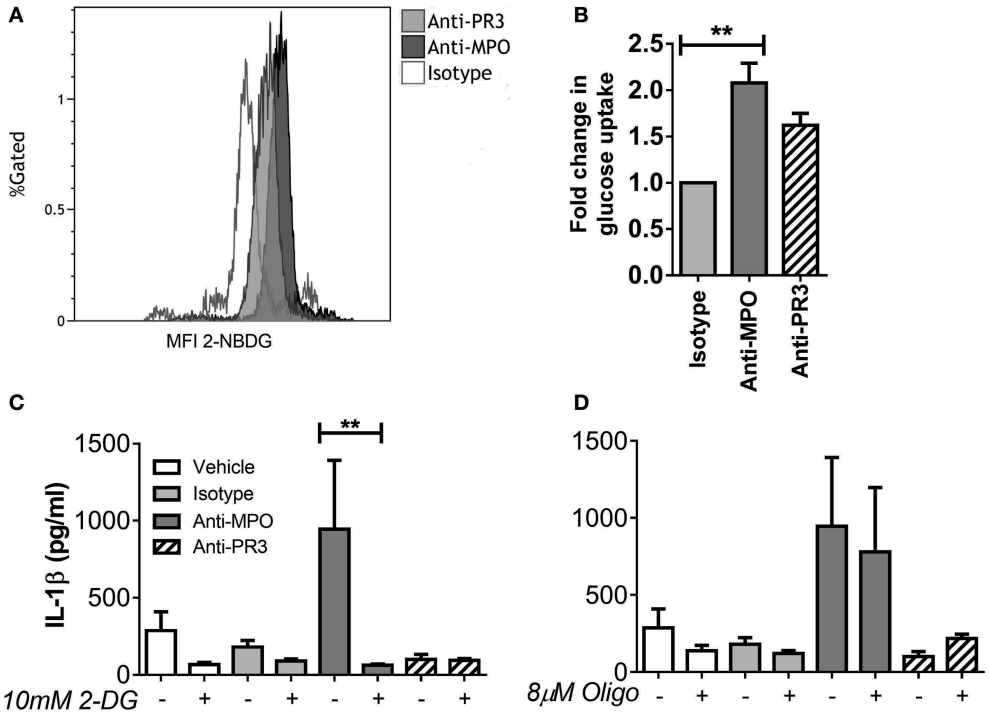

FIGURE 1 | IL-1β production from anti-MPO stimulated monocytes requires glycolysis, but not oxphos. CD14+ monocytes were stimulated with mAb directed against MPO, PR3 or an isotype control, followed by incubation with 2-NBDG. Median fluorescence intensity (MFI) was compared between treatments. Representative overlays are shown for anti-MPO and anti-PR3 treatments **(A)**. Fold change in 2-NBDG uptake after antibody stimulation is shown for three independent donors **(B)**. CD14+ cells were incubated with either 10 mM 2-DG **(C)** or 8 μM oligomycin **(D)** and then stimulated with 5 μg/ml mAb directed against MPO, PR3 or isotype control antibody. IL-1β was measured in supernatants by ELISA. Data are presented as the mean and SEM. Statistical analysis was performed by students *T*-test **(B)** (**$p < 0.01$) or one-way ANOVA with Friedman's post-test **(C,D)** (**$p < 0.01$) ($n = 6$).

due to non-mitochondrial oxygen consumption, such as through reactive oxygen species production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. We addressed this question using the oxphos inhibitor rotenone, which specifically inhibits mitochondrial oxygen consumption. Rotenone reduced overall OCR following both anti-MPO (**Figure 4B**) and anti-PR3 (**Figure 4C**) monocyte stimulation, although not completely to control levels, thereby confirming that much of the observed oxygen consumption was due to oxphos, with a small contribution from NADPH oxidase (**Figure 4D**). Interestingly, we found an immediate increase in ECAR in the absence of glucose following addition of both antibodies, indicating an increase in non-glycolytic acidification (**Figure 4E**). Upon glucose addition, ECAR increased in response to both antibodies by a similar amount. This increase was followed by a rapid decline in anti-MPO treated cells followed by a subsequent increase by 4 h post glucose addition while anti-PR3 stimulated cells showed a steady decline from their peak after glucose injection (**Figure 4E**).

Mitochondrial Reactive Oxygen Species Induced by Anti-MPO Stimulation Are Required for IL-1β Production

We have found that monocytes stimulated with anti-MPO antibodies increase oxphos in parallel with glycolysis, but only inhibition of glycolysis blocks IL-1β production (**Figure 1**). The increased oxidative metabolism may be required for the production of biosynthetic precursors or to generate ROS. We tested the hypothesis that mitochondrial ROS (mROS) contribute to the pro-inflammatory effect of anti-MPO antibodies on monocytes. Using the general oxidative stress indicator CM-H_2DCFDA we detected an increase in cellular ROS in anti-MPO treated cells (**Figure 5A**). Similar experiments employing the mROS-specific probe MitoSOX also indicated an increase in mROS in response to anti-MPO (**Figure 5B**). In order to examine the importance of this increase in the inflammatory activation of monocytes by anti-MPO we pre-treated cells with the mROS-specific scavenger MitoTempo. We found that the IL-1β produced by monocytes in response to anti-MPO was inhibited by MitoTempo in a dose-dependent manner (**Figure 5C**), indicating a role for mROS in this pro-inflammatory pathway. ANCA stimulation did not alter monocyte mitochondrial membrane potential as measured by JC-1 staining (**Supplementary Figure 2**).

DISCUSSION

The fundamental importance of metabolic changes in immune cell activation is becoming a key area of interest. Cellular metabolic pathways are complex and often interconnected, with

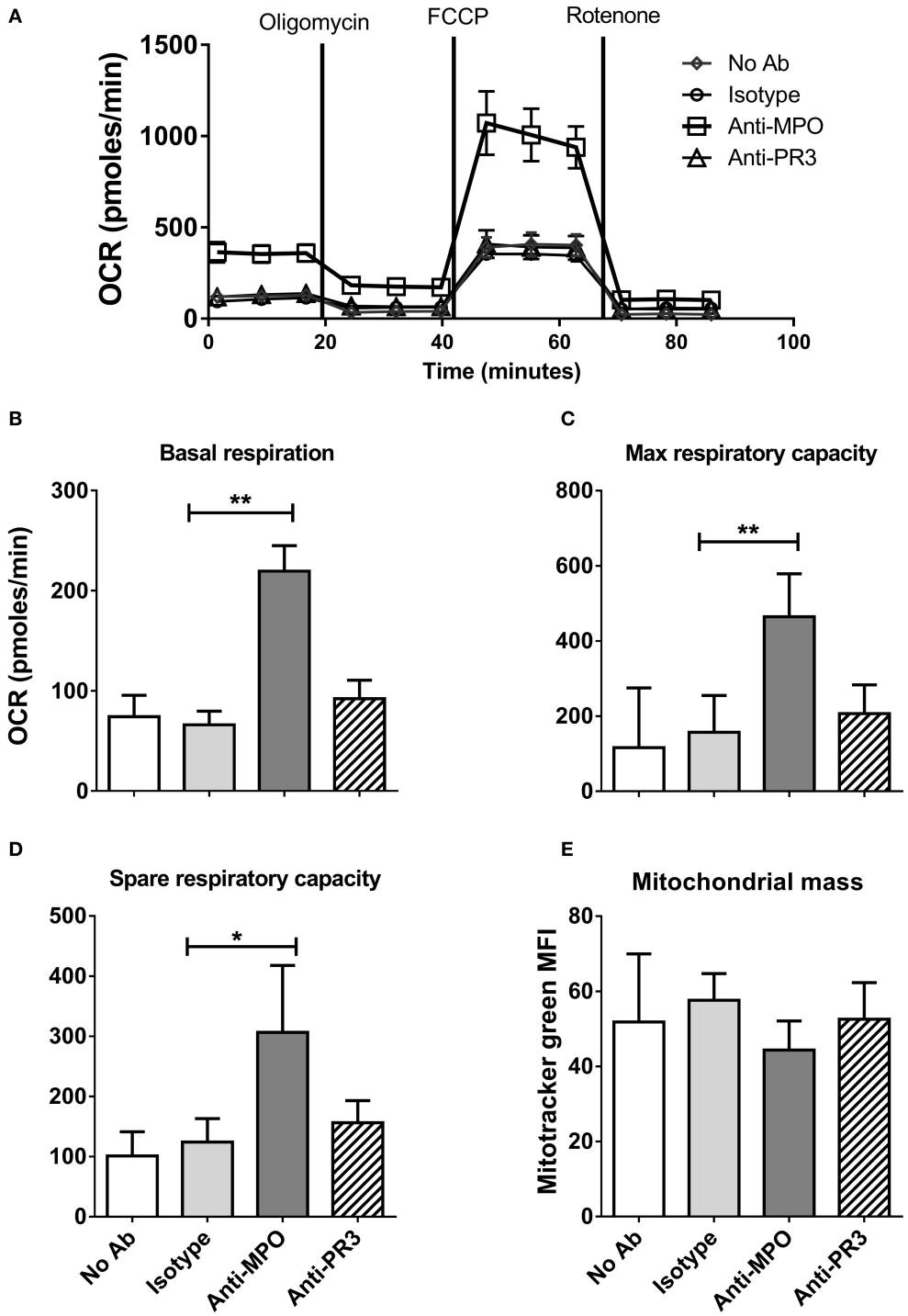

FIGURE 2 | Anti-MPO, but not anti-PR3 stimulation of monocytes leads to increased oxidative respiration and respiratory capacity. CD14+ monocytes were stimulated with 5 μg/ml mAb directed against MPO, PR3 or isotype control antibody. Initial oxygen consumption rate (OCR) was measured followed by addition of 4 μM oligomycin, 2 μM FCCP and 4 μM rotenone **(A)**. Basal respiration levels were calculated by subtracting the non-mitochondrial respiration (post rotenone addition) from the initial OCR readings **(B)**. Maximum respiratory capacity was calculated by subtracting non-mitochondrial respiration from OCR values following the addition of FCCP **(C)**. Spare respiratory capacity was calculated by subtracting basal from maximum respiration **(D)**. Mitochondrial mass was measured by flow cytometry of cells incubated with 50 nM MitoTracker Green following stimulation as above **(E)**. Data are presented as the mean and SEM. Statistical analysis was performed by one-way ANOVA with Friedman's post-test (*$p < 0.05$, **$p < 0.01$) (**A–D** $n = 6$, **E** $n = 3$).

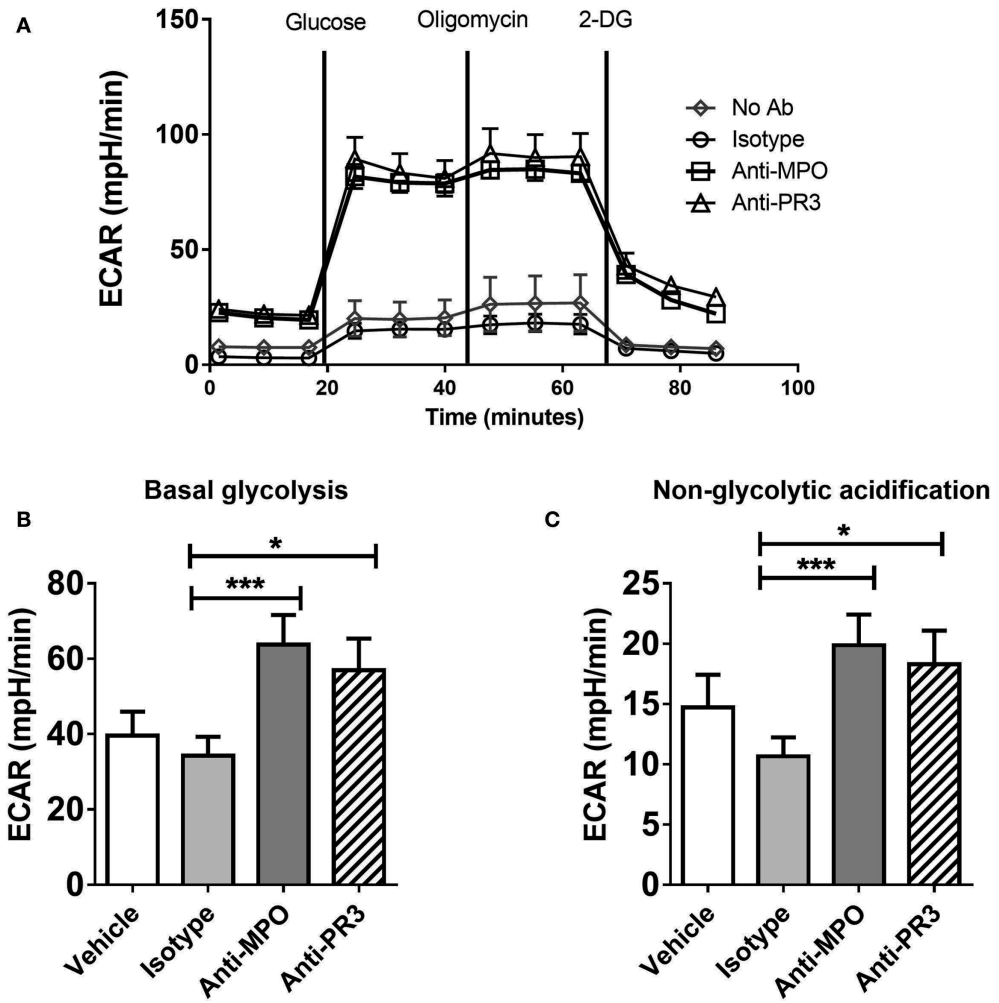

FIGURE 3 | Anti-MPO and anti-PR3 stimulation results in an upregulation of glycolysis in monocytes. CD14+ monocytes were stimulated with 5 μg/ml mAb directed against MPO, PR3 or isotype control antibody. Extracellular acidification rate (ECAR) was measured at basal levels and following the addition of 4.5 mM D-glucose, 4 μM oligomycin and 10 mM 2-DG **(A)**. Glycolytic rate was calculated by subtracting non-glycolytic acidification (post 2DG treatment) from the rate of acidification following the addition of glucose **(B)** Non-glycolytic acidification was defined as the ECAR rate after 2-DG addition **(C)**. Data are presented as the mean and SEM. Statistical analysis was performed by one-way ANOVA with Friedman's post-test (*$p < 0.05$, ***$p < 0.001$) ($n = 11$).

multiple pathways changing in a particular cell type in response to a specific stimulus (20). Macrophages have become the subject of intense study in terms of their metabolic profile in response to pro-inflammatory stimuli such as LPS. These studies have predominantly focused on murine bone marrow derived macrophages (BMDMs) and tissue resident macrophages (10, 21, 22) and have largely neglected blood-borne myeloid lineage cells, specifically monocytes. While monocytes can translocate to tissues and differentiate into macrophages or dendritic cells, they also have effects while still in the circulation (23, 24). These functions may be particularly important in diseases where the mechanism of action is not localized to a specific tissue, but rather to the vasculature, as is the case in AAV, and where the autoantigens are located within the monocyte.

18-F-Fluorodeoxyglucose Positron Emission Tomography with Computed Tomography (PET) which quantifies glucose uptake, has shown increased rates of glucose uptake in the affected organs of patients with AAV (25), indicating probable upregulation of cellular metabolism in these immune cell rich areas. Here, we have shown that ANCA treatment of monocytes results in increased glucose uptake as measured by the fluorescent glucose analog 2-NBDG. This compound has been shown to be a useful measure of glucose uptake (19) although some recent studies suggest that uptake of this glucose analog may be lower than that of radiolabelled glucose (26). As we found increased 2-NBDG uptake in response to both anti-MPO and anti-PR3 stimulation, any artifactual diminished uptake of this compound implies an even greater increase in glucose uptake in monocytes in response to ANCA.

We investigated the hypothesis that altered cellular metabolism, in response to increased glucose uptake, is involved in the response of monocytes to ANCA stimulation. The current

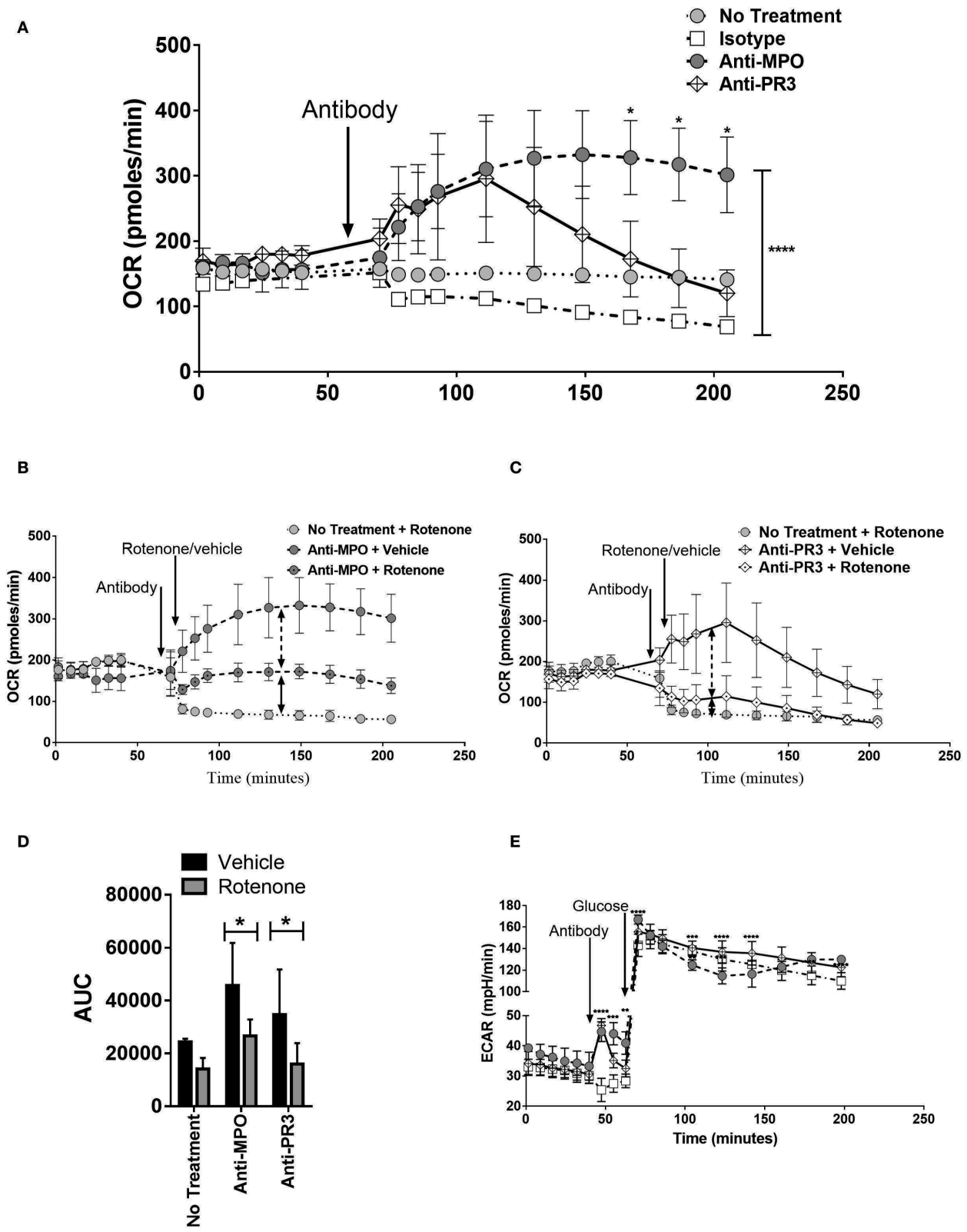

FIGURE 4 | Anti-MPO and anti-PR3 stimulated monocytes have differing OCR and ECAR kinetic patterns. OCR of CD14+ monocytes was measured at regular intervals following the addition of 5 μg/ml mAb directed against MPO **(A,B)**, PR3 **(A,C)** or isotype control antibody **(A–C)** by Seahorse extracellular flux analysis. Cells

(Continued)

FIGURE 4 | were subsequently treated with rotenone (black dot in symbol) or vehicle **(B,C)**. The area under the curve (AUC) from the point of antibody injection was calculated **(D)**. ECAR was measured at regular intervals following the addition of 5 μg/ml mAb directed against MPO **(A,B)**, PR3 **(A,C)** or isotype control antibody **(A–C)** by Seahorse extracellular flux analysis **(E)**. Statistical analysis was performed by two-way ANOVA with Tukey's multiple comparisons test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$) ($n = 3$). Light gray circles, no treatment; white boxes, isotype control; dark gray circles, anti-MPO; white diamond, anti-PR3.

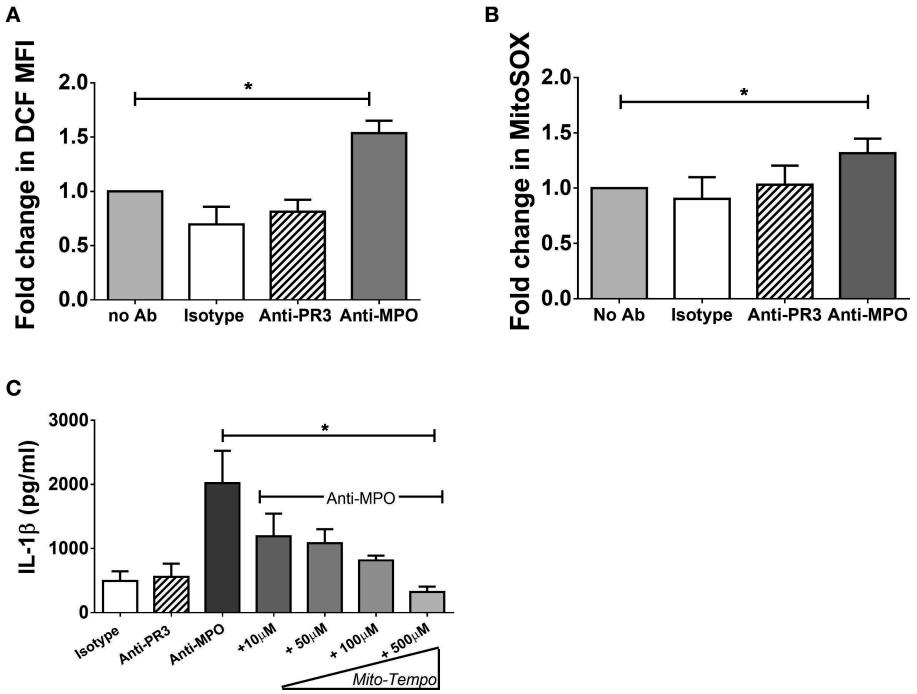

FIGURE 5 | Anti-MPO induced IL-1β production is abrogated by the mitochondrial ROS scavengers MitoTempo. CD14+ monocytes were incubated with increasing concentrations of MitoTempo followed by stimulation with 5 μg/ml mAb directed against MPO, PR3, or isotype control antibody. Supernatants were removed and IL-1β was measured by ELISA **(A)**. For flow cytometry experiments, monocytes were stimulated as above. CM-H_2DCFDA **(B)** or MitoSOX Red **(C)** was then added to the cells **(B)** and the MFI was determined by flow cytometry. Statistical analysis was performed by one-way ANOVA with Friedman's post-test (*$p < 0.05$) ($n = 5$).

paradigm in pro-inflammatory immune cell activation is that these cells shift to a more glycolytic phenotype. While this is the case in most cell types, it is also true that some plasticity between pathways exists in cells depending on their level of activation. Treg cells for example, require oxphos for long term survival (27) but exhibit enhanced glycolysis in the initial stages of activation (28). Macrophages have increased reliance on specific metabolic pathways, depending on their polarization. Contrary to this, we have demonstrated that ANCA treatment of monocytes results in broad upregulation of glucose metabolism. This may be the result of monocytes carrying out pro-inflammatory effector functions while also undergoing the process of differentiation into the macrophages found in lesions of AAV patients (29).

One of the most interesting aspects of this study are the similarities and differences in response to anti-MPO and anti-PR3 stimulation. In our earlier work, we showed that only anti-MPO treatment resulted in IL-1β production. Here we show that the initial metabolic response to both antibodies is remarkably similar with both glycolysis and oxphos being increased. However, only anti-MPO treatment resulted in sustained oxphos upregulation and this, along with increased mROS, correlated with the pro-inflammatory IL-1β production. Some of the metabolic effects of ANCA on monocytes mimic those seen in pro-inflammatory macrophages. In both cases, blocking glycolysis or mitochondrial ROS (mROS) results in a downregulation of the secretion of proinflammatory cytokines (15). In LPS stimulated macrophages, which have switched to predominantly glycolytic metabolism, mROS are produced through complex I (30). This mROS production has been shown to be dependent on an increase in mitochondrial membrane potential (15). It has been hypothesized that one reason for the switch to glycolysis in activated macrophages is the need to maintain mitochondrial membrane potential to allow for activation of this pathway (31). In response to TCR activation, some activated T cells also increase both glycolysis and oxidative respiration upon activation (32–34) and it is thought that the oxidative respiration may be needed in order to produce ROS (35). Our findings, that inhibition of glycolysis and mROS abrogate IL-1β production, while oligomycin had no effect, suggest that monocytes following ANCA stimulation may require glycolysis feeding into oxphos in order to produce ROS, rather than altering membrane potential to drive these

pro-inflammatory pathways. ANCA activated monocytes may therefore be more similar to T cells than macrophages in this regard.

Patients with anti-PR3 and anti-MPO often display differing disease phenotypes with anti-PR3 disease being particularly associated with granuloma formation. The differences between the overall metabolic phenotype of PR3 vs. MPO stimulated cells may be an important factor in these cells forming granulomas. Granuloma formation has been shown to be reliant on glycolytic metabolism in Mycobacterium Tuberculosis infection (36). The upregulation of these pathways alone in anti-PR3 stimulated monocytes may therefore play a role in granuloma formation in PR3-ANCA vasculitis. The upregulation of both oxidative phosphorylation and glycolysis seen in anti-MPO treated monocytes indicates a more pro-inflammatory phenotype. This suggests that these cells may be having increased effector functions to both recruit other immune cells and to directly damage tissue. This difference is emphasized by the increase in pro-inflammatory cytokines from these cells along with their increased ROS production. Both IL-1β and ROS have been shown to result in tissue damage which may help drive the glomerulonephritis that is observed in some patients with MPO-ANCA vasculitis (37).

How the extent of metabolic changes correlates with the role of monocytes in PR3 and MPO ANCA vasculitis is not known. We hypothesize that the short kinetics of anti-PR3 stimulated metabolic changes in monocytes prevents these cells from effectively upregulating ox phos and allows them to remain glycolytic without enhancing their inflammatory cytokine production. This in turn provides the potential for these cells to form granulomas associated with PR3 ANCA vasculitis. In contrast, the prolonged metabolic changes seen in MPO treated monocytes results in profound a pro-inflammatory phenotype which may result in tissue damage often found in patients with MPO-ANCA vasculitis.

This study has provided initial data into the response of these pathways to ANCA. We have shown how ANCA stimulation leads to shifts in monocyte cellular metabolism and how anti-MPO and anti-PR3 antibodies have differing effects on the metabolism of these cells. The links we have shown between pro-inflammatory cytokine production in anti-MPO treated monocytes and these changes in metabolism suggest that the overall differences between anti-MPO and anti-PR3 treated cells, and clinical phenotypes, may be at least partially explained by altered metabolic phenotypes. These alterations in metabolism may provide future targets for clinical interventions in an antibody type stratified treatment approach.

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 human participants were reviewed and approved by School of Medicine Research Ethics Committee Trinity College Dublin. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

EO'B designed and performed experiments, analyzed data, and wrote the manuscript. CW and EL performed experiments. JW performed statistical analysis. RP conceptualized experiments and edited the manuscript. ML and FH designed experiments, analyzed data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00553/full#supplementary-material

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Trend of Survival of a Cohort of Chinese Patients With Systemic Lupus Erythematosus Over 25 Years

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Objectives: To revisit the trend of survival of systemic lupus erythematosus in a cohort of Chinese patients over 25 years.

Methods: Patients who fulfilled the 1997 ACR criteria for SLE and were followed in our hospital since 1995 were included. Patients were stratified into two groups according to the year of diagnosis: (1) 1995–2004 and (2) 2005–2018. Survival of patients was studied by Kaplan–Meier analysis. Organ damage as assessed by the Systemic Lupus International Collaborating Clinics (SLICC) damage index (SDI) and causes of death in the first 10 years of SLE onset was compared between the two groups. Cox regression was used to study factors associated with survival.

Results: A total of 1,098 SLE patients were registered in our database. After excluding 157 patients diagnosed outside the time period of 1995–2018, 941 patients were studied (92% women). All were ethnic Chinese. The mean age of SLE onset was 35.1 ± 14.4 years, and the mean duration of observation was 13.1 ± 6.6 years. Seventy-seven (8.2%) patients were lost to follow-up. Groups 1 and 2 consisted of 364 and 577 patients, respectively. The mean SDI score at 10 years of disease onset was significantly higher in group 1 than group 2 patients (1.01 ± 1.43 vs. 0.57 ± 0.94; $p < 0.01$), particularly in the neuropsychiatric, musculoskeletal, and gonadal domains. Within 10 years of SLE onset, 32 (8.8%) patients in group 1 and 25 (4.3%) patients in group 2 died ($p = 0.005$). The 5- and 10-year cumulative survival rates were 93.6 and 91.0% in group 1 and 96.5 and 94.2% in group 2 patients, respectively (log-rank test $p = 0.048$). Infection accounted for more than half of the deaths in both groups. More group 1 than group 2 patients died of vascular events, but the difference was not statistically significant. Cox regression showed that the age of SLE onset and damage score accrued at 10 years, but not the time period in which SLE was diagnosed, were significantly associated with mortality.

Conclusions: The improvement in survival of our SLE patients is probably related to the accrual of less organ damage in the past 15 years.

Keywords: damage, lupus, mortality, time trend, morbidity

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that predominantly affects younger women. The disease course is characterized by periods of remission and exacerbation that are largely unpredictable (1). The consequence of this fluctuating disease course is organ damage, as a result of persistent disease activity or treatment-related complications. Organ damage in SLE is a major risk factor for further organ damage accrual, impaired quality of life (QOL), and mortality (2, 3).

With earlier referral and diagnosis, availability of renal replacement therapy, more potent antimicrobial drugs, less toxic immunosuppressive regimens, and improved supportive care for organ complications, the survival of SLE has improved tremendously in the past few decades (4). A recent meta-analysis (5) of 125 studies showed that survival of adult SLE improved gradually from the 1950s to the mid-1990s in both affluent and less affluent countries. However, the survival rate has plateaued since the mid-1990s despite a reduced proportion of patients deceased due to active SLE. A substantial proportion of SLE patients still succumbed of complications related to refractory disease or therapies (6). The mortality of SLE is increased by at least three- to 4-fold when compared with the age- and gender-matched population (7). The commonest cause of death is infection, followed by vascular complications and cancer. Thus, there are unmet needs to introduce more effective but less toxic therapies in SLE and reduce long-term complications such as atherosclerosis, osteoporosis, and malignancies in order to improve the survival of the disease further.

Although there have been a number of survival studies of SLE in the past decade, not too many were performed in the Asian populations (8–25). Analysis of the survival of hospitalized patients (9, 22), those admitted to the intensive care unit (12, 19) or referred to tertiary centers (23), subsets of patients with nephritis (14), neuropsychiatric manifestations (17), or pulmonary hypertension (15) in some studies would create bias in the mortality rate. Moreover, the follow-up of most of these Asian studies was not long enough to look at the trend of survival over time. A large cohort of Chinese SLE patients in our hospital was followed by the same group of physicians since 1995. We hereby report the secular trend of survival of these patients in the past 25 years.

PATIENTS AND METHODS

Tuen Mun Hospital is a large regional public hospital in Hong Kong providing medical services to a population of 1.2 million residing in the vicinity. All citizens are entitled to receive a full range of medical services from government hospitals by payment of a nominal fee. Patients diagnosed to have SLE after 1990 in our outpatient clinics or during hospital stay or referred from other hospitals are captured in a longitudinal database. Adult patients under the care of all specialists such as rheumatologists, nephrologists, geriatricians, hematologists are included. All are ethnic Chinese with their family origin in southern part of China. All patients fulfill four or more 1997 American College of Rheumatology (ACR) criteria for the classification of SLE (26) and are being followed by the same group of physicians at an usual interval of 12–16 weeks. More frequent clinic visits are arranged for those with active/unstable disease or complications.

The demographic characteristics, cumulative manifestations of SLE, and autoantibodies of the patients are captured. The clinical status of the SLE patients in our registry is updated every 6 months.

Assessment of Organ Damage and Mortality

Organ damage of SLE is assessed by the Systemic Lupus International Collaborating Clinics (SLICC) damage index (SDI) (27), a validated instrument consisting of 41 items that measure irreversible organ damage not caused by active inflammation in 12 organ systems. Each item should be present for at least 6 consecutive months in order to be scored. The SDI score is updated annually.

For patients who died during their disease course, we analyzed the causes of death according to the documentation of their attending specialists in the medical records based on investigation results or best clinical judgment. Autopsy would be performed for uncertain cause of death, academic interest, or medico-legal purpose. For those who succumbed due to any causes, data were censored at the time of death.

Trend of Survival Over Time

To study the trend of survival over time and the causes of death, we divided our patients arbitrarily into two groups: (1) group 1: SLE diagnosed between 1995 and 2004; and (2) group 2: SLE diagnosed between 2005 and 2018. Organ damage, mortality, and causes of death in the first 10 years of SLE diagnosis were also compared between the two groups.

Statistical Analyses

Unless otherwise stated, values in this study were expressed as mean ± SD (standard deviation). Comparison of continuous variables between two groups was performed using the independent sample Students' *t*-test. Categorical variables were compared by the chi-square test. When the frequency was <5 in any cell of the contingency table, the Fisher exact test was used. Correction for multiple comparisons was made by Bonferroni's method. The cumulative probability of survival of the patients over time was studied by Kaplan–Meier analysis. For those who died or were lost to follow-up, data were censored at the time of death and last clinic visits or hospitalization, respectively. Comparison of survival between two groups was made by the long-rank test. Cox regression was used to analyze the hazard ratio (HR) and 95% confidence interval (CI) for survival. Covariates included in the model were age of SLE onset, sex, SDI score, renal involvement, ever use of hydroxychloroquine (HCQ) within 10 years of diagnosis, and time period in which patients were diagnosed. Statistical significance was defined as a p-value of <0.05, 2-tailed. All statistical analyses were performed using the SPSS program (version 18.0 for Windows 10).

RESULTS

Study Population and Clinical Manifestations

Up to March 2020, a total of 1,098 SLE patients were registered in our cohort database. One hundred and fifty-seven patients were excluded as the diagnosis of SLE was made before 1995 or after 2019. Finally, 941 patients were included (862 women; 92%). All were ethnic Chinese. The mean age of onset of SLE was 35.1 ± 14.4 years, and the mean follow-up of these patients was 13.1 ± 6.6 years. Seventy-seven (8.2%) patients were lost to follow-up, and their data were censored at the last clinic visits.

There were 364 patients in group 1 and 577 patients in group 2. The cumulative manifestations of these patients are shown in **Table 1**. The age of onset of SLE was significantly higher in group 2 patients. The prevalence of anti-ENA antibodies was also significantly higher in this group. Regarding clinical manifestations, arthritis and lymphopenia were significantly more frequent in group 1 patients. The frequencies of other

TABLE 1 | Cumulative manifestations and therapies within 10 years of diagnosis.

Clinical manifestations	Group 1 (*N* = 364)	Group 2 (*N* = 577)	*P*	***P*
	N (%); mean ± SD			
Age of onset, years	32.4 ± 13.6	36.8 ± 14.6	<0.001	<0.03
Women	328 (90.1)	534 (92.5)	0.19	NS
Duration of follow-up, years	20.2 ± 2.7	8.6 ± 3.9	<0.001	<0.03
Arthritis	263 (72.2)	339 (58.8)	<0.001	<0.03
Malar rash	173 (47.5)	243 (42.1)	0.10	NS
Discoid rash	44 (12.1)	50 (8.7)	0.09	NS
Mucosal ulceration	58 (15.9)	78 (13.5)	0.31	NS
Photosensitivity	100 (27.5)	110 (19.1)	0.003	NS
Hemolytic anemia	92 (25.3)	135 (23.4)	0.51	NS
Leukopenia	147 (40.4)	185 (32.1)	0.009	NS
Thrombocytopenia	92 (25.3)	132 (22.9)	0.40	NS
Lymphopenia	264 (72.5)	344 (59.6)	<0.001	<0.03
Lymphadenopathy	60 (16.5)	85 (14.7)	0.47	NS
*Neuropsychiatric manifestations	43 (11.8)	50 (8.7)	0.12	NS
Renal	211 (58.0)	282 (48.9)	0.007	NS
Serositis	73 (20.1)	117 (20.3)	0.93	NS
Myositis	13 (3.6)	21 (3.6)	0.96	NS
Gastrointestinal	25 (6.9)	60 (10.4)	0.07	NS
Autoantibodies				
Anti-dsDNA	252 (69.2)	400 (69.3)	0.98	NS
Anti-Sm	46 (12.6)	160 (27.7)	<0.001	<0.03
Anti-Ro	205 (56.3)	384 (66.6)	0.002	0.06
Anti-La	50 (13.7)	152 (26.3)	<0.001	<0.03
Anti-nRNP	95 (26.1)	220 (38.1)	<0.001	<0.03
Medications ever used ≥1 month				
Prednisolone	280 (76.9)	468 (81.1)	0.12	NS
Hydroxychloroquine	213 (58.5)	452 (78.3)	<0.001	<0.03
Methotrexate	23 (6.3)	64 (11.1)	0.01	NS
Mycophenolate mofetil	66 (18.1)	227 (39.3)	<0.001	<0.03
Tacrolimus/	78 (21.4)	136 (23.6)	0.45	NS
Cyclophosphamide	87 (23.9)	47 (8.1)	<0.001	<0.03
Azathioprine	200 (54.9)	276 (47.8)	0.03	NS

N, number; SD, standard deviation; NS, non-significant.

**Only included manifestations that required immunosuppression (e.g., psychosis, acute confusional state, myelitis, neuropathy, myasthenia gravis).*

***P-values adjusted by Bonferroni's method.*

TABLE 2 | Organ damage within 10 years of SLE onset in patients studied.

Organ/system	Group 1	Group 2	*P
	N (%)		
Ophthalmological	19 (5.2)	24 (4.2)	NS
Neuropsychiatric	54 (14.8)	50 (8.7)	0.042
Renal	39 (10.7)	37 (6.4)	NS
Pulmonary	18 (4.9)	34 (5.9)	NS
Cardiovascular	16 (4.4)	23 (4.0)	NS
Peripheral vascular	10 (2.7)	13 (2.3)	NS
Gastrointestinal	3 (0.8)	2 (0.3)	NS
Musculoskeletal	50 (13.7)	40 (6.9)	0.014
Dermatological	22 (6.0)	31 (5.4)	NS
Gonadal	17 (4.7)	2 (0.3)	<0.014
Endocrinological	9 (2.5)	15 (2.6)	NS
Malignancy	8 (2.2)	14 (2.4)	NS
Total SDI	167 (45.9)	206 (35.7)	0.028
SDI ≥ 5	10 (2.7)	3 (0.5)	NS
Mean time to first organ damage, months	41.1 ± 40	18.9 ± 28.1	<0.001

SD, standard deviation; N, number; SDI, systemic lupus; NS, non-significant; Erythematosus international collaborating clinics (SLICC) damage index.
**P-values adjusted by Bonferroni's method.*

TABLE 3 | Causes of death within 10 years of onset of SLE.

Cause of death	Group 1	Group 2	P
	Number (%)		
Infection	18 (56.3)	14 (56.0)	0.99
*Vascular	5 (15.6)	1 (4.0)	0.22
Pulmonary hypertension	1 (3.1)	3 (12.0)	0.31
Malignancy	2 (6.3)	3 (12.0)	0.65
Refractory and uncontrolled SLE	2 (6.3)	1 (4.0)	1.00
Suicide	1 (3.1)	1 (4.0)	1.00
Sudden death without obvious causes	3 (9.4)	2 (8.0)	1.00
Total	32 (100)	25 (100)	0.005

**Included cerebrovascular accident, acute coronary syndrome, and aortic dissection.*

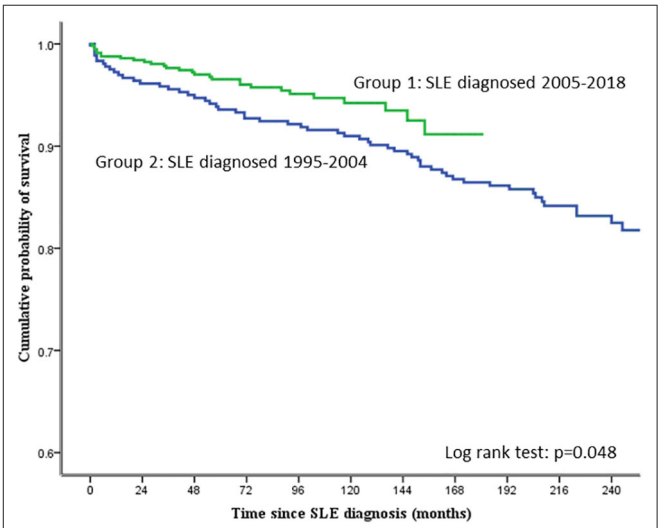

FIGURE 1 | Cumulative probability of survival in the SLE patients studied.

manifestations were similar between the two groups. Regarding treatment of SLE during the first 10 years of diagnosis, more group 2 patients had received HCQ and mycophenolate mofetil (MMF) whereas more group 1 patients were ever treated with cyclophosphamide (CYC).

Organ Damage

Table 2 shows the organ damage scores in the two groups of patients within 10 years of disease onset. The mean total SDI score was significantly higher in group 1 than group 2 patients. Among the 12 organ systems, the SDI scores in the neuropsychiatric, musculoskeletal, and gonadal domains were significantly higher in group 1. The proportion of patients with organ damage in these three systems was also significantly higher in this group of patients. In those patients with organ damage, the mean time to first damage was 41.1 ± 40 months in group 1 and 18.9 ± 28.1 months in group 2 ($p < 0.001$), suggesting that late damage was more common in group 1 patients.

Mortality

Within 10 years of SLE onset, 32 (8.8%) patients in group 1 and 25 (4.3%) patients in group 2 died ($p = 0.005$). **Table 3** shows the causes of death in these patients. Infection was the main cause of death in both groups, accounting for more than half of the deaths. Vascular causes of death were more common in group 1 than group 2 patients, but the difference was not statistically significant.

The cumulative survival rate of all the 941 patients studied was 95.3, 92.9, 88.5, and 84.5% at 5, 10, 15, and 20 years, respectively. **Figure 1** shows the cumulative probability of survival of the two groups of patients. The 3-, 5-, and 10-year survival in group 1 patients was 95.6, 93.6, and 91%, respectively. The corresponding figures for group 2 were 97.7, 96.5, and 94.2%, respectively. The difference in survival between group 1 and group 2 was statistically significant (log-rank test; $p = 0.048$).

Table 4 shows the causes of death of all the 941 patients studied according to the duration of SLE. In addition to the 57 patients who died within 10 years of SLE onset, 30 other patients in the cohort died beyond 10 years of SLE onset (total 87 deaths). Patients who died beyond 10 years of disease onset were less likely to be caused by infection but more likely to be contributed by pulmonary hypertension, chronic cardiopulmonary disease, and malignancies.

Table 5 shows the Cox regression analysis for factors associated with mortality. Univariate analysis showed that age of onset, male sex, renal involvement, and SDI score at 10 years were associated with mortality. The use of HCQ was negatively associated with mortality. Group 1 patients showed a higher mortality rate than group 2, but statistical significance

was borderline (hazard ratio 0.61 [0.38–1.001]; $p = 0.05$). Multivariate analysis revealed only the age of onset (1.06 [1.04–1.08] per year; $p < 0.001$), SDI at 10 years (1.65 [1.47–1.85] per point; $p < 0.001$), and ever use of HCQ (0.54 [0.34–0.85]; $p = 0.008$) were significantly associated with mortality. The time period in which SLE was diagnosed was not a significant factor determining this outcome.

DISCUSSION

This is a survival study of an inception cohort of SLE patients diagnosed since 1995. Our results showed that the overall 10-year survival of our SLE patients was 92.9%, which is similar to those reported in Asian studies after the 2000s (9–11, 16, 18, 21, 24). However, direct comparison is not feasible because the cumulative survival rate computed by the Kaplan–Meier method was not reported in most of these studies. In our cohort, there was an improvement in survival of patients diagnosed between 2005 and 2018 compared to those diagnosed between 1995 and 2004. Organ damage was also significantly less common in patients diagnosed in the more recent period. Univariate Cox regression analysis showed that survival of the group of patients diagnosed after 2004 (group 2) was better than those who diagnosed earlier (group 1) ($p = 0.05$; borderline significance). However, statistical significance was lost when damage score at 10 years, sex, age of SLE onset, renal involvement, and ever use of HCQ were put into the multivariate model. As group 1 patients had younger onset of SLE (favorable factor for survival), the worse prognosis of this group was likely due to more organ damage accrued at 10 years when compared to group 2 patients.

An interesting observation was noted when we compared the clinical manifestations of the two groups of patients. Patients diagnosed after 2004 were significantly older at the time of SLE diagnosis, and they were less likely to have arthritis and renal disease during the course of their illness. The mean age of SLE diagnosis has increased from 32.4 years to 36.8 years in the period of 2005–2018. This is consistent with our clinical impression that more SLE patients in the recent decade were diagnosed in the middle age range with the presentation of hematological in the absence of musculoskeletal or dermatological symptoms. The reason for this observation is unclear, but it is unlikely to be the effect of increased referrals or awareness of SLE by primary care physicians as our hospital has been the only specialty referral hospital for SLE in the areas covered by public medical service in the past 2–3 decades. Moreover, there has not been any change in the pattern of inter-specialty referral and our cohort of SLE patients has included all the patients seen by different specialists in our department. One postulation is the increased use of HCQ in our SLE patients beyond dermatological and articular indications in the past decade, which might protect against the development of joint and renal manifestations. However, further clinical trials of HCQ are needed to confirm this postulation.

As shown in **Table 1**, the prevalence of antibodies to the anti-extractable nuclear antigens (anti-ENA) has increased from the period 1995–2004 to 2005–2018. This can be explained by the change in the methodology of anti-ENA assay in our laboratory from counter immune-electrophoresis (CIEP) to enzyme-linked

TABLE 4 | Cause of death of the whole cohort of SLE patients.

Causes of death	0–5 years of onset	>5–10 years of onset	>10 years of onset
Number of deaths	***N* = 40**	***N* = 17**	***N* = 30**
Infection	24 (60%)	8 (47%)	6 (20%)
Vascular	4 (10%)	2 (11.8%)	6 (20%)
Pulmonary hypertension	2 (5%)	2 (11.8%)	5 (16.7%)
Malignancy	3 (7.5%)	2 (11.8%)	5 (16.7%)
Sudden death without obvious causes	2 (5%)	3 (17.6%)	5 (16.7%)
Suicide	2 (5%)	0 (0%)	0 (0%)
Refractory/uncontrolled SLE	3 (7.5%)	0 (0%)	1 (3.3%)
Pulmonary fibrosis	0 (0%)	0 (0%)	2 (6.7%)

SLE, systemic lupus erythematosus.

TABLE 5 | Cox regression analysis of factors affecting mortality.

Covariates	Univariate	*P*	Multivariate	*P*
	Hazard ratio (95% confidence interval)		Hazard ratio (95% confidence interval)	
Age of SLE onset, per year	1.06 (1.05–1.08)	<0.001	1.06 (1.04–1.08)	<0.001
SDI score at 10 years, per point	1.45 (1.32–1.59)	<0.001	1.60 (1.42–1.80)	<0.001
Male sex	2.29 (1.31–4.00)	0.004	1.30 (0.73–2.31)	0.38
Renal involvement	2.04 (1.37–3.02)	<0.001	1.36 (0.86–2.17)	0.19
Ever use of HCQ at 10 years	0.42 (0.28–0.63)	<0.001	0.54 (0.34–0.85)	0.008
Group 2 (vs. group 1)	0.61 (0.38–1.001)	0.05	0.76 (0.45–1.28)	0.30

SDI, systemic lupus erythematosus international collaborating clinics (SLICC) damage index; HCQ, hydroxychloroquine.

immunosorbent assay (ELISA) followed by confirmation with Western blotting in the year 2005.

Another observation from the current study is that patients diagnosed after 2004 had accrued less organ damage at 10 years, which is the most important factor for the improved survival. In particular, reduced damage in the neuropsychiatric, musculoskeletal, and gonadal domains has made the difference. In a cohort study from the US John Hopkins University, glucocorticoid use was a major risk factor for organ damage accrual (28). The risk of organ damage was increased by more than 3-fold when the mean daily dosage of prednisone was >20 mg. The multicenter Asia Pacific lupus collaboration group also reported that the time-adjusted mean prednisolone dose was independently associated with damage accrual in SLE patients (29). In a subset of patients with no disease activity over time, the mean prednisolone dose remained an independent risk factor for damage accrual. Thus, every attempt should be made to limit the dosage and duration of glucocorticoid use in SLE. The more judicious use of high-dose glucocorticoids in our cohort has led to the observed reduction in avascular necrosis of the bone and osteoporotic fractures. For instance, the standard dosage of prednisolone for severe lupus nephritis has reduced from 1 mg/kg/day to around 0.6–0.8 mg/kg/day in recent years with the early use of glucocorticoid-sparing agents such as azathioprine and mycophenolate mofetil (MMF). Moreover, the duration of high-dose prednisolone has been limited to <8 weeks. On the other hand, the substitution of cyclophosphamide (CYC) pulses to MMF as first-line induction therapy of major organ disease in most patients is linked to the lower incidence of premature ovarian failure in our patients. With the increased awareness of the cardiovascular complications, regular surveillance of traditional risk factors is being performed in recent years, which serves to explain the lower incidence of vascular complications such as ischemic stroke in our patients.

As shown in our data, infection remains the main cause of death of our SLE patients regardless of the time period of diagnosis. Thus, more effort has to be done to minimize the risk of infection in SLE patients. Apart from the use of the minimally effective doses of immunosuppressive medications, measures to prevent common viral and bacterial infections are equally important. Influenza and pneumococcal vaccines, which are safe and efficacious in SLE (30), are not routinely administered to our patients. These vaccinations should be more encouraged in the future by a standard protocol facilitated by our rheumatology nurses. Personal hygiene and social distancing are crucial as reflected in the COVID-19 global epidemic. Antibiotic prophylaxis for certain opportunistic infections such as pneumocystis jirovecii pneumonia (31) is not yet a routine practice in Asian countries but should be considered for SLE patients with multiple risk factors such as renal dysfunction, severe lymphopenia, and treatment with combination of multiple immunosuppressive agents, particularly high-dose glucocorticoid and CYC.

There are several limitations in this study. First, we did not have data on the serial disease activity score over time and therefore it is uncertain if better disease control in recent years has contributed to the improved survival. Second, data on the cumulative dosages of medications, particularly prednisolone and HCQ, are unavailable for evaluation of their contribution to SLE prognosis. Finally, we did not have information of all the patients on the duration of symptoms before SLE diagnosis and their drug compliance, which might also be factors affecting the long-term prognosis.

CONCLUSION

We have presented the data of a large cohort of SLE patients treated in a service hospital over 25 years and confirmed the improved survival. The improvement in SLE prognosis is mainly contributed by a significant reduction in organ damage accrual, which is linked to the more judicious use of glucocorticoids, early use of glucocorticoid-sparing agents, and the regular surveillance and treatment of traditional vascular risk factors. Despite the decrease in the mortality figures, further reduction should be the target. As infection remains the main cause of death, protocol-based vaccination programs against common viral and bacterial infections, as well as antibiotic prophylaxis for opportunistic infections in high-risk patients, should be adopted in the future. It is hoped that SLE patients can continue to live longer with less end organ damage accrual so that they can enjoy a better quality of life.

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 human participants were reviewed and approved by Ethics committee, Tuen Mun Hospital, Hong Kong. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors have contributed to the interpretation of the data and approved the final manuscript.

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CASE REPORT
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Gout in the Chest Misdiagnosed as Ankylosing Spondylitis

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Gout is a crystal-related joint disease caused by single sodium urate deposition in the joints or in soft tissues. In recent years, the incidence of gout has increased, but cases of urate crystals deposited in the chest–ribs are rare. Here, we describe a 39-year-old man who complained of frequent pain and a feeling of tightness in chest–ribs and was misdiagnosed as ankylosing spondylitis. In addition, treatment with non-steroidal anti-inflammatory drugs (NSAIDs) and etanercept for 6 months showed no improvement, which confirmed the misdiagnosis. After physical examination, blood examination, and dual-energy CT examination, the patient was diagnosed with gout and received 50 mg benzbromarone once a day with treatment of low serum uric acid. In conclusion, gout in the chest and ribs is an unusual manifestation and has rarely been reported in the literature. This case highlights an important but overlooked history of hyperuricemia in the diagnosis, and dual-energy CT is the preferred method for differential diagnosis of chest–ribs gout.

Keywords: gout, hyperuricemia, ankylosing spondylitis, dual energy CT (DECT), case report (source: MeSH NLM)

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CASE PRESENTATION

We report a case of a 39-year-old man whose main complaints were chest pain and tightness in the past 2 years. He had been taking non-steroidal anti-inflammatory drugs (NSAIDs) orally over the past year, but the relief was not obvious. Six months ago, he was diagnosed as ankylosing spondylitis (AS) and treated with etanercept, but the symptoms have not been alleviated, and chest and back discomfort was worse. He has no other history of disease, although hyperuricemia has been reported in previous hematological tests. The patient has no history of trauma, uveitis, psoriasis, inflammatory bowel disease, or pain in other joints. There was no obvious abnormality in the chest, heart, and abdomen. Thoracic mobility was normal. There was no movement restriction of the spine. He had tenderness of the fourth to seventh thoracic vertebrae but no tenderness in the sacroiliac joints. The straight-leg elevation test was negative. The results of the blood test showed high uric acid (9.9 mg/dL, reference value: 3.4–7.0 mg/dL) and positive human leukocyte antigen B27 (HLA-B27). Erythrocyte sedimentation rate, C-reactive protein (CRP), blood

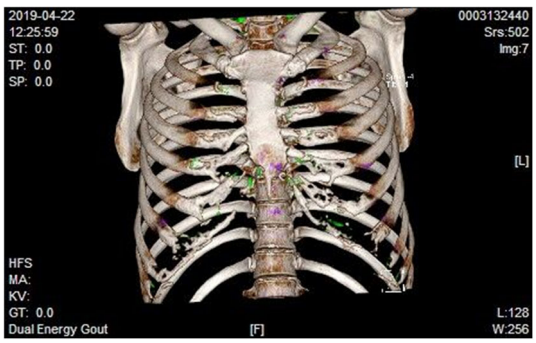

FIGURE 1 | Dual-energy CT examination of the chest. Urate crystal deposition is indicated in green.

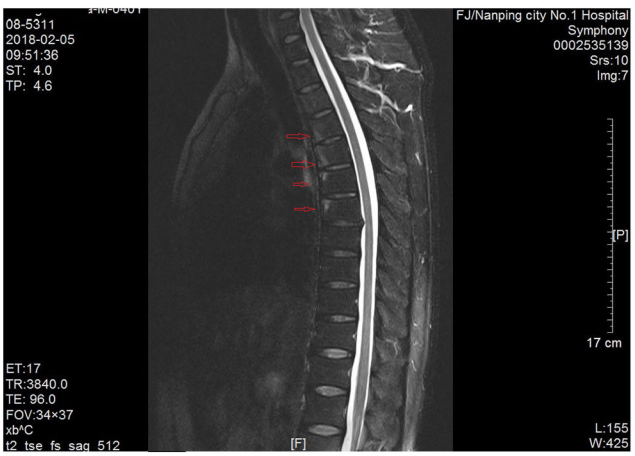

FIGURE 2 | MRI examination of thoracic vertebrae. Abnormal signal of T4–T7 was indicated by a red arrow.

tumor markers, and other blood tests were normal. Electrocardiogram, cardiac color Doppler ultrasound, full abdominal color Doppler ultrasound, and chest CT excluded heart disease and tumor. Both CT and MR of sacroiliac joints suggested mild degeneration of bilateral sacroiliac joint and no bone edema. When the patient visited our department, we considered the possibility of uric acid crystal deposition and performed dual-energy CT (DECT) examination of the chest. Urate crystal deposition was found in the bilateral chest; costal joint; costal cartilage; third left, first bilateral, and second costal vertebrae; costal head joint; first and second lateral transverse process; and left upper scapula (**Figure 1**). According to his medical history, physical examination, laboratory examination, and DECT examination, urate crystals may be the main culprit.

DISCUSSION

Gout is a metabolic rheumatism that is common in middle-aged and elderly men. The main manifestations are crisis of joint pain, swelling, tenderness, and elevated skin temperature. In the vast majority of patients, there is hyperuricemia, and urate is mainly deposited in the joints and periarticular tissue. Deposit in facet joints is a very rare event (1). Pain and tightness in the chest and ribs may indicate diseases such as inflammation of the ribs, inflammatory low back pain, heart disease, or chest tumors, but gout should also be considered, despite its rare occurrence.

This is an interesting case of a man who has experienced recurrent chest and back pain and chest tightness over the past 2 years. Electrocardiogram, cardiac color Doppler ultrasound, total abdominal color Doppler ultrasound, lung CT, and related blood tests were performed to exclude heart diseases, tumors, and inflammatory diseases. Both CT and MR of thoracic vertebrae showed abnormal signal changes of the thoracic vertebrae in T4–T7, so it is necessary to consider the possibility of AS. However, no sacroiliitis was found in CT and MR. ESR and CRP were normal. In addition, he has no family history of psoriasis and AS. The patient consulted many doctors and was misdiagnosed as AS because of HLA-B27(+) and abnormal signal changes of T4–T7 (**Figure 2**).

AS is a progressive chronic inflammatory disease that affects the axial skeleton, leading to structural damage and dysfunction. Clinical manifestations of AS usually begin in late adolescence or early adulthood and rarely after the age of 40. The characteristic clinical symptoms of AS are inflammatory back pain, IBP, and morning stiffness, but they are often not well-recognized at the first visit (2). In addition, although 90% of AS patients are HLA-B27 positive, in our clinical practice, we often encounter the situation of HLA-B27-positive patients being misdiagnosed as AS.

Our case was HLA-B27 positive, with repeated chest and back pain for 2 years. However, the most common site of AS is the sacroiliac joint. We performed CT and MRI of the sacroiliac joint and found no sacroiliitis. ESR and CRP were normal. He has no family history of psoriasis and AS. Therefore, the diagnosis of AS is not accurate. In addition, treatment with NSAID and etanercept for 6 months showed no improvement, which confirmed the misdiagnosis of AS. He had a history of hyperuricemia, and the patient was never treated seriously. His serum uric acid level provided an important clue to the diagnosis of the disease. We conducted chest dual-energy CT and found that the thoracic vertebrae and chest–ribs were covered with green urate crystals. In conclusion, despite all new diagnostic tools, establishing a correct diagnosis of gout remains one of the daily challenges of clinical rheumatologists.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Fujian Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

WX designed the study. SZ, QW, WQ, and SX performed the study. All authors contributed to the article and approved the submitted version.

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Fulminant Course of Neuromyelitis Optica in a Patient With Anti-MDA5 Antibody-Positive Dermatomyositis: A Case Report

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Anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibody is a myositis-specific marker detected in clinically amyopathic dermatomyositis (DM). DM with anti-MDA5 antibody can be accompanied by rapidly progressive interstitial lung disease (RP-ILD) and other autoimmune disorders. Until now, only one case of neuromyelitis optica (NMO) with anti-MDA5-positive DM has been reported worldwide, in which the patient achieved a favorable outcome with intensive immunotherapy. We report a case of NMO in a patient with anti-MDA5-positive DM complicated by ILD and rheumatoid arthritis. Our patient experienced a fulminant course of NMO, rather than RP-ILD, in the presence of hyperferritinemia, which resulted in profound neurological sequelae despite immunotherapy including rituximab.

Keywords: neuromyelitis optica, clinically amyopathic dermatomyositis, interstitial lung disease, antibody, rituximab, ferritin

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INTRODUCTION

Anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibody is a myositis-specific marker detected in clinically amyopathic dermatomyositis (DM) (1). Anti-MDA5-positive DM is known to be complicated by rapidly progressive interstitial lung disease (RP-ILD), resulting in high mortality (2, 3). Serum ferritin level is a disease activity biomarker in interstitial lung disease (ILD) with anti-MDA5-positive DM (4). Neuromyelitis optica (NMO) is an immune-mediated inflammatory disease of the central nervous system whose pathogenesis is linked to anti-aquaporin-4 (anti-AQP4) antibody (5). To the best of our knowledge, only one case of NMO with anti-MDA5-positive DM has been reported worldwide, in which the patient achieved a favorable outcome with intensive immunotherapy (6). Herein, we report a case of NMO with high disease activity in the presence of anti-MDA5 antibody and persistent hyperferritinemia, which resulted in profound neurological sequelae.

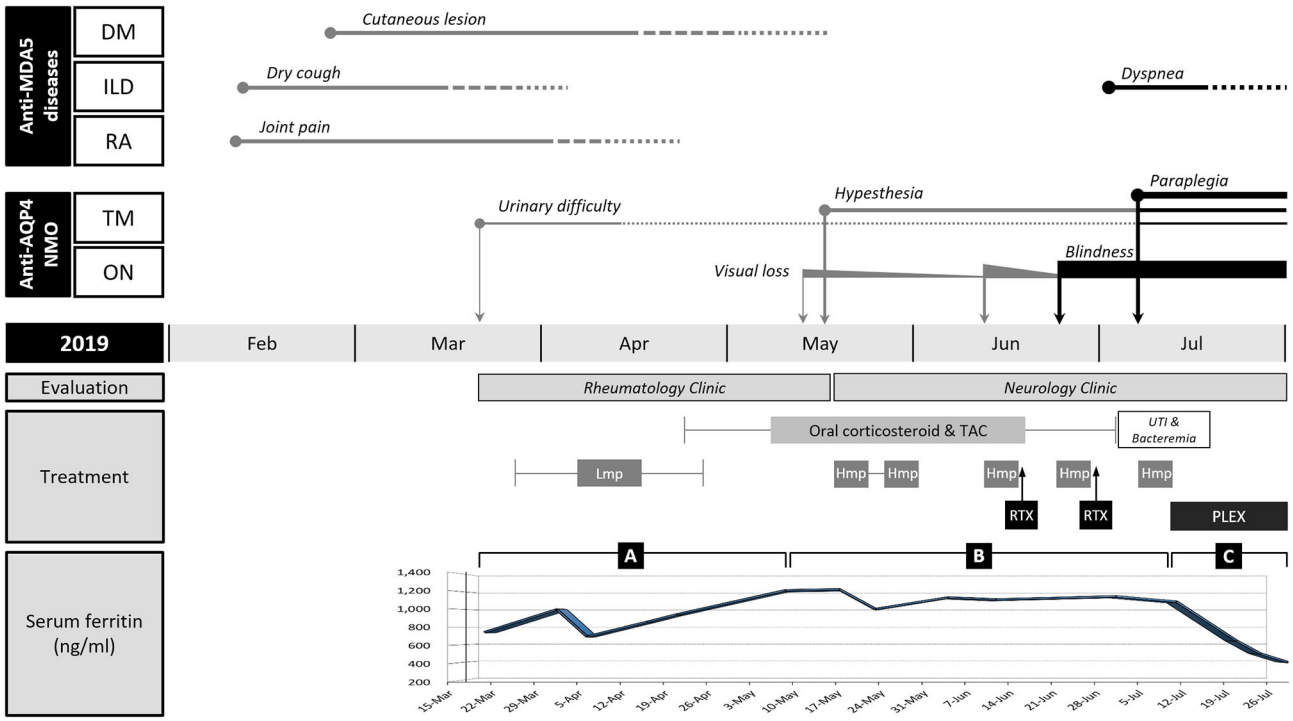

FIGURE 1 | Clinical course and changes in the serum ferritin level of the patient. Anti-MDA5-positive dermatomyositis (DM), interstitial lung disease (ILD), and rheumatoid arthritis (RA) were improved after immunotherapy with corticosteroids and tacrolimus (TAC), but neuromyelitis optica (NMO) frequently relapsed from mid-May (in 2019). NMO and ILD were exacerbated after rituximab (RTX) induction. Hyperferritinemia persisted throughout the course of illness. The change of serum ferritin was divided into three segments based on the inflection points: rising **(A)**, plateau **(B)**, and falling **(C)** phases. **(A)** Serum ferritin level initially decreased by 30% at the beginning of immunotherapy but kept increasing despite symptomatic improvement of ILD. **(B)** Serum ferritin level remained high in the period of frequent relapses of NMO. **(C)** Serum ferritin level drastically dropped after plasma exchange (PLEX). The length of the straight line indicates the duration of symptoms or signs. The thickness of straight lines and the size of circles or rectangles directly indicate clinical severity. The solid line and dotted lines reflect the presence and alleviation of the symptoms, respectively. TM, transverse myelitis; ON, optic neuritis; Lmp, intravenous low-dose methylprednisolone; Hmp, intravenous high-dose methylprednisolone; UTI, urinary tract infection.

CASE PRESENTATION

A 35-year-old man who had a 2-month history of urinary difficulty was referred to our neurology clinic for evaluation of decreased visual acuity of the right eye and paresthesia of both legs. Within the last 2 months, he had been diagnosed with rheumatoid arthritis (RA), ILD, and clinically amyopathic DM.

When the patient first visited the rheumatology clinic, he had a 4-week history of non-productive cough and joint pain with morning stiffness in both hands. The detailed clinical course of the patient is shown in **Figure 1**. On physical examination, erythematous or purpuric patches with crusts on the anterior chest, knuckles, and elbows (**Figures 2A,B**) were observed. Ultrasonography of joints showed synovitis and effusion involving multiple small joints (both wrist joints and five small joints of both hands) and both knee joints. The three-phase bone scintigraphy revealed inflammation in the affected joints (**Figure 2C**). Computed tomography (CT) of the chest showed perilobular consolidations and fibrosis in the posterior aspect of both lower lungs (**Figure 4A**). In pulmonary function test (PFT), forced vital capacity (FVC) was decreased (3.05 L, 61% of predicted value), and forced expiratory volume in 1 s (FEV1)/FVC ratio was increased (93%), which were suggestive of restrictive lung disease. The serologic test showed positivity for antinuclear antibody (titer 1:40), rheumatoid factor (59.4 IU/ml, normal range < 14 IU/ml), and anti-cyclic citrullinated peptide antibody (9.5 U/ml, normal range < 5 U/ml). The levels of acute phase reactants including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum ferritin were elevated as follows: ESR 66 mm/h (normal range < 20 mm/h), CRP 1.03 mg/dl (normal range < 0.3 mg/dl), and ferritin 743 ng/ml (normal range 4–275 ng/ml). Skin biopsy of the anterior chest showed superficial perivascular inflammation (**Figure 2D**).

Under the diagnosis of RA-ILD, the patient started intravenous (IV) methylprednisolone therapy. A week after hospitalization, a sudden elevation (2,187 IU/L, normal range 56–244 IU/L) of serum creatine kinase (CK) was observed. Though the patient did not complain of muscle weakness and myalgia, magnetic resonance imaging (MRI) of the thigh showed contrast-enhancing asymmetric and patchy T2-hyperintensity in hip and thigh muscles (**Figure 2E**). Anti-synthetase antibodies and anti-Mi-2 antibodies were negative. The pathologic finding of muscle biopsy led to additional diagnosis of DM (**Figure 2F**).

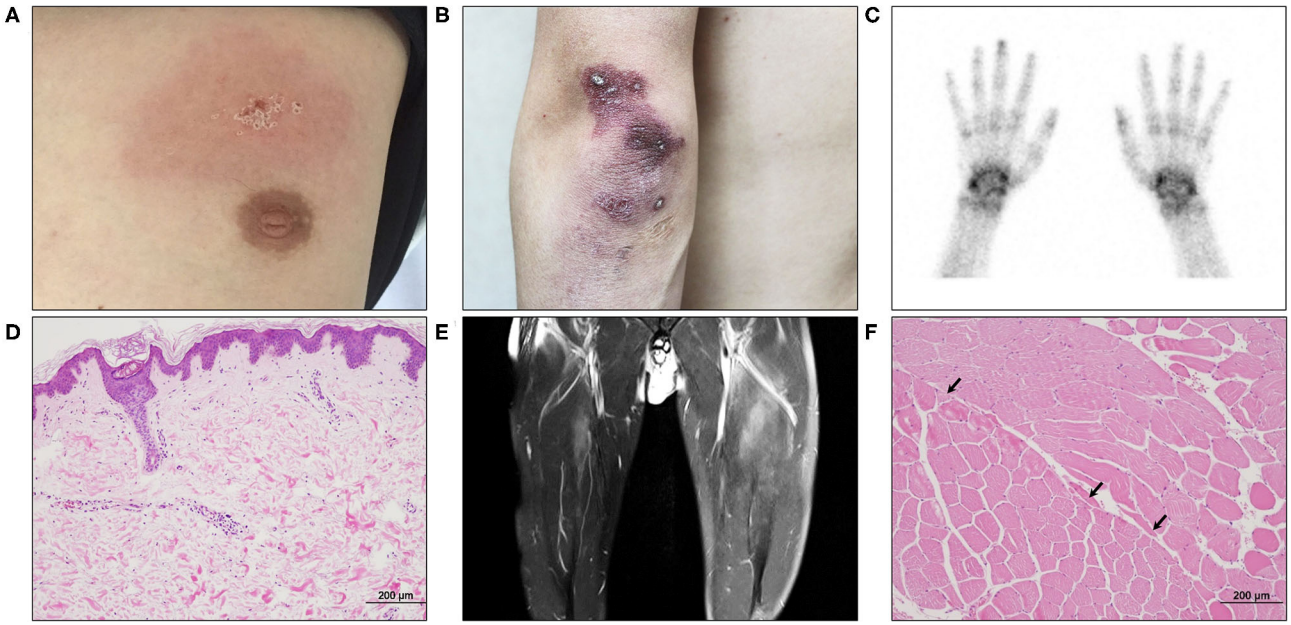

FIGURE 2 | The initial cutaneous, imaging, and pathological findings of the patient. **(A)** An erythematous patch with scales was seen in the anterior chest. **(B)** A diffuse purpuric patch with crusts was seen in the left elbow. **(C)** The three-phase bone scintigraphy revealed increased uptake in both wrists and interphalangeal joints. **(D)** Skin biopsy of the chest displayed a couple of small vessels with perivascular inflammatory cell infiltration in the upper dermis (H&E, ×100). **(E)** Thigh MRI showed multifocal infiltrations in the anterior thigh muscles with left side dominancy. **(F)** Biopsy of the left vastus lateralis muscle revealed a small number of atrophic angular myocytes (arrows) in the perifascicular area (H&E, ×100).

The patient continued IV methylprednisolone therapy (20–40 mg daily) for a month and took oral deflazacort (24 mg daily) and tacrolimus (1–2 mg daily) for the next month. The symptoms and signs including arthralgia and cough almost improved, and the levels of muscle enzymes normalized. Nevertheless, serum ferritin level has been gradually increasing up to 1,210 ng/ml after a transient decrease at the beginning of immunotherapy (*Segment A* in **Figure 1**).

When the patient presented to a neurology clinic with visual and sensory symptoms, it had been 3 months since the onset of symptoms of RA and ILD. Neurological examination showed dilated pupil of the right eye with responsiveness, paresthesia below T4 dermatome, and bilaterally positive Babinski sign. Visual field (VF) test showed altitudinal hemianopia involving the lower hemisphere (**Figure 3A**). Brain MRI showed retrobulbar neuritis of the right optic nerve (**Figure 3C**) without abnormality in the brain. Spine MRI showed multifocal contrast-enhancing T2-hyperintensity involving the thoracic spinal cord (**Figure 3D**). CSF analysis showed elevated protein levels (72.8 mg/dl, normal range < 45 mg/dl) without pleocytosis. Serologic study revealed positivity (1+) for anti-AQP4 antibody (measured by cell-based indirect immunofluorescence assay) and strong positivity (2,875 U/ml, normal value < 32 U/ml) for anti-MDA5 antibody (measured by enzyme-linked immunosorbent assay). Ultimately, the patient was diagnosed with anti-AQP4-positive NMO combined with anti-MDA5-positive DM with ILD and RA. Two cycles of IV pulsed methylprednisolone (1,000 mg daily for five consecutive days) were injected a week apart, and the dose of tacrolimus was increased to 3 mg daily. The visual acuity got better gradually (**Figure 3B**), but sensory disturbance did not. Although pulmonary fibrosis seemed to be slightly progressed on the regular follow-up chest CT, his respiratory symptoms and FVC were rather improved (**Figure 4B**). Meanwhile, serum ferritin level constantly remained high at above 1,000 ng/ml (*Segment B* in **Figure 1**).

A month after the first attack of optic neuritis, his vision of the right eye suddenly worsened again. VF exam showed severe visual disturbance with periphery involvement (**Figure 3E**), and rituximab (IV, 1,000 mg each, 2 weeks apart) was decided to be administered to prevent further relapse of NMO. However, his VF defect was exacerbated after the first rituximab injection (**Figure 3F**), and orbit MRI demonstrated the relapse of optic neuritis (**Figure 3G**). Furthermore, the patient developed mild dyspnea (oxygen demand, 2 L/min via nasal prong) followed by complete paraplegia and lack of voiding after a subsequent rituximab injection. Spine MRI showed longitudinally extensive transverse myelitis involving the thoracic cord (**Figure 3H**). FVC declined to 2.83 L (56% of the predicted) in PFT, and ILD was aggravated on chest CT (**Figure 4C**). Fluorescence intensity of anti-AQP4 antibody significantly increased to 4+, even though $CD19^+$ B-cells constituted 0.1% of the total lymphocytes. Oral immunosuppressants (prednisone 20 mg and tacrolimus 3 mg) were discontinued owing to recurrent urinary tract infection and subsequent *Klebsiella aerogenes* bacteremia, and the patient underwent eight sessions of therapeutic plasma exchange (PLEX). Serum ferritin level drastically decreased

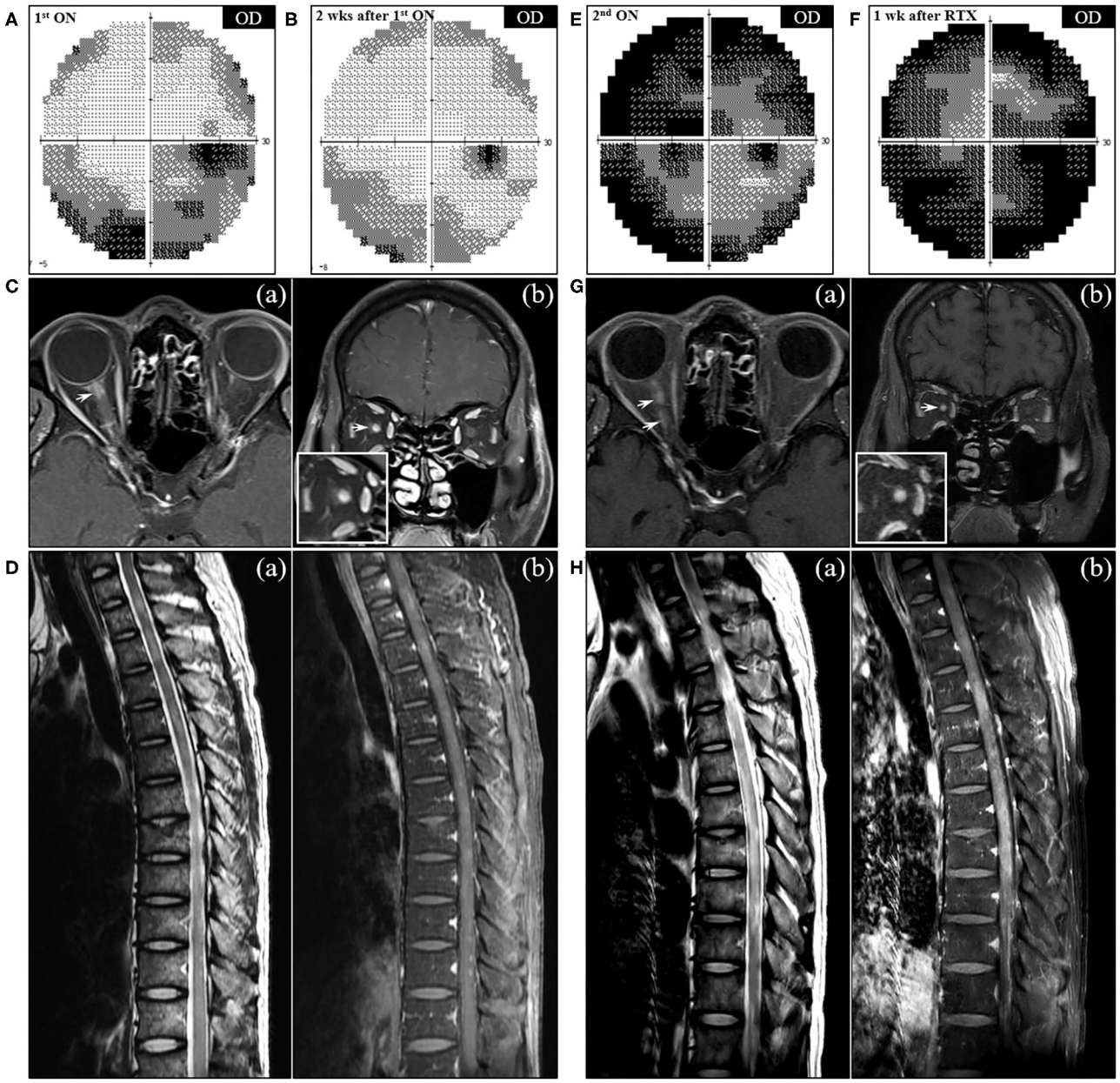

FIGURE 3 | Serial visual field tests and MRI findings of NMO in the patient. **(A)** The initial visual field (VF) test demonstrated the incomplete loss of the inferior and peripheral vision in the right eye, which partially improved after corticosteroid therapy **(B)**. **(C)** Axial (a) and coronal (b) T1-weighted orbit MR images showed contrast enhancement in the right optic nerve at the back of the eye (arrow). **(D)** Sagittal MRI of the thoracic spine showed multifocal T2-hyperintensity (a) with patchy contrast enhancement (b). **(E)** VF test performed at the second attack of ON showed near-total blindness of the right eye, which was exacerbated to complete blindness a week after rituximab injection **(F)**. **(G)** Follow-up orbit MRI showed the contrast-enhancing lesions (arrows) affecting the entire right optic nerve on the axial (a) and coronal (b) T1-weighted images. **(H)** Follow-up spine MRI after the second rituximab injection showed long segmental T2-hyperintensity (a) with diffuse enhancement (b) in the thoracic cord extending from T4 to T11 vertebral levels. OD, oculus dexter.

by 64% (from 1,109 to 399 ng/ml) after PLEX (*Segment C* in **Figure 1**). The patient showed no clinical deterioration for the next 4 months, and his respiratory distress slowly improved. However, prior neurological disability including blindness of the right eye, paraplegia, and bladder dysfunction failed to improve.

DISCUSSION

This case showed a rare combination of NMO and overlapping rheumatic diseases associated with anti-MDA5 antibody, which resulted in serious neurological disability. In the previous report of NMO with anti-MDA5-positive DM, the patient had a good

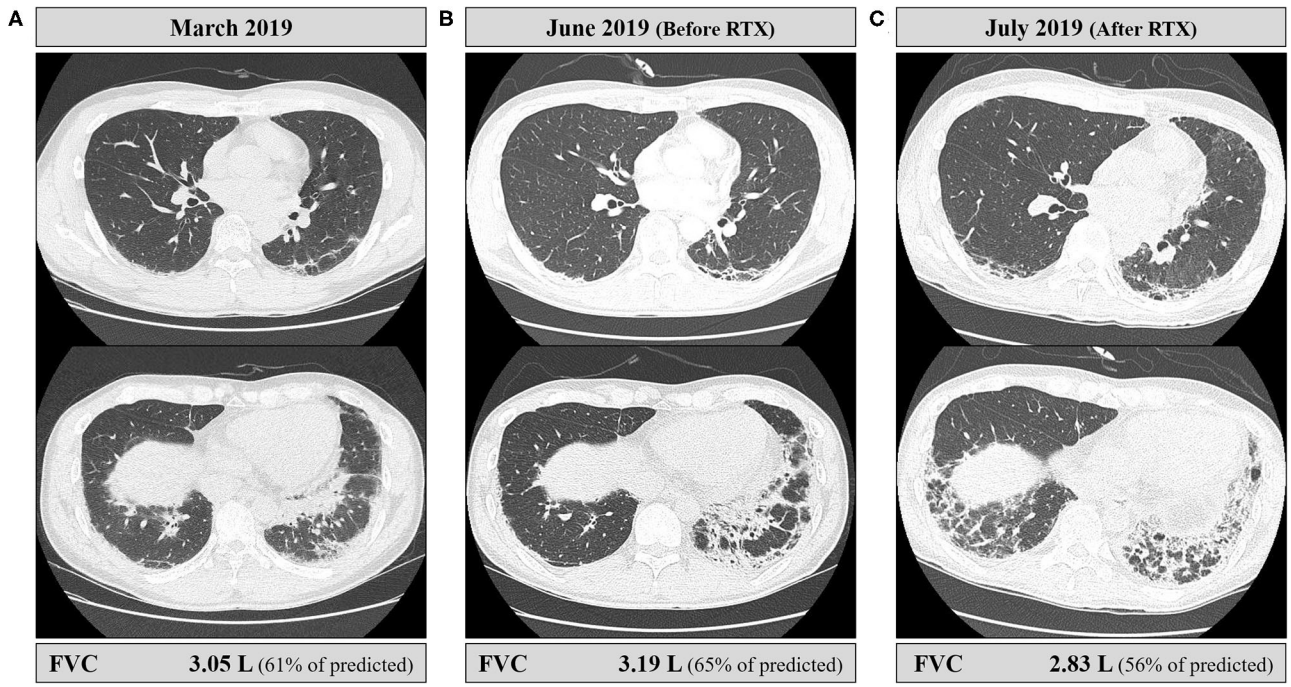

FIGURE 4 | Serial chest CT findings and pulmonary functions of the patient. **(A)** Initial CT at the time of diagnosis of ILD showed perilobular consolidations and fibrosis in posterior aspects of the lower lobes, and forced vital capacity (FVC) was 3.05 L. **(B)** CT performed just before the rituximab injection showed the increased extent of fibrosis. However, the patient did not complain of respiratory symptoms including cough or dyspnea, and FVC was slightly improved by 4.6% compared to baseline. **(C)** CT performed immediately after the second rituximab injection showed an increased extent of fibrosis and perilobular consolidations. The patient developed mild dyspnea, and FVC declined by 11% from the latest one.

outcome after combined immunotherapy with corticosteroids, rituximab, cyclophosphamide, and PLEX (6). Our current patient also appeared to have a favorable course during the first 2 months after the initiation of immunotherapy. However, NMO relapsed frequently within the following 2 months, whereas symptoms or signs of anti-MDA5-associated diseases were rather silent. In addition, the patient experienced an unexpected exacerbation of NMO and ILD after rituximab induction.

Though the pathogenesis of anti-MDA5-positive DM is largely unknown, the involvement of the hyperactivated interferon system has been suggested (7). Serum level of interferon-alpha (IFN-α) was aberrantly elevated in patients with anti-MDA5-positive DM and shown to be correlated with disease activity (7, 8). Although serum IFN-α level was not measured, it was expected to be elevated in this study. Meanwhile, growing evidence indicates that the elevation of serum IFN-α level might also take part in the pathogenesis or disease activity of anti-AQP4-positive NMO (9–11). There are cases of NMO induced by the therapeutic use of recombinant IFN-α for other diseases (10, 11). Moreover, there was a case of anti-AQP4-positive NMO in a child with increased endogenous IFN-α level (12). Taken together, elevation of IFN-α in the presence of anti-MDA5 antibody might also affect the pathogenesis or high disease activity of NMO in this study.

Serum ferritin is an acute phase reactant induced by various cytokines in inflammatory responses (13, 14). In our case, hyperferritinemia persisted throughout the course of illness without the occurrence of RP-ILD. Moreover, serum ferritin level kept increasing even in the period of symptomatic improvement of ILD, which is not consistent with the fact that serum ferritin is a useful marker for evaluating the therapeutic response of ILD in patients with anti-MDA5-positive DM (4, 15). This discrepancy meant that hyperferritinemia just indicates insufficient control of overall inflammatory conditions, not specifically ILD. Rather, serum ferritin level remained high during the period of frequent relapses or exacerbation of NMO, and it was not until the patient underwent PLEX that serum ferritin level dramatically decreased and there was no more relapse of NMO. Hyperferritinemia and its persistence appeared to be associated with the highly active state of NMO in our patient, though the paucity of the case of NMO with anti-MDA5-positive DM and lack of previous literature made it difficult to determine the relevance between them. Further data on ferritin level in NMO with or without anti-MDA5 antibody are needed.

Rituximab is well-known to be effective in reducing the frequency of relapse and severity in patients with NMO (16, 17). ILD complicated with anti-MDA5-positive DM is often unresponsive to conventional corticosteroid therapy (18). Rituximab might be a promising therapeutic option, as several cases showing its efficacy against refractory ILD with anti-MDA5 antibody have been reported (18–20). However, in our case, the patient experienced unexpected exacerbation of both NMO

and ILD in a more severe manner after induction treatment with rituximab. It may just be a natural worsening of both unstable diseases regardless of rituximab infusion. However, the concurrent flare-up of his autoimmune diseases might be related to rituximab induction, given paradoxical exacerbation of NMO, called post-rituximab relapse, has been often reported (21). The presumed mechanism of the phenomenon is associated with B-cell activating factor (BAFF), a crucial regulator of B-cell maturation and antibody production (21, 22). Rituximab can lead to the transient elevation of BAFF within the induction period with a lack of therapeutic efficacy, which is followed by the elevation of anti-AQP4 antibody (22). That is, elevated serum BAFF may stimulate the already existing plasma cells to produce antibodies, thereby contributing to rebound of disease activity (21). This hypothesis is consistent with the finding that the fluorescence intensity of anti-AQP4 antibody was stronger than that measured before rituximab injection in our case. In terms of ILD in our case, what caused the unexpected worsening of ILD after rituximab injection is unclear, with the rarity of similar cases. It is assumed that the post-rituximab elevation of serum BAFF level may be responsible for the exacerbation of ILD, as in post-rituximab NMO relapse, given that BAFF was recently suggested to play a major role in the development of ILD in patients with anti-MDA5-positive DM (7, 23).

In summary, NMO can overlap with anti-MDA5-positive DM and ILD, and its relapse or exacerbation may occur with high disease activity in the presence of anti-MDA5 antibody or hyperferritinemia. In addition, it is worthy to consider that rituximab may contribute to the transient worsening of both NMO and ILD during the early post-induction period.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study was approved by the Institutional Review Board at Chonnam National University Hospital (CNUH-EXP-2020-005). The patient provided written informed consent for the publication of the case report.

AUTHOR CONTRIBUTIONS

T-SN: conceptualization and supervision. Y-RK, K-HK, K-HL, KK, and GC: data curation. Y-RK, K-HK, S-JL, and S-YC: formal analysis. Y-RK and T-SN: investigation and visualization. Y-RK, K-HK, and T-SN: writing—original draft. All authors contributed to the article and approved the submitted version.

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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.

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ORIGINAL RESEARCH
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Polypharmacy in Middle-European Rheumatoid Arthritis-Patients: A Retrospective Longitudinal Cohort Analysis With Systematic Literature Review

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Objective: To assess polypharmacy and related medication aspects in Middle-European rheumatoid arthritis (RA) patients, and to discuss the results in view of a systematic literature review.

Methods: In this retrospective cohort study, charts were reviewed from RA-patients consecutively recruited between September 27, 2017 and April 29, 2019. Drugs were assigned to the Anatomical Therapeutic Chemical (ATC) groups as proposed by the World Health Organization (WHO). Results were compared to those of a systematic literature review.

Results: One hundred seventy-five consecutive RA-patients were included. The mean number of drugs was 6.6 ± 3.5, with 2.4 ± 1.2 drugs taken specifically for RA—compared to 2.6 in the literature. 33.7% of patients experienced polypharmacy defined by ≥5 drugs, compared to 61.6% in the literature–with women affected more frequently than men. After 7 years of follow-up, the number of drugs increased in all ATC-groups by an average of 12.7 %, correlating with age (Corrcoeff = 0.46) and comorbidities (Corrcoeff = 0.599). In the literature, polypharmacy is not always defined precisely, and has not been considered in management guidelines so far.

Conclusion: Polypharmacy is a frequent issue in RA-management. With an increasing number of comorbidities during the course of the disease, polypharmacy becomes even more relevant.

Keywords: arthritis (including rheumatoid arthritis), clinical pharmacology, polypharmacy (source: MeSH, NML), coding, comorbidities, drug intake, gender differences

INTRODUCTION

Several definitions exist for polypharmacy (1), including the number of medications (usually above 4) and their inappropriateness (2, 3). The number of patients affected by polypharmacy considerably varies when using different definitions (3). Polypharmacy may result in unwanted drug interactions (4), and/or lead to an increase of adverse and serious adverse events, with more frequent admissions to hospitals, thus extending the costs of health care (5). In elderly people over 65 years, more than 50% are prescribed more than 6 medications, and almost 20% receive an inappropriate drug (5). An increasing amount of comorbidities is directly linked to the number of medications advised (6). Especially elderly patients are affected and more endangered by the prescription of unnecessary medications due to their usual higher number of comorbidities (7).

Over the last decades, the incidence of rheumatoid arthritis (RA) remained constant but shifted over the years with a decrease in seropositive and an increase of seronegative RA (8). In RA, life expectancy is shortened by 2–3.5-fold compared to the general population (9). Modern treatment medications and approaches enable to lower disease activity, but mortality still remains unchanged higher than in control groups (10). The increased standardized mortality ratio of 50%–independent from age and gender (11)—is related to cardiovascular events, infections, extra-articular manifestations, with a possible (but still not clearly defined) role of treatments such as glucocorticoids. The excess in cardiovascular risk is not fully explained by conventional risk factors like age and arterial hypertension. Comorbidities can decrease life quality and physical functioning and are even important in patients close to remission (12), other comorbidities might not affect or be affected by RA at all (13). Since comorbidities usually need additional treatment, patients with comorbidities are expected to be more exposed to polypharmacy than those patients without comorbidities. Some diseases seem to appear more likely before RA diagnosis and might predispose for RA, such as other autoimmune diseases and epilepsy. Before diagnosis, RA patients do not have more comorbidities than controls (7).

According to a systematic literature review non-compliance is reported in up to 55% of elderly patients with polypharmacy (14), although the percentages of elderly US veterans feeling to have too many medications are surprisingly low with 4% (15). This discrepancy may be result from different definitions of treatment in administrative and clinical settings.

This observational longitudinal cohort-study retrospectively assessed polypharmacy and comorbidities in consecutive Middle-European RA-patients to estimate the frequency and to identify possible causes of polypharmacy, and to discuss local data with results of a systematic literature review (SLR).

PATIENTS AND METHODS

Literature Review

A literature review was performed with PICO questions on polypharmacy "as defined by number of medications," "as defined by number of inappropriate medications according to Beers criteria from 2012" and "depending on number of comorbidities" in RA compared to controls. Search items are listed in the (**Appendix 1–3**). Publications were included with patients diagnosed with RA, without geographical limits and in English language up to February 2020. Both risk factors and indicators for higher prevalence of polypharmacy were considered as outcome of observational studies published until February 2020. Literature from PubMed and the Cochrane databases was included if written in English language. Additional hand searches were performed in the publications cited for this work. The PRISMA guidelines were applied, Mendeley Desktop (Version 1.19.3) used for citation purposes. Search items are listed in **Table 1**, and the selection process summarized in a flow diagram (**Figure 1**).

Cohort Study and Chart Review

The study is designed as a retrospective, longitudinal cohort study in the setting of a Middle-European secondary/tertiary referral center (project name: SolutionX). After informed and written consent, consecutive patients are recruited by a single investigator (M.S.), and all RA patients recruited between September 27, 2017 and April 29, 2019, were included. A positive vote was obtained from the ethical committee of the Medical University of Innsbruck (September 15, 2017, AN 2017-0041 317/4.18). Less than 1% of the patients denied recruitment or could not be recruited because of a psychiatric disease.

The chart review was performed following the STROBE recommendations for cohort studies. Data were selected from the physician's reports stored in the hospital information system (Cerner), if follow-up data were available. The Disease-Activity-Score-28 (DAS28) was calculated using the Clinical Disease Activity Index (CDAI), together with the erythrocyte sedimentation rate (ESR). Medication was classified according to the Anatomical Therapeutical Chemical (ATC) Classification System of the World Health Organization Collaborating Center for Drug Statistics Methodology (WHOCC). Medication was sorted as prescribed daily, weekly, monthly, every few months, and on demand. The absolute number of drugs with two or more effective ingredients was counted once, and then listed in the different ATC classifications of their active agents. In case there was no information about the frequency of intake, the frequency given in the recommendation was used. Local treatments, Chinese and other herbs, homeopathy, and micronutrients were not considered. Obesity was defined using the body mass index (BMI) >30 kg/m^2, and anemia was defined as hemoglobin (Hb) of <12 g/l in female and <13 g/l in male patients.

After pseudonymization, data were analyzed using the SPSS program (Version 26, October 2019, IBM). Descriptive statistics included means and standard deviations as well as frequencies of different characteristics. Box plots were used to visualize comparisons between groups. The Shapiro–Wilk-test was used to test for normal distributions to decide for further test choices. To compare groups, the Wilcoxon test was used for not normal distributed dependent variables and the Mann–Whitney-*U*-test for independent not normal distributed variables to detect significant increases or decreases in the number of medications

TABLE 1 | Study characteristics from the literature review with Beers criteria as available (ordered according to year of publication).

Publi-cation	Study size	Study design	Female (%)	Age (years)	Disease duration	Beers criteria	Number of comorbidities	Refernces
1985	108 clinical + 153 outpatient	CS	68.8 % clinical 73.7 % outpatient	N/A	N/A	N/A	N/A	(16)
1999	1975: 148 1995: 164	C	1975: 79.1 % 1995: 76.8 %	1975: 46.3 y 1995: 48.6 y	14 y 13.3 y	N/A	1975: 10 % 1995: 15 %	(17)
2007	348	C	71.8%	61.4 y	13.1 y	N/A	Mean 2, 17.2 % 1 21.8 % 2 21.3 % 3 27 % > 3	(6)
2011	295 (∼50 % RA)	C	55.6 % >65 y 67.3 % <65 y	73 >65 y 49 <65 y	N/A	N/A	N/A	(18)
2016	54	CS	100%	Without PP: 39 y with PP: 45 y	3 y	N/A	Without PP: 43.3 % with PP: 76.2 %	(19)
2017	1,101	C	78.8%	61.3	10.4 y	N/A	N/A	(20)
2019	200	CS	86%	64	N/A	12x inappropriate-4.2 % 2 duplications-0.7 % 3 contraindications −1 % 2× missing-0.7%	3.1 comorbidities (56.5 % ≥ 3 comorbidities)	(21)
2019	792	CS	89%	56.6	12.7 y	N/A	59 % 1–3 24.5% >3	(22)
2019	22,005	C	76%	57	10	N/A	N/A	(23)

C, cohort study; CS, cross-sectional study; N/A, not assessed; y, years.

between first and last visits for different ATC-Groups and men and women, as well as estimate the significance in differences of gender. For the estimation of possible correlations between the number of medications and factors such as age, disease duration, and -activity, comorbidities and RF, the Pearson correlation coefficient (PCC), and for comparisons between different age groups, the Kruskal–Wallis-Test was used, with additional *post hoc* analysis using Dunn–Bonferroni-tests to exclude possible confounders during the test.

Assessments of the risk of bias to the manuscript were low for patients' selection (>98% of consecutive patients were included), there was no relevant performance or detection bias (with retrospective design). There may be an attrition bias (as patients may have switched to another rheumatologist outside the hospital—with affected the study only if it was the last visit, the manuscript, however, only described the visits in this hospital).

Anonymized data are available by the authors on request.

RESULTS

Polypharmacy in Rheumatoid Arthritis Literature

A total of nine studies are included into this review (**Figure 1**, **Table 1**). Overall, polypharmacy is common and considerable in RA-patients (6). Polypharmacy has been associated with age, female gender, multimorbidity, disease activity, disease duration, and functional impairment, resulting in a higher risk of hospital admissions (20, 23).

In 24.446 RA-patients from these studies, treatment included an average of 5.3 medications (**Table 2**). Some of these studies showed that 45.1% of RA-patients have additional comorbidities, and 61.6% of all RA-patients are affected with polypharmacy (**Table 2**). Polypharmacy was significantly associated with comorbidities and the use of corticosteroids, MTX and bDMARDs (22). Additional aspects were that polypharmacy had a negative impact on health-related quality of life (19), is common also in hospitalized RA-patients (16) with specific RA-medications increasing between 1978 and 1995 (17). RA-patients older and younger than 65 years are treated differently (18), and polypharmacy is associated with drug-related problems [Odds Ratio = 2.96 (1.48–5.91); $p = 0.003$] (21). Besides, a non-compliance rate of 7.6% (drugs being "not taken or administered at all") is reported with a correlation between non-compliance and polypharmacy ($p = 0.027$) (21).

Comorbidities and Side-Effects of RA as Cause of Polypharmacy in the Literature

A mean of 2.8 comorbidities and side effects of RA is reported in 2 studies, and considered responsible for polypharmacy and drug-to-drug interactions (20, 22). More specifically, increasing numbers of comorbidities have been related to the number of drugs (20), with a significant correlation with a standardized

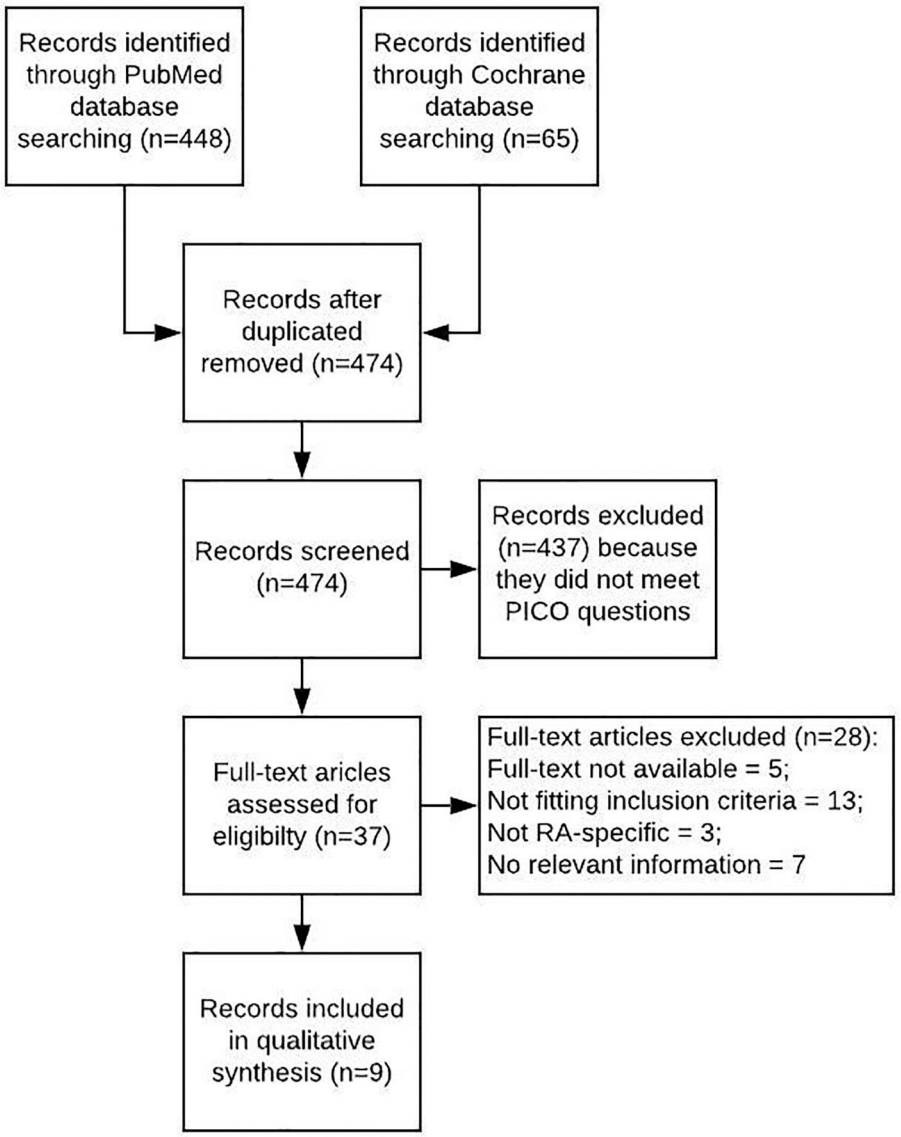

FIGURE 1 | Flowchart picturing the process of the SLR.

regression weight of 0.54 in another study (6). Others describe a correlation between the number of medications and the Rheumatic Disease Comorbidity Index (RDCI), one of various implemented indexes to account comorbid illnesses (23). These authors discuss a possible effect of the severity of comorbidities, as severe diseases may need more medications.

Adverse Events and Polypharmacy in the Literature

Reporting of adverse events widely varies between the studies. Adverse reactions have been described in 38.8 % of RA-patients, most of them associated with DMARDs, even associated with polypharmacy (21). Another study reported a "non-linear association" between the number of medications and acute hospitalisations, especially for those patients taking more than 10 drugs (20). 44.5% of these hospitalisations happened due to a possible severe adverse event (SAE), and a RA-specific drug was involved in 51.9% of these patients. An increased rate of SAEs was reported together with an increased number of medications, with infection as the most common SAE. The authors' calculation provides an Hazard ratio (HR) of 1.13 as additional risk for SAEs per drug (23).

Polypharmacy in Rheumatoid Arthritis Cohort

For this Middle-European longitudinal observational study, datasets from the first and the last visit at the

TABLE 2 | Polypharmacy, total number of medications, and frequency of prescribed RA-related medications (DMARDs, glucocorticoids and NSAIDs) in the literature.

Number of patients [n]	Polypharmacy [%]	Number of medications	DMARDs [% of patients]	csDMARD [%]	bDMARD [%]	Glucocorticoids [%]	NSAIDs [%]	References
348	69.5 % (>3)	5.4 (2.4 for RA)	86.8	MTX: 56.3 %	N/A	31.3	19.5	(6)
54	44.4 %	N/A	N/A	N/A	N/A	N/A	N/A	(19)
1,101	N/A	5.2	79	45 % Mono 26 % Double 8 % Triple	22	16	N/A	(20)
200	64.5 %	5.5	94.5	59 % Mono 26.5% Double 7 % Triple MTX: 67 %	1	50	28.5	(21)
792	67.9 % (>5)	5.5 (2.8 for RA)	N/A	90.9 % MTX: 68 %	35.7	47	9.1	(22)
22,005	N/A	5 for others	N/A	N/A	N/A	38	N/A	(23)
			82.5 ± 5.4	84.8 ± 6.1 %	25.2 ± 10.2	37.3 ± 5.1	14.7 ± 7.3	

N/A, not assessed. Last row mean ± SD, standard deviation, weighted for number of patients.

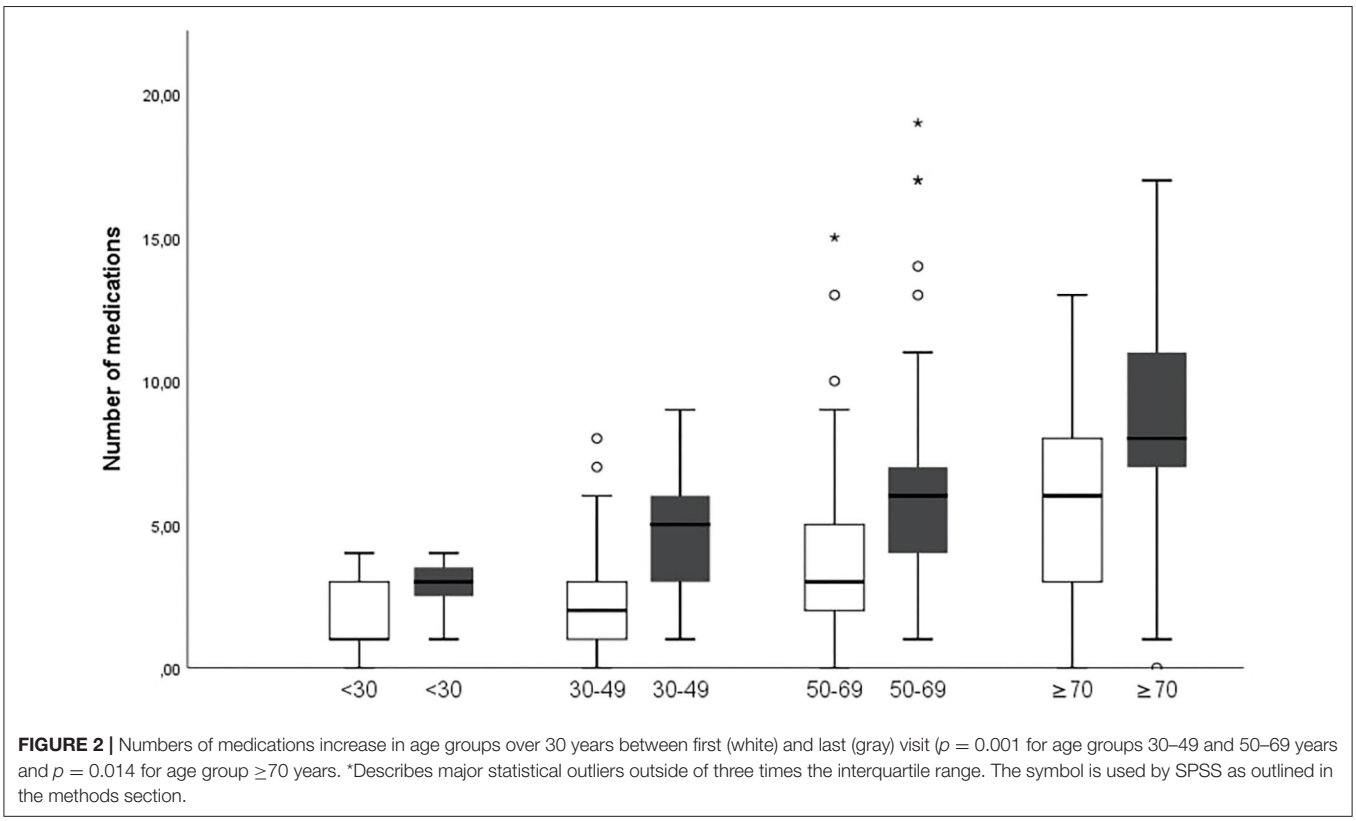

FIGURE 2 | Numbers of medications increase in age groups over 30 years between first (white) and last (gray) visit ($p = 0.001$ for age groups 30–49 and 50–69 years and $p = 0.014$ for age group ≥70 years. *Describes major statistical outliers outside of three times the interquartile range. The symbol is used by SPSS as outlined in the methods section.

rheumatological outpatient clinic (with 6.78 ± 5.42 years apart) are available for 175 RA-patients. Depending on the definition used for polypharmacy ($n \geq 4$, ≥5, or ≥6 daily, regular medications), 26.3, 21.1, 14.9% of patients are affected by polypharmacy at the first visit and 45.1, 33.7, and 28.0% of the patients at the last visit, respectively. These percentages for polypharmacy increase from the first to last visit independently from the definition applied. Accordingly, the average number of medications increases from 3.5 ± 2.9 medications at the first to 6.6 ± 3.5 medications at the last visit.

At first visit, women took more drugs than men (3.8 ± 2.9 vs. 2.9 ± 2.8 medications, respectively; $p = 0.022$, using the Wilcoxon test). At last visit, the number of medications increased both for women (to 6.8 ± 15.2; $p = 0.001$, Mann–Whitney-U-Test) and men (to 6.2 ± 12.3; $p = 0.001$,

TABLE 3 | Patient's demographics, disease characteristics and medications at first and last visit (including on request medication, descriptive statistics include means, and standard deviations; percentages in parentheses calculated from total number).

	First visit	Last visit	*p*-values
Female gender [%]	73.1	73.1	n.s.
Age [years]	54.5 ± 14.9	61.5 ± 14.4	0.001**
Current Smoker [%]	19.4	18.3	n.s.
-Ex-smoker [%]	9.1	14.3	n.s.
Alcohol, occasionally [%]	33.1	45.7	n.s.
Disease duration [months]	32.9 ± 76.3	114.6 ± 101.9	0.001**
CDAI	12.7 ± 9.8	5.6 ± 6.9	0.001**
DAS28	3.5 ± 1.3	2.7 ± 4.8	0.001**
Rheumatoid factor [U/L]	146.2 ± 361.8	77.8 ± 159.7	n.s.
No. of medications	3.5 ± 2.9	6.6 ± 3.5	0.001**

***Highly significant with p < 0.01.*

Mann–Whitney-*U*-Test), but no longer differed significantly between women and men.

The number of drugs increases from first to last visit in all age groups over 30 years (**Figure 2**). Also, the number of medications correlates with age both at the first visit and the last visit [Pearson Correlation Coefficient (PCC) = 0.457 and 0.460, respectively; $p = 0.001$]. The correlation holds true when male and female gender are analyzed separately, the number of medications correlated with age both in female patients at the first and last visit (PCC = 0.471 and 0.467, respectively; $p = 0.001$), and in male patients (PCC = 0.467 and 0.450, respectively; $p = 0.002$).

Analysis of ATC-Coded Medication Related to Comorbidities in Rheumatoid Arthritis Cohort

Sorted by the group of ATC codes, medications of 14 ATC-groups and specific classes were taken more frequently at last visit (**Table 3**). Not all of them were directly related to RA. RA-specific medications include immunosuppressants of the ATC-group L04, hydroxychloroquine, sulfasalazine and glucocorticoids. The number of the RA-specific medications increased from 0.7 (19.7% of all 3.5 medications) at the first visit to 2.4 (36.1% of all 6.6 medications) at the last visit. Analgesics add another 1.0 medications (27.4% of all medications) at the first visit and 0.8 (12.4% of all medications) at the last visit.

At the initial visit, 83.4% of all patients showed any comorbidity in addition to RA (**Table 4**). This percentage further increased during the following 7 years to 96% of all patients having at least one comorbidity or side-effect of RA. The number of all different types of comorbidities increased, except anemia was reduced from 8.0 to 4.6% during follow-up.

At the first visit, 32% of the patients had a single comorbidity, exceptions occurred with one exemplary patient who presented with 7 different comorbidities beside RA at first visit. Only 6.78 years later, most of the patients (25.1 %) had 4 comorbidities.

Comorbidities and RA side-effects are listed in **Table 5** according to the ATC-list. Therefore, osteoarthritis is listed despite being a possible complication of RA after long-standing disease. Other frequent comorbidities involve the cardiovascular system. Accordingly, medications of the ATC-group for cardiovascular medication, as well as antithrombotic medications increased. For example, the number of patients suffering from arterial hypertension increased from 18.3 to 34.9% during follow-up. Also, the number of patients with muscular dysbalances more than doubled from 15.4% at the first to 28% at the last visit. Most comorbidities and RA-related side-effects increased. Using an increasing number of antianaemic agents (ATC-code B03, as numbered in **Table 5**), only anemia was less frequent at the last compared to the first visit (with 8.0 vs. 4.6%, respectively, **Table 4**).

A linear correlation exists between the number of medications and the number of comorbidities and side-effects at the first visit (PCC = 0.458; $p < 0.001$; **Figure 3**). At the last visit, this linear correlation is even more prominent with a relevant PCC of 0.599 ($p < 0.001$).

Other Potentially Underlying Factors of Polypharmacy in Rheumatoid Arthritis Cohort

Further analysis was performed for a possible correlation between the number of medications with disease activity, frequency of rheumatologic visits, rheumatoid factor and disease duration. These parameters did not correlate with polypharmacy (data not shown).

DISCUSSION

This observational study shows polypharmacy (defined as ≥5 medications) in 33.7% of RA-patients, especially in female patients older than 50 years. This number is low in comparison with current literature, reporting polypharmacy in as many as 44.4 to 67.9% of all RA-patients (19, 21, 22). The reason for this difference may be differences of the insurance systems, or the fact that definitions used for polypharmacy are still not consistent (2, 6). In the systematic literature review, the definition of more than 5 drugs was applied in one trial (22), the definitions of more than 3 drugs in another one (6), while all other studies did not provide a specific definition used. In order to make observations and documentations more comparable throughout the literature, a consensus will be helpful for the future. Nevertheless, the average patient had more medications in this cohort compared to the literature (6.6 vs. 5.3, respectively). This indicates different subgroups of patients with more comorbidities and up to 19 medications.

The approach of this study to use ATC-codes for analyses of medication groups is new and has not been applied for the approach to polypharmacy in RA so far. Indeed, ATC-coding does not only allow the comparison between disease-specific treatment and treatment of comorbidities, but also comparison of polypharmaceutical aspects during follow-up. Using the ATC-codes may reduce a possible reporting bias. This can be an

TABLE 4 | Number of comorbidities at the first visit and months later, assorted to the ATC-group of medication that they are requiring.

Comorbidity	ATC-Code	First visit %	Last visit	*p*-value
Anemia	B03	8.0	4.6	n.s.
Cardiovascular morbidities	C	28.0	47.4	0.001**
Diabetes mellitus	A10	6.9	10.9	0.035*
Lung diseases	R	2.9	4.6	n.s.
Tendon rupture	M01/M02	2.3	10.9	0.001**
Osteopenia/osteoporosis	M05	13.7	41.1	0.001**
Osteoarthritis	M01/02	64.0	90.3	0.001**
Eye involvement	S01	5.1	12.6	0.002**
Thyroid disease	H03	9.1	12.6	n.s.
Muscular disbalances/back pain	M	18.1	33.0	0.001**
Neoplasms	L01/L02	9.7	18.3	0.001**

*Anemia is defined as Hb<12 for female and Hb<13 for males. *significant with $p < 0.05$, **highly significant $p < 0.01$.*

TABLE 5 | Percentages of ATC-coded medication groups at first and last visit (in alphabetical order).

ATC code	ATC group	% first visit	% last visit	p-value
A	Alimentary and metabolism	54.3	79.4	0.001**
A11/A12	Vitamins and trace elements	28.6	62.3	0.001**
B1	Antithrombotic	13.7	25.1	0.001**
B03	Antianaemic	14.3	62.9	0.001**
C	Cardiovascular	28.6	44.6	0.001**
G	Genito-urinary system	1.7	6.3	0.011**
H02AB	Glucocorticoids	33.1	4	n.s.
H03	Thyroid gland	20.0	26.3	0.005**
J	Antibiotics	1.7	1.7	n.s.
L04	Immunosuppressants	22.9	79.4	0.001**
M	musculoskeletal	60.0	64	n.s.
-M01	NSAIDs (regular and on request)	54.9	49.1	n.s.
	NSAIDs (regular intake only)	37.1	12.6	0.001**
-M03	Muscle relaxants	0.6	0	n.s.
-M04	Gout	1.7	6.3	0.011*
-M05B	Bone diseases	9.1	18.3	0.002**
N	Nervous system	20.0	27.4	0.042*
N02	Analgesics	9.1	14.9	n.s.
P01	Antimalaria	4	13.1	0.001**
R	Respiratory	1.1	5.7	0.011*
V	Various	1.1	6.9	0.004**

*n.s., not significant; *significant $p < 0.05$; **highly significant $p < 0.01$.*

advantage for future studies, as new medications like tsDMARDs and bDMARDs can be attributed to existing ATC-codes for comparisons independent from the specific drugs used. Whereas, some newer tsDMARDs and bDMARDs are used on a daily basis, others are applied only every other week or month. 3.4% of RA-patients had more than 4 medications on a weekly basis, although a majority of patients consider "forgetfulness" as an explanation for non-compliance in weekly dosing (24).

All ATC-assorted groups of medications were prescribed more frequently at the last visit compared to the first visit. However, taking a closer look on the different subgroups, major increases were observed for immunosuppressants (L04), treatments for osteopenia/osteoporosis (M05B), and cardiovascular treatment (C). Only the number of analgesics like NSAIDs (M01) was reduced, and more than two thirds of patients with a prescribed NSAID at the first visit did not need further regular prescriptions. These observations went along with the increasing number of medications (from 3.5 to 6.6 over 6.8 years, with 0.9 newly prescribed medications per year of observation). With about 5.3 prescribed medications per patient, the mean number of medications in the few analyzed studies of the SLR was still only slightly lower than at the last visit in our cohort. Only the use of NSAIDs was reduced, in accordance with the intensified treatment for RA. Whereas, at the first visit women took

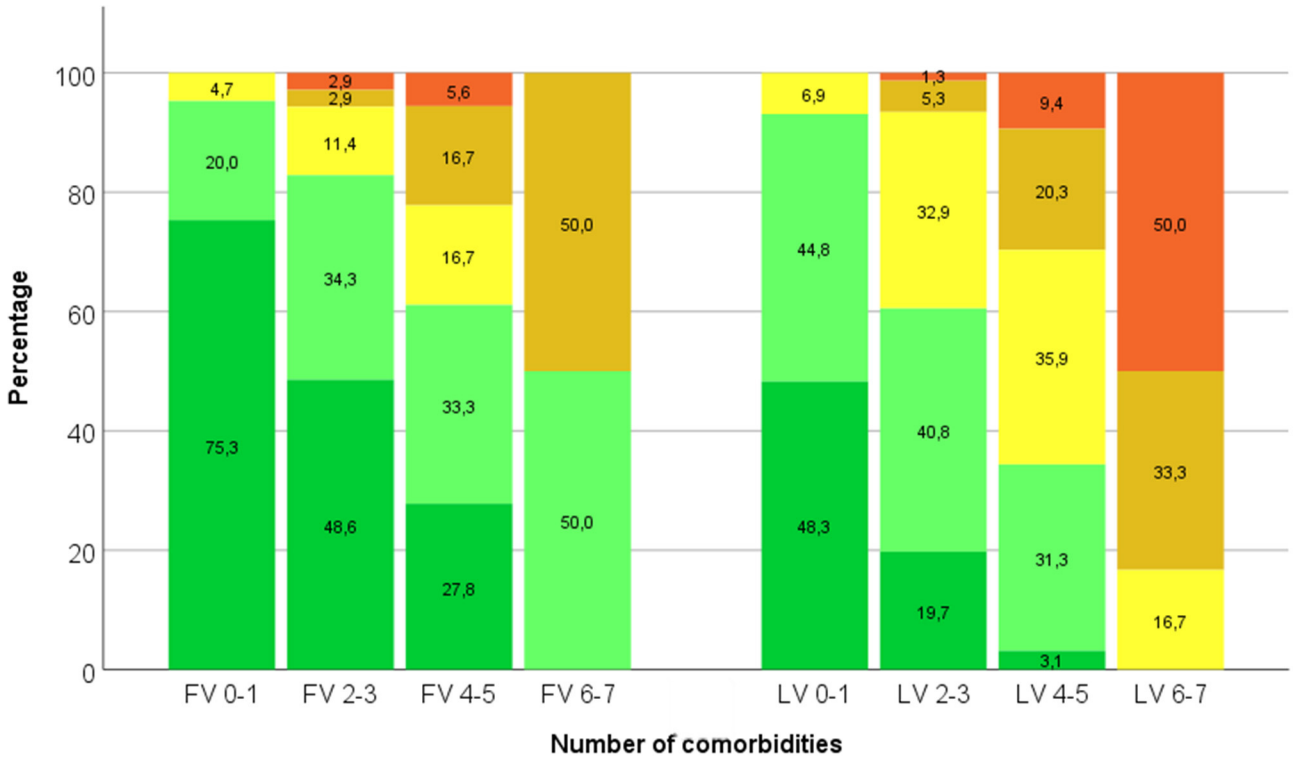

FIGURE 3 | Percentages of patients with increasing number of medications (grouped into ≤3—dark green–, 4–6—light green–, 7–9—yellow–, 10–12—amber–, and >12—red–) depend on number of comorbidities (0–1, 2–3, 4–5, and 6–7) both at first visit (FV) and last visit (LV).

more medications than men, both women and men had more medications at the last visit. Also in the SLR, polypharmacy is reported to be associated with age, whereas results are inconclusive for the gender association (6, 20, 23).

The increasing number of medications strongly correlates with the number of comorbidities (**Figure 3**). This observation is expected but becomes relevant at the last visit with 95% of the RA-patients suffering from one or more diseases other than RA. Almost every RA-patient needs co-medication together with the RA-treatment, thus easily facing the problem of polypharmacy. As at the same time polypharmacy is suspected as a risk factor for mortality and morbidity, polypharmacy is therefore recommended to be avoided especially in the elderly (1). In this Middle-European cohort, RA-patients over 70 years of age had 8.6 medications if they had already fulfilled the criteria of polypharmacy with 5.8 medications at the first visit. Indeed, the number of medications correlated with age groups. These findings correspond to the literature in general but could be specifically confirmed for RA-patients in this cohort now. Taken together, comorbidities requiring additional medication can be related to the disease course itself or to RA-treatment (for example arterial hypertension after NSAIDs). Comorbidities, however, should not lead to hesitance of prescribing DMARDs to achieve the treat-to-target goals. Certainly not age itself but the comorbidities make a difference between the vital and the frail patient, as mentioned by many rheumatologists (25). Furthermore, comorbidities are one of the most frequent reasons, that cause a difficult-to-treat RA, alongside with extra-articular manifestations (26). In this Middle-European cohort the number of prescribed medications did not correlate with disease activity and disease duration, which had been proposed earlier in other studies (6, 20).

The question, how much the intake of multiple medications with possible interactions harms the patient more than provides a benefit, and reduces the patients' compliance, is still unanswered. Bechman et al. (23) addressed two aspects of polypharmacy in his paper. The aspect of serious adverse events (SAE) could not be assessed in the Innsbruck cohort, as SAEs did not occur within the observational period. A certain bias because of admission to different local hospitals cannot be excluded. Occurrence of SAEs is certainly another important aspect of polypharmacy. The EULAR good response rate was observed to be lower with polypharmacy in his work. In the Innsbruck cohort, disease activity scores and polypharmacy showed no correlation and further detailed study would be needed to assess individual improvements compared to number of medications prescribed.

The most important limitation of this study is its retrospective design with incomplete datasets (resulting e.g., in heterogeneity of times from first to last visit) and the relatively small sample size. Also, more detailed information on the type of increase in number of medications (early or late, linear or logarithmic) could not be answered in this retrospective study. In the literature review, one study included only 50% of the 295 patients with RA (18), and not all studies provide exact numbers of patients

with comorbidities. Second, there is no consistent definition which comorbidities to be assessed in clinical studies with RA. In this Middle-European study osteoarthritis is considered as comorbidity, although osteoarthritis could also be secondary to RA. Also, anemia can be related to RA itself, to its medication or occur independent from both (but still is summarized as ATC-code B03). Age and the number of comorbidities certainly are confounders for this study. With this approach, it cannot be excluded, that the number of medications does not only correlate with the number of comorbidities, but purely depends on the increased age. Furthermore, the female gender could be a confounder because of similar age changes. Concerning the SLR, the exclusion of Embase and a biased rob of the studies have to be considered as additional limitations.

Improved studies on polypharmacy have to rely on detailed data sets, including all parameters possible relevant for assessment of risk factors and consequences of polypharmacy. Disease-specific treatment may prevent additional prescription of analgesics, thus avoiding unnecessary polypharmacy and further supporting the strategy of T2T (27). In RA-patients, knowledge about the underlying diagnosis, risk of disease complications and comorbidities will be critical to improve patients' adherence, especially in aged women with comorbidities. If the patient needs more pain medication than expected, diagnosis and treatment should be reconsidered. Drug indications, contraindications, and doses have to be re-evaluated on a regular basis, to assure an optimal pharmacological treatment without unnecessary polypharmacy.

CONCLUSION

In rheumatoid arthritis, polypharmacy affects more than a third of RA-patients and increases with age and number of comorbidities. Only a few RA-studies focus on polypharmacy in the literature so far. In future studies the definitions of polypharmacy should be reported, and a consensus be reached on the most relevant definition of polypharmacy.

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 human participants were reviewed and approved by Ethics committee of the Medical University Innsbruck, AN 2017-0041 370/4.18. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JD and RM: data collection. JD and SM: statistical analysis. JD and MS: concept and design of the study, drafting the manuscript. All authors: final corrections and approval.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.573542/full#supplementary-material

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REVIEW
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Autoimmunity in Focal Segmental Glomerulosclerosis: A Long-Standing Yet Elusive Association

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Focal segmental glomerulosclerosis (FSGS) is a histological term that describes a pathologic renal entity affecting both adults and children, with a wide array of possible underlying etiologies. Podocyte damage with scarring, the hallmark of this condition, leads to altered permeability of the glomerular barrier, which may result in massive proteinuria and relentless renal function deterioration. A definite cause of focal segmental glomerulosclerosis can be confirmed in a minority of cases, while most forms have been traditionally labeled as primary or idiopathic. Despite this definition, increasing evidence indicates that primary forms are a heterogenous group rather than a single disease entity: several circulating factors that may affect glomerular permeability have been proposed as potential culprits, and both humoral and cellular immunity have been implicated in the pathogenesis of the disease. Consistently, immunosuppressive drugs are considered as the cornerstone of treatment for primary focal segmental glomerulosclerosis, but response to these agents and long-term outcomes are highly variable. In this review we provide a summary of historical and recent advances on the pathogenesis of primary focal segmental glomerulosclerosis, focusing on implications for its differential diagnosis and treatment.

Keywords: FSGS, immunity, permeability factor, podocytopathy, idiopathic nephrotic syndrome

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Focal segmental glomerulosclerosis is a histological term describing the presence of partial tuft sclerosis ("segmental") in some of the glomeruli ("focal") from a renal biopsy specimen. As such, FSGS does not identify a specific disease, but rather a lesion with a wide array of possible underlying etiologies that may lead to protean clinical manifestations. FSGS may affect both children and adults, and currently represents one of the most frequent pathologic entities associated with nephrotic syndrome (1). The pathogenic mechanisms leading to FSGS share a common cellular target, the podocyte, a terminally differentiated cell whose foot processes act as structural parts of the glomerular filtration barrier. Podocyte damage may result from systemic diseases, drug exposure, infections or mutations of genes encoding structural podocyte proteins. Nevertheless, a definite etiology cannot be identified in up to 80% of FSGS cases (2), which historically fall under the classification of "idiopathic" or "primary." Despite this unifying definition, increasing evidence indicates that primary forms may be caused by several distinct pathogenic processes and could therefore benefit from a targeted treatment. Autoimmunity has been consistently reported as a pivotal player in the pathogenesis of these forms, and recent studies suggest that both humoral and cellular immunity may be involved. In this review, we focus on the immune and molecular aspects of podocyte damage associated with a FSGS pattern of injury and discuss current and novel therapeutic options for patients presenting with this condition.

PATHOGENESIS

FSGS is now considered as part of the podocytopathy spectrum of diseases, a term that includes all entities in which the podocyte is the primary target of the underlying pathogenic process (3). Podocytes are terminally differentiated epithelial cells that possess foot processes with a highly organized actin cytoskeleton. Interdigitation of podocyte foot processes is fundamental for the integrity of glomerular architecture and concurs in maintaining glomerular permselectivity to macromolecules. The first manifestation of podocyte injury consists in actin cytoskeleton disorganization, increased podocyte motility and foot process effacement (4), which may be followed by podocyte hypertrophy, detachment, and loss. As podocyte regeneration is limited, this process is often insufficient to compensate large podocyte losses, and frequently results in scar formation (5, 6). Such changes lead to a severe alteration in the glomerular structure, loss of filtration barrier selectivity and variable degrees of proteinuria.

Several factors may concur in causing podocyte damage and ultimately lead to FSGS, many of which have been extensively described. Primary FSGS still remains a diagnosis of exclusion and requires ruling out definite etiologies. Accordingly, the pathogenesis of primary FSGS remains poorly defined. The search for a unifying pathogenic mechanism for these forms has been largely unsuccessful, and it is now evident that primary FSGS entails many different diseases with a common phenotype.

TABLE 1 | Secondary causes of FSGS.

Genetic	Mutations in genes coding for podocyte proteins Mutations in syndromal genes (including collagen) Risk allele variants (APOL1)
Infections	Human Immunodeficiency Virus Cytomegalovirus SARS-CoV-2 Parvovirus B19 Epstein-Barr Virus Simian virus 40
Drugs and toxins	Heroin and Cocaine Anabolic Steroids Interferon Lithium Pamidronate Sirolimus Calcineurin Inhibitors
Maladaptive—reduced nephron number	Reflux nephropathy Surgical renal ablation Renal dysplasia Unilateral renal agenesis Oligomeganephronia
Maladaptive—normal nephron number	Obesity Hypertension Sickle-cell disease Atheroembolic disease Primary/secondary glomerular disease

Maladaptive, Genetic, Infectious and Toxic Risk Factors

Secondary causes of FSGS (**Table 1**) include all those conditions that result in a low nephron number and/or single-nephron hyperfiltration, which are generally categorized as "maladaptive" FSGS (7). In these forms, glomeruli are submitted to an increased mechanical stress that eventually results in hemodynamic alterations, dysfunctional reparative processes and focal sclerosis (8).

A constantly increasing number of mutations in genes encoding for podocyte proteins has been described in both *de novo* and hereditary forms of FSGS (3). In addition, susceptibility genes such as the APOL1 variant are important risk factors for FSGS in selected populations (9, 10).

Interferon (11) and bisphosphonates (12, 13) have been shown to induce severe forms of FSGS, which may sometimes respond to drug cessation and glucocorticoids. Cases of FSGS associated with severe tubulointerstitial lesions were also reported in patients taking cocaine, heroin, calcineurin inhibitors, or lithium (14–16). Podocytopathies with an FSGS pattern can be also caused by HIV, SARS-CoV-2, Parvovirus B19, cytomegalovirus and Epstein–Barr virus (17–20).

Circulating Permeability Factors

Several lines of evidence indicate that one or more molecules that directly or indirectly alter glomerular permeability may be responsible for FSGS in most primary forms (**Figure 1**). The existence of such "permeability factors" has been supported by rapid FSGS recurrence within hours from renal transplantation (21), by the efficacy of plasma exchange and selective apheresis methods in treating this condition (22–24), and by disease resolution after graft re-transplantation from a patient with FSGS recurrence to a diabetic recipient (25). Consistently, exposure to serum from patients with post-transplant recurrence was shown to increase glomerular permeability both *in vitro* and in animal models (26, 27). Another indirect evidence came from the observation of transient proteinuria in a child from a mother with FSGS, which suggested transplacental transmission of a permeability factor that was eventually cleared by the newborn (28).

The existence of a permeability factor in idiopathic nephrotic syndrome was first hypothesized in the early 1970's with reference to minimal change disease (MCD), another glomerular disorder that falls under the podocytopathy classification. At that time, observations such as the absence of immunocomplex deposition, disease sensitivity to steroids and cyclophosphamide, as well as spontaneous remission following measles infection (which suppresses cellular immunity), led to the hypothesis of a pivotal role for T cells in the disease pathogenesis (29). Subsequent studies showed that a glomerular permeability factor was secreted by human T cells from patients with MCD, but this factor could not be conclusively identified (30–32).

Later on, proteomic analysis of sequentially fractionated plasma from patients with FSGS recurrence after renal transplantation led to the identification of cardiotrophin-like cytokine factor-1 (CLCF-1) as a plausible permeability factor candidate (33–35). CLCF-1 is a 22 kDa B-cell stimulating cytokine from the IL-6 family, expressed in secondary lymphoid organs, bone marrow and lymphocytes. This molecule binds with

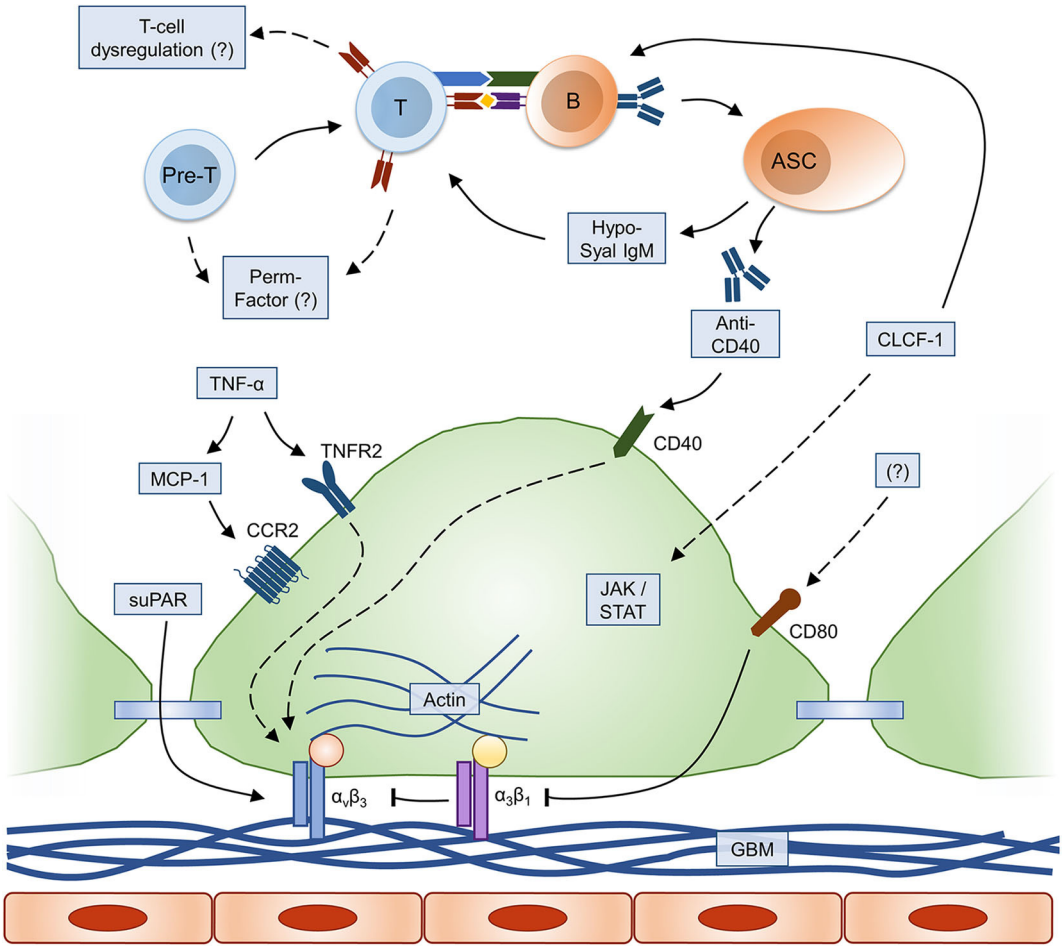

FIGURE 1 | Immune and molecular mechanisms of FSGS pathogenesis. Relevant immune and inflammatory pathways leading to alterations in podocyte foot process architecture are summarized (dashed lines: hypothetical/incompletely understood pathway); please refer to text for explanation. ASC, antibody-secreting cell; B, B cell; CCR2, C-C chemokine receptor type 2; CLCF-1, cardiotrophin-like cytokine factor-1; GBM, glomerular basement membrane; JAK/STAT, Janus kinases (JAK) and signal transducer and activator of transcription proteins (STAT) signaling pathway; MCP-1, monocyte chemoattractant protein-1; Pre-T, T-cell precursor; suPAR, soluble urokinase plasminogen activator receptor; T, T cell; TNF, tumor necrosis factor; TNFR2, TNF receptor 2.

high affinity to protein A and is present at concentrations 100 times higher in plasma from FSGS patients compared to plasma from healthy controls. CLCF-1 has been shown to increase glomerular permeability to albumin *in vitro*, and to reduce nephrin expression in cultured podocytes. Notably, these effects are blocked by CLCF-1-specific antibodies, which supports a direct pathogenic effect; interestingly, CLCF-1 activity is also inhibited by galactose and normal serum (36–38). Despite these promising results, confirmation by other research groups has not been reported to date. External validation of CLCF-1 as a permeability factor in independent patient cohorts is definitely required to clarify the relative impact of this molecule on FSGS pathogenesis and to plan targeted interventions in the future.

A seemingly major breakthrough in FSGS pathogenesis ignited the renal community in 2011, when Wei and colleagues identified the soluble urokinase plasminogen activator receptor (suPAR) as a possible circulating permeability factor; suPAR is the cleaved form of a membrane-bound glycoprotein (uPAR) that interacts with podocyte αvβ3 integrins, membrane proteins that connect actin filaments with the extracellular matrix. The authors showed that suPAR was increased in most patients with FSGS, and that this molecule activated αvβ3 integrins in cultured podocytes (39), which induced actin filament reorganization and proteinuria (40, 41). Consistently, serum samples obtained before transplant recurrence promoted αvβ3 integrin activation, an effect that was reduced by suPAR removal through plasmapheresis (39), and the extent of podocyte effacement correlated with suPAR levels (42). Moreover, uPAR-null mice treated with high doses of recombinant suPAR developed proteinuria and early FSGS lesions (39). Elevated suPAR levels in 84.3 and 55.3% of FSGS patients were confirmed also in the FSGS CT and PodoNet international cohorts, respectively (43).

Initial enthusiasm was however curtailed by reports from other investigators, who failed to replicate these findings in independent patient cohorts. Serum suPAR concentrations were

found to inversely correlate with glomerular filtration rate, and after adjustment for this confounder suPAR lost its ability to discriminate between FSGS and other proteinuric nephropathies (44–49). In addition, subsequent attempts to elicit FSGS changes in wild-type mice were unsuccessful, suggesting that the uPAR-null background could be at least in part responsible for the disease phenotype observed (49–51). Since suPAR can be cleaved into several shorter molecules, some authors suggested that a hypoglycosylated fragment not readily detected by standard assays, rather than full-length suPAR, could be responsible for FSGS pathogenesis (52). In a recent study, a novel method able to identify both full-length and suPAR fragments outperformed the conventional ELISA assay in discriminating FSGS cases from other proteinuric nephropathies in a single-center cohort (53), but external validation has not been reported yet.

Notwithstanding the frequent lack of immune deposits in renal biopsies from FSGS patients, several autoantibodies against selected antigen specificities (actin, adenosine triphosphate synthase, aldose reductase, and angiotensin II type 1 receptor) have been described in anecdotal cases (54, 55). Delville and colleagues analyzed pre-transplant sera from patients with and without post-transplant FSGS recurrence using protein array data. A panel of seven autoantibodies was found to predict disease recurrence with 92% accuracy in a larger validation set. In this panel, autoantibodies against CD40, a costimulatory molecule of the TNF receptor superfamily highly expressed by antigen-presenting cells, bore the strongest impact on the prediction of FSGS recurrence (56). CD40 was found to be expressed by cultured podocytes *in vitro* and in renal biopsies from patients with recurrent FSGS, but not in normal kidneys. Anti-CD40 antibodies isolated from these patients disrupted podocyte architecture *in vitro* and induced proteinuria in wild-type mice, effects that were reversed by a CD40 blocking antibody. Interestingly, blocking either suPAR or αvβ3 integrin activation ameliorated podocyte injury *in vitro* (56), whereas co-administration of suPAR enhanced proteinuria *in vivo* (51, 56, 57), thus suggesting that anti-CD40 antibodies and suPAR may synergize in inducing αvβ3 integrin activation and FSGS lesions. Studies to assess the pathogenicity of the other autoantibodies identified by Delville and colleagues and to validate anti-CD40 antibodies as a permeability factor in additional patient cohorts are eagerly awaited.

Adaptive Immunity

As previously discussed, the involvement of T cells in the pathogenesis of idiopathic nephrotic syndrome was theorized more than 40 years ago. Since then, multiple studies have evaluated T-cell phenotype and function in these patients, which highlighted differences in the relative abundance of T-cell subsets, skewed polarization toward a T_H2 phenotype, enhanced mobilization of hematopoietic stem cells, along with increased T_H17 effector and reduced regulatory T cell frequencies (58–64). Notably, most of these studies were conducted in small cohorts of patients and by pooling histologically disparate podocytopathy cases, thus potentially increasing the risk of simultaneously analyzing the immune phenotype of highly diverse conditions. Interestingly, adoptive transfer of hematopoietic stem cells obtained from patients with FSGS to immunodeficient mice induced foot process effacement and proteinuria; however, these effects were not observed after infusion of peripheral blood mononuclear cells from the same donors, suggesting that immature cells rather than differentiated T cells could be involved in the pathogenesis of the disease (65).

The efficacy of B cell-depleting anti-CD20 monoclonal antibodies in maintaining the remission of steroid-sensitive idiopathic nephrotic syndrome underscored the potential role of B cells in the pathogenesis of MCD- and FSGS-associated podocytopathies (66, 67). Even though the CD20 molecule is not expressed by most antibody-secreting cells (ASC), the depletion of mature and memory B-cells has been shown to hamper the generation of new short-lived ASC, thus potentially impacting on autoantibody production (68). Notably, faster memory B-cell reconstitution after treatment was shown to predict nephrotic syndrome relapse (69), and continuous B cell depletion has been proposed as a strategy to maintain disease remission (70). Within the classification limits of a study in a pediatric cohort with unavailable renal pathology assessment, the production of hypo-sialylated IgM antibodies binding to T-cell surface has been reported as a possible mechanism of steroid dependence in idiopathic nephrotic syndrome (71). This work suggests the existence of a pathogenic link between B and T cells in MCD and FSGS, which can be favorably affected by anti-CD20 therapy. Aside from antibody production, B cell-targeted treatment might also affect the autoreactive T-cell pool, since B cells can efficiently present antigens and provide costimulatory signals to T cells. Moreover, proximity due to interaction with antigen-specific B cells has been proposed as a potential mechanism of autoreactive T-cell depletion following anti-CD20 therapy (72).

The CD80 (B7-1) molecule, which is expressed by antigen-presenting cells and provides costimulatory signals to T cells, has been also implicated in the pathogenesis of FSGS. Lipopolysaccharide-mediated induction of CD80 in podocytes caused actin cytoskeleton reorganization *in vitro* and nephrotic range proteinuria *in vivo* (73). These effects were linked to β1 integrin inactivation, which normally anchors podocyte foot processes to the glomerular basement membrane, and were completely restored by CD80 silencing or pharmacologic blockade (74). CD80 staining in native and post-transplantation renal biopsies identified a subset of patients with FSGS in whom this mechanism seems to be relevant (74), even though these findings were not corroborated by other groups (75, 76). Notably, urinary excretion of CD80 assessed in two large patient cohorts correlated with disease activity and was able to discriminate between primary and secondary FSGS forms, although the highest values were observed for MCD cases (77).

It has also been speculated that podocyte immunological functions could play an important role in damage progression. Indeed, podocytes react to injury by changing their phenotype and setting aside their barrier function in favor of an immunological one; these events determine the alteration of the glomerular barrier and lead to proteinuria. However, in some forms of FSGS, adaptive immunity may also stimulate an autoimmune response that becomes itself an additional source of injury and sensitizes podocytes to circulating factors (78).

Tumor Necrosis Factor Pathway

Elevated serum concentrations of tumor necrosis factor (TNF)-α have been reported in some patients with FSGS (79), and stimulation of the TNFR2 receptor with TNF-α evoked robust downstream signaling in cultured podocytes (80). However, a subsequent study showed that activation of the TNF pathway in cultured podocytes exposed to serum from FSGS patients occurred in 21% of cases, irrespective of circulating TNF-α levels (81). This once again underscored the heterogeneity of FSGS pathogenesis and identified intrarenal activation of the TNF pathway as a potential convergence point of multiple pathogenic mechanisms in this disease. Similarly to what described for suPAR and anti-CD40 antibodies, TNF pathway stimulation in podocytes induces αvβ3 integrin activation and actin filaments reorganization (82). Evidence of early glomerular TNF pathway activation was also obtained with an unbiased approach, i.e., by microarray analysis of glomerular RNA isolated from pre- to post-transplant biopsies of FSGS patients, suggesting that one or more permeability factors may induce FSGS through this mechanism (83). Consistent with these data, pharmacologic inhibition of TNF-α might be beneficial in some but not all of patients with FSGS (84, 85). Unpublished observations from the NEPTUNE network investigators suggest that patients with activation of the TNF pathway might be at higher risk for rapid renal function deterioration (86). Urinary levels of two downstream components of the TNF pathway, MCP-1 (also known as CCL2) and TIMP-1, were found to be associated with TNF activation in the same cohort (86). Elevated urinary MCP-1 concentrations were also confirmed in FSGS patients by other investigators, and correlated with the degree of proteinuria (87). Moreover, glomerular expression of MCP-1 and its receptor CCR2 were increased in patients with FSGS and in mouse models of the disease (88). CCR2 knockout and antagonism with a small molecule inhibitor reduced glomerular injury and proteinuria *in vivo* (89), thus identifying a possible additional therapeutic target.

PATHOLOGY

The pathognomonic features of FSGS initially affect only a few glomeruli and are characterized by tuft sclerosis, which is limited to a portion of the otherwise normal glomerulus. These lesions are initially predominant in juxtamedullary glomeruli, but progressively spread to the outer cortex. Hence, if renal biopsy is performed early in the course of the disease, FSGS diagnosis may be missed, particularly when the number of sampled glomeruli is small and the biopsy specimen contains only superficial cortex tissue.

The Columbia classification proposed to subdivide FSGS lesions in 5 histologic categories include the tip, cellular, perihilar, collapsing, and the not otherwise specified (NOS) variants (90). However, that classification was based exclusively on light microscopy findings. Different types of lesion may coexist in the same biopsy sample and histologic features can also change over time, with all subtypes usually evolving to a NOS phenotype as renal function deteriorates toward end-stage renal disease (ESRD) (91). The process of segmental sclerosis and capillary collapse progresses to a gradual obliteration of glomeruli, which may undergo complete "reabsorption," leaving behind non-functioning aglomerular tubules (92). These changes are strongly associated with a progressive form of interstitial fibrosis, tubular atrophy and vascular damage. Some authors also proposed that the collapsing variant may be a completely distinct disease from other FSGS forms, due to its highly unfavorable outcome, peculiar pathology findings and typical association with HIV infection (93).

Immunofluorescence studies are typically negative, but deposits of IgM and C3 may be observed in mesangial and sclerotic areas (9). Strassheim and colleagues hypothesized that natural IgM could bind to neoantigens exposed in the glomerulus due to non-immune injury, activating the complement system and promoting further damage. Consistent with this hypothesis, B cell depletion reduced IgM deposition and attenuated renal injury in a mouse model of FSGS (94). In addition, in a subset of patients with primary FSGS, colocalization of IgM with C3 suggested complement activation following recognition of a cognate antigen (94). However, the clinical significance of these deposits remains controversial (95–97).

On electron microscopy (EM), foot process effacement, podocyte detachment, and segmental sclerosis with podocyte loss are the most common findings. Foot process effacement is often diffuse (>80%) and severe in tip, cellular and collapsing variants, while this feature can be more variable in NOS and less severe in the perihilar variant (2, 98). As discussed later, the degree of foot process effacement has important implications for the differential diagnosis of FSGS causes and for the correct identification of primary forms.

CLINICAL PRESENTATION AND OUTCOME

Proteinuria is the most common feature at presentation in FSGS, and may range from sub-nephrotic levels to full-blown nephrotic syndrome with hypoalbuminemia, hypercholesterolemia, and diffuse edema.

Newborns with congenital nephrotic syndrome are usually premature with low birth weight, and severe nephrotic syndrome is diagnosed soon after birth. FSGS is due to genetic mutations in most of these cases, and ESRD typically develops in infancy. These patients have a comparable mortality, growth, and time to transplantation as infants with other primary renal diseases (99).

Children and adolescents frequently present with signs and symptoms of nephrotic syndrome such as periorbital and dependent edema. Owing to the high frequency of steroid-sensitive MCD in this age group, renal biopsy is usually not performed in patients presenting with isolated nephrotic syndrome. Histologic evaluations are usually reserved for those patients with atypical characteristics (syndromic features, rapid renal function deterioration, positive autoimmune panel) and for steroid-resistant cases.

Adults may be asymptomatic, but even sub-nephrotic proteinuria may eventually increase to the nephrotic range over time. Microscopic hematuria is found initially in about half of cases, while gross hematuria is rare. Hypertension is frequent in

adults, and impaired renal function may be already present at the time of referral in up to 25% of patients with FSGS. Unless medically contraindicated, renal biopsy should be performed in all adult patients to confirm the diagnosis and to guide future management.

The natural course of primary FSGS unresponsive to treatment is frequently relentless, with 50% of patients progressing to ESRD within 3–8 years from diagnosis (100). In a few cases, FSGS is characterized by a rapidly progressive course marked by massive proteinuria and severe hypertension. Many patients may develop complications due to the nephrotic syndrome, including infection, thrombotic complications, and cardiovascular disease. However, patients who achieve and maintain asymptomatic non-nephrotic proteinuria have a significant improvement in the overall natural history of FSGS, both in term of renal disease progression and extra-renal complications (100, 101).

The best predictor of a favorable outcome is complete remission, which has been defined as proteinuria <0.2–0.3 g/day (or a urinary protein to creatinine ratio <200–300 mg/g) associated with stable glomerular filtration rate (102, 103). Unfortunately, spontaneous complete remission is exceptional, but can be achieved with treatment in a similar proportion of pediatric and adult patients (104). A number of studies pointed out that children (105, 106) and adults (107–109) who achieve complete remission maintain normal renal function over the time, while most of non-responders progress to ESRD. The same investigators outlined that also a partial remission, defined as a proteinuria <2.0–3.5 g/day (variable definition among studies) with stable renal function can improve outcomes in comparison with non-responders. Nevertheless, the length of exposure to proteinuria may be more important than single time-point proteinuria values: time-varying proteinuria has been proposed as a reliable metric to capture the risk of a 50% reduction in glomerular filtration rate or progression to ESRD (110).

Additional clinical factors may provide useful information regarding the prognosis of patients with FSGS. Impaired renal function at presentation indicates a poor prognosis, unless the increase of serum creatinine is the consequence of acute kidney injury, due to diuretic-induced hypovolemia and/or severe hypoalbuminemia. Arterial hypertension can also contribute to the development of renal failure in FSGS: as autoregulation of glomerular pressure in FSGS is impaired, the increase in systemic blood pressure leads to a rise in glomerular pressure, which results in glomerular capillary wall stretch, endothelial damage, and increased filtration of proteins (111) along with microvascular lesions leading to renal ischemia and interstitial fibrosis (112).

Histological findings may also help in assessing renal outcomes. The prognosis is usually severe in patients with diffuse interstitial fibrosis and tubular atrophy (113, 114). In addition, mesangial proliferation at renal biopsy was associated with a 4.6 relative risk of serum creatinine doubling in some series (107). Diffuse and multiple segmental sclerotic areas at the initial biopsy and, even more importantly, an increase in the number of globally sclerotic glomeruli in follow-up biopsies, correlate with chronic kidney disease development.

The Columbia classification may also provide useful prognostic information. The tip lesion variant has been associated with low pathologic scores and rate of progression to ESRD, also due to a high response rate to treatment. Compared to NOS, the collapsing variant usually displays more severe nephrotic syndrome and lower renal function at diagnosis. Overall, 7% of tip, 47% of collapsing and 20% of NOS variant patients progressed to ESRD at 3 years from diagnosis (113). Other studies confirmed that patients with tip lesions display a favorable outcome, while patients with collapsing FSGS have a worse prognosis (115–117).

After renal transplantation, primary FSGS has a high rate of recurrence in the allograft, which significantly reduces long-term graft survival (118). There is considerable variability among case series, but recent data from pediatric and adult cohorts indicate that the overall recurrence rate is similar across age groups, affecting approximately one third of patients (23, 119). These reports may however underestimate the true incidence of primary FSGS recurrence, because most studies defined primary forms irrespective of the presence of nephrotic syndrome or the degree of foot process effacement on EM, likely including secondary FSGS forms in the analysis. Disease remission with treatment can be achieved only in half of cases, which makes recurrent FSGS a largely unmet medical need.

DIAGNOSIS

A correct differential diagnosis between primary and secondary FSGS forms is paramount to guide management. Even though light microscopy alone cannot differentiate primary from secondary FSGS, primary forms share some typical features, including the presence of a full-blown nephrotic syndrome (proteinuria >3.5 g/day, albuminemia <3.0 g/dL) and EM ultrastructural findings consistent with diffuse (>80%) foot process effacement (**Table 2**). However, since primary FSGS is still a diagnosis of exclusion, maladaptive, infectious, toxic, and genetic forms should be always ruled out.

Hemodynamic maladaptation to a congenital or acquired reduction of nephron mass is responsible for most cases of secondary FSGS. Maladaptive FSGS can be frequently suspected from medical history and renal imaging. Clinically, these forms are characterized by non-nephrotic or nephrotic-range proteinuria in the absence of hypo-albuminemia, hypercholesterolemia, and edema. Maladaptive FSGS frequently shows perihilar hyalinosis involving >50% of hypertrophic glomeruli with segmental lesions. Ultrastructure analysis usually reveals segmental foot process effacement (<80%) instead of the diffuse pattern observed in primary forms, indicating podocyte mechanic injury rather than a circulating pathogenic mediator (98). Infections should be identified by an appropriate work-up and treated accordingly, as remission with disease-specific treatment can be frequently achieved. In drug-induced FSGS, prompt identification and removal of the offending agent are paramount.

The frequency of genetic mutations is high in pediatric patients and tends to reduce with higher age at onset. Genetic

TABLE 2 | Characteristics of primary and secondary FSGS forms.

	Primary	Genetic	Maladaptive
Clinical presentation	Acute, full-blown nephrotic syndrome in most cases (proteinuria >3.5 g/day, albumin <3.0 g/dL); may develop gradually in some cases	Variable from sub-nephrotic proteinuria to nephrotic syndrome	Gradual development of sub-nephrotic proteinuria (<3.5 g/day), sometimes progressing to nephrotic-range; nephrotic syndrome is extremely uncommon (albumin >3.0 g/dL)
Light microscopy	Can be associated with any variant, glomerulomegaly uncommon	Can be associated with any variant	Often peri-hilar variant, glomerulomegaly is common
Electron microscopy	Diffuse (>80%) foot process effacement	Either diffuse or segmental foot process effacement	Segmental (<80%, often <50%) foot process effacement
Treatment and outcome	Steroids are effective in ∼60% of cases, other IS may be used as steroid-sparing agents or for steroid-resistant cases. Lack of response to treatment predicts progression to ESRD	Immunosuppression is typically ineffective, most cases progress to ESRD within a few years from diagnosis	Immunosuppression contraindicated, often good response to RAS-inhibitors; slow progression toward ESRD

ESRD, end-stage renal disease; IS, immunosuppressive agents; RAS, renin-angiotensin system.

testing is usually advised in all patients with congenital nephrotic syndrome and in those presenting with syndromic features and/or a positive family history. Moreover, it was recently proposed that any mismatch between clinical features and ultrastructural findings (i.e., nephrotic syndrome with segmental foot process effacement, or non-nephrotic proteinuria with diffuse foot process effacement) should also trigger genetic testing (7). Nevertheless, the most compelling indication for genetic analyses remains the resistance to an adequately long and correctly dosed steroid course, since a positive result can be obtained in a significant fraction of both pediatric and adult patients in this case (120–122). Further immunosuppressive treatment should be avoided in patients with genetic mutations, as the risk-benefit ratio is unfavorable. In such cases, supportive therapy should be optimized to lessen the impact of additional risk factors (e.g., hypertension) and to manage accompanying symptoms. Despite such measures, most of these patients progress to ESRD over a relatively brief period, but FSGS recurrence after transplantation is virtually non-existent (7), except in cases associated with NPHS1 mutations (123).

TREATMENT OF PRIMARY FSGS

Asymptomatic patients with non-nephrotic proteinuria and stable renal function usually do not progress to ESRD and are not exposed to the potential complications of nephrotic syndrome. Based on these considerations, no specific treatment besides conservative management (including salt restriction and inhibition of the renin-angiotensin system) is strictly necessary. However, proteinuria, serum creatinine and blood pressure should be monitored over time. Edema, arterial hypertension, dyslipidemia, and hypercoagulability are frequent complications in patients with nephrotic syndrome, whose treatment is critical to improve life-expectancy and quality of life.

The baseline specific treatment for patients with primary FSGS and nephrotic syndrome rests on glucocorticoids. These agents have well-known genomic and non-genomic immunomodulatory properties, which result in a general suppression of cellular and humoral immunity. In addition, glucocorticoids may also directly promote podocyte survival and actin cytoskeleton stabilization, increasing resistance to injury (124, 125). Response to glucocorticoids is crucial to define the prognosis and to guide further management; based on treatment response, patients are classified as steroid-sensitive or steroid resistant (**Table 3**).

TABLE 3 | Clinical definitions in FSGS.

Steroid-sensitive Nephrotic syndrome	Remission of nephrotic syndrome after therapy with glucocorticoids
Frequently-relapsing Nephrotic syndrome	Two or more relapses of nephrotic-range proteinuria within 6 months after initial response to glucocorticoids
Steroid-dependent nephrotic syndrome	Two or more relapses during or within 2 weeks from completion of glucocorticoid therapy
Steroid-resistant nephrotic syndrome	Remission not achieved after adequately dosed glucocorticoid therapy for 4–6 weeks (children) or 16 weeks (adults)

In children, glucocorticoid therapy is started without histologic confirmation and is usually maintained for 2–3 months, with most patients achieving remission within the end of the first month of treatment (126). Of note, the majority of these children have podocytopathies associated with MCD rather that FSGS lesions, which are more likely to respond to steroids. Adult patients may experience a significant delay in the response to glucocorticoids compared to pediatric patients, thus justifying the need of prolonged therapy (prednisone 1 mg/Kg/day or 2 mg/Kg every other day for up to 16 weeks) before being defined as steroid-resistant (SR) (103, 107, 127–129). This label is often mistakenly attributed to patients who are given insufficient doses of glucocorticoids for far too short periods of time. In adult patients who achieve remission (47–66% of cases),

glucocorticoids are slowly tapered over the following 6 months (103, 130).

Up to 70–80% of pediatric and adult patients who achieve remission, however, may experience one or more relapses, that are usually treated with the same steroid schedule. These patients may unfortunately become steroid-dependent (SD) or experience frequent relapses (FR) (**Table 3**), leading to increased exposure to glucocorticoids and their adverse effects. Measures to reduce the risk of steroid toxicity include dose reduction in the elderly and in obese subjects, use of steroid-sparing immunosuppressive agents, and administration of a single dose of a short-acting glucocorticoid in the morning between 7 and 9 a.m., in order to mimic the circadian rhythm of cortisol. Patients should be counseled to maintain regular physical activity to prevent myopathy and obesity, and to follow a low-calorie and low-salt diet to prevent hypertension, edema, obesity and cardiovascular disease (131, 132). *P. Jiroveci* prophylaxis and use of biphosphonates in women over 50 years should be also considered.

Calcineurin inhibitors (CNI) is an additional option for both SR and SD/FR patients, as well as for patients with contraindications to prolonged steroid courses. CNI activity relies on the inhibition of IL-2 signaling essential for T-cell activation; moreover, these agents directly stabilize podocyte synaptopodin, which regulates the actin filament cytoskeleton, and protect against podocyte injury (133, 134). Two small randomized trials (135, 136) and several observational studies (137–140) demonstrated that cyclosporine can significantly reduce proteinuria in SR patients, and similar or even better results have been reported with tacrolimus (141–144). Moreover, cyclosporine efficacy was also demonstrated in SD/FR patients as a steroid-sparing agent (145), even though the relapse rate upon discontinuation can be excessively high. In addition, although the anti-proteinuric effects of CNI are well-demonstrated, there is no established evidence that these agents can prevent FSGS progression in the long-term. Rather, long-term CNI use has been avoided due to fear of development or aggravation of tubular atrophy, interstitial fibrosis, and glomerular sclerosis. This risk may largely depend on the doses used and, although some individuals are particularly prone to CNI toxicity (perhaps because of altered pharmacodynamics), progressive renal damage is less likely to occur in patients with normal kidney function using low CNI doses (<2.5–3.0 mg/Kg/day for cyclosporine and <0.05 mg/Kg/day for tacrolimus).

Cyclophosphamide, an alkylating agent that affects multiple components of the immune system, has showed efficacy when given as treatment of first episodes or in FR patients (146–149), but proved ineffective, at standard doses, in patients with SR nephrotic syndrome (150). Concerns for gonadal and systemic toxicity have discouraged the use of alkylating agents in FSGS, and only a single course is usually recommended (103).

Mycophenolate inhibits *de novo* purine synthesis, preferentially affecting T- and B-cell expansion, but also has non-immune effects that result in prevention of mesangial cell proliferation, inhibition of podocyte apoptosis and preservation of nephrin and podocin expression (151). Mycophenolate showed comparable effectiveness to levamisole in maintaining remission in SD/FR children (152), but was inferior to cyclosporine in another randomized trial (153). Observational studies reported a low rate of remission with mycophenolate in SR patients (154–156). A randomized controlled trial showed that mycophenolate associated with high-dose dexamethasone achieved remission in only one third of patients (157), even though a significant fraction of patients enrolled in the study did not have nephrotic syndrome and were likely affected by secondary FSGS forms. Another trial found that this immunosuppressive agent was inferior to tacrolimus in SR nephrotic syndrome (158). Nevertheless, the use of mycophenolate as maintenance treatment after remission induction with cyclosporine in SR patients might prevent relapses and reduce the risk of nephrotoxicity (159).

Adrenocorticotrophic hormone (ACTH) is a melanocortin peptide that activates melanocortin receptors and controls steroidogenesis, thus inducing immunomodulating and anti-inflammatory effects. Moreover, ACTH has been shown to reduce foot process effacement and podocyte apoptosis, to prevent the downregulation of podocyte-specific proteins and to increase catalase activity, thereby reducing oxidative stress in podocytes (160, 161). Anecdotal reports outlined the possibility of obtaining complete or partial remission with the use of ACTH, either in its synthetic or natural gel form (162–164).

The chimeric monoclonal antibody rituximab and its fully human counterpart ofatumumab selectively deplete B cells through targeting of the CD20 molecule, thus influencing humoral immunity as well as B- and T-cell crosstalk. Moreover, rituximab may also have direct effects on podocytes through SMPDL-3b stabilization, which was shown to prevent the disruption of actin cytoskeleton and podocyte apoptosis (165, 166), although the specificity of this binding has been questioned (167). Rituximab proved to be effective in preventing relapses in both adult and pediatric SD/FR patients (66, 67). On the other hand, poor results from observational studies have been reported with the use of rituximab in SR nephrotic syndrome (168–170), and the only randomized clinical trial available so far failed to detect any additional benefit from rituximab over CNI (171). Ofatumumab efficacy was reported in anecdotal cases of SR nephrotic syndrome (172, 173), but a recent randomized controlled trial in pediatric patients resistant to multiple therapeutic lines was terminated early for futility (174).

Abatacept, a CTLA4-Ig fusion protein with high affinity for CD80, inhibits T-cell costimulation and prevents podocyte β1 integrin inactivation induced by CD80 expression. Abatacept induced partial or complete remission of proteinuria in 4 patients with FSGS recurrence after kidney transplantation and in one patient with FSGS in the native kidney (74). While these data were confirmed by some investigators (175), others were unable to find any beneficial effect with this drug (176, 177). A randomized controlled trial was designed to clarify the effect of abatacept in treatment-resistant nephrotic syndrome (NCT02592798), but was reportedly terminated early due to poor enrolment (85).

Several attempts at non-selective removal or specific blockade of putative circulating factors have been performed, especially in case of post-transplant FSGS recurrence, which represents a

particularly challenging and largely unmet medical need. The use of plasma exchange, immunoadsorption and LDL-apheresis methods in patients who did not respond to available therapies have been reported with variable outcomes (178), but methods to selectively remove circulating permeability factor candidates have not been reported yet. A phase 2 randomized clinical trial is under way to assess the effect of the anti-CD40 monoclonal antibody Bleselumab, which blocks the interaction between CD40 and its ligand, in preventing FSGS recurrence (NCT02921789). Based on evidence from *in vitro* experiments of galactose efficacy in antagonizing the effects of CLCF-1, and after preliminary results from anecdotal cases (179), a small randomized controlled trial assessed the effects of oral galactose supplementation in patients with multi-resistant FSGS. Serum galactose concentration did not significantly differ between pre- and post-treatment assessments, as galactose is rapidly metabolized after absorption. A 50% proteinuria reduction with stable renal function was observed in 2 of 7 patients treated, a proportion that was virtually identical to the control arm (180). The same investigators also evaluated TNFα antagonism with adalimumab, reporting an overall response in 4 of 17 patients treated (pooled from the phase 1 pharmacokinetic study and the randomized controlled trial) (84, 180). As activation of the TNF pathway may be a prerogative of only a subgroup of FSGS cases, a trial to assess whether adalimumab can normalize the urinary concentration of TNF pathway activation biomarkers (MCP-1 and TIMP-1) in these patients has been planned (NCT04009668). Moreover, an open label, dose-escalation study to test the efficacy of an oral inhibitor of the MCP-1 receptor CCR2 in adult patients with primary FSGS is currently ongoing (NCT03703908).

CONCLUSIONS

The history of primary FSGS has been characterized by the rise and fall of biomarkers and potential therapeutic targets like very few other disorders in nephrology. Since the term FSGS merely indicates a pathologic entity shared by a wide array of diseases, it is imperative that data from novel therapeutic strategies are obtained from adequately powered trials that appropriately differentiate between primary and secondary FSGS forms (181). In addition, as primary FSGS itself is likely a broad group of disorders with distinctive pathologic mechanisms, efforts should be aimed to the search of novel biomarkers and to the validation of those already proposed in small patient cohorts. In the long run, this may help to define a personalized treatment strategy for each of these patients that could finally surpass the outdated concept of "one-therapy-fits-all."

AUTHOR CONTRIBUTIONS

MP and CP revised the literature, wrote the first draft, and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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SYSTEMATIC REVIEW
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A Systematic Review of Treatment Options and Clinical Outcomes in Pemphigoid Gestationis

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Background: Treatment regimens for pemphigoid gestationis (PG) are non-standardized, with most evidence derived from individual case reports or small series.

Objectives: To systematically review current literature on treatments and clinical outcomes of PG and to establish recommendations on its therapeutic management.

Methods: An a priori protocol was designed based on PRISMA guidelines. PubMed, Scopus, and Web of Science databases were searched for English-language articles detailing PG treatments and clinical outcomes, published between 1970 and March 2020.

Results: In total, 109 articles including 140 PG patients were analyzed. No randomized controlled trials or robust observational studies detailing PG treatment were found. Systemic corticosteroids ± topical corticosteroids and/or antihistamines were the most frequently prescribed treatment modality ($n = 74/137$; 54%). Complete remission was achieved by 114/136 (83.8%) patients. Sixty-four patients (45.7%) were given more than one treatment modality due to side effects or ineffectiveness. Leaving aside topical corticosteroids as monotherapy ± antihistamines in patients with mild disease, systemic corticosteroids ± topical corticosteroids and/or antihistamines led to complete remission in the highest proportion of patients (83%), while steroid-sparing treatments ± topical corticosteroids and/or antihistamines were associated with the lowest proportion of flares (55.5%).

Limitations: The review has been drafted based on a limited number of single case reports and small case series. Underreporting/underdiagnosis of patients with mild-to-moderate PG, partial/absent follow-up, absence of precise description of neonatal outcomes and lack of validated objective scores for measuring disease severity are other limitations of our study. Our systematic review was affected by publication bias.

Conclusion: Systemic corticosteroids are the most frequently used treatment for PG. Whilst most patients achieve complete remission, many of them have

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refractory/persistent disease requiring multiple lines of therapy. Therefore, we provided an algorithm for PG treatment integrating the results of this systematic review with current knowledge available for bullous pemphigoid. High-quality studies will further help assess the effectiveness of different treatment options for PG.

Keywords: pemphigoid gestationis, autoimmune bullous diseases, herpes gestationis, pregnancy, systematic review, treatment

INTRODUCTION

Pemphigoid gestationis (PG), formerly known as herpes gestationis, is a rare subepidermal autoimmune blistering disease belonging to the group of specific dermatoses of pregnancy (1). It is characterized by intensely pruritic urticarial plaques and/or vesiculobullous lesions typically starting in the periumbilical region. Although PG usually occurs in late pregnancy or puerperium, it may also be rarely associated with gestational trophoblastic disease (GTD), including choriocarcinoma and hydatiform mole (2). Linear deposits of complement fraction 3 (C3) ± immunoglobulin (Ig) G along the dermal-epidermal junction on direct immunofluorescence (DIF) of perilesional skin are mandatory to confirm the clinical suspicion (3). PG may have a chronic-relapsing course, with flares usually occurring after delivery, during menses, or in association with the use of hormonal contraceptives. Recurrences in subsequent pregnancies are common (4). Systemic and topical corticosteroids are empirically recognized as a cornerstone of PG treatment, especially during the gestation period and in mild-to-moderate cases. On the other hand, a wide variety of therapeutic approaches, including steroid-sparing agents such as intravenous immunoglobulin therapy, azathioprine, and dapsone, have been reported for persistent cases refractory to first-line regimens or patients with intolerance to (or medical inadvisability of) systemic corticosteroids. Moreover, the management of this disease may be challenging owing to the safety concerns during pregnancy or lactation of some immunosuppressive drugs (5). Nevertheless, except for the recommendations of the French Society of Dermatology (6), no specific guidelines have been developed for the treatment of PG. In addition, no systematic review has been carried out on therapeutic options for PG to date and the current data available on PG treatment are largely based on case reports and case series. This systematic review aimed at providing a comprehensive and up-to-date analysis of treatment options employed for PG and developing a therapeutic algorithm for this disease.

MATERIALS AND METHODS

Protocol and Literature Search

The recommendations contained in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (7) were followed. The literature review was conducted using PubMed, Scopus and Web of Science databases. The search strings were the following: "pemphigoid AND gestationis" and "herpes AND gestationis." Publications between 1 January 1970 and 24 March 2020 were searched independently and cross-checked by two researchers (GG and DF) (**Supplementary Table 1**).

Selection of Articles

Articles were screened by title and abstract and those deemed relevant were reviewed in full text. Any disagreements regarding article suitability were solved by a third independent author (AVM). The articles were included in the qualitative synthesis if (i) PG diagnosis was based on a clinical picture suggestive of subepithelial autoimmune bullous disease occurring during pregnancy, post-partum or in association with GTD, a histopathologic image of subepidermal detachment and a DIF test showing linear deposition of C3 ± IgG along the dermal-epidermal junction, (ii) they were published in English, (iii) they documented in detail the treatment and clinical outcome.

Outcomes

Primary outcome measures were different treatment regimens and response to therapy, defined as complete remission (CR), partial response (PR), and active disease at the end of follow-up. Secondary outcome measures were side effects, occurrence of flares, and pregnancy-related outcomes in mothers and children.

Data Extraction and Analysis

Two authors (GG and FD) critically reviewed the included articles and independently extracted the following variables onto a Microsoft Excel spreadsheet: age at onset, age at main treatment initiation, gestational age at PG onset, gestational age at first treatment initiation, number of pregnancies, PG recurrence in different pregnancies, first treatment initiation, first treatment, main treatment initiation, main treatment, lines of therapy, modality of treatment, prednisone-equivalent dose of systemic corticosteroids (initial and maximum), maternal disease outcome, gestational age at delivery, mode of delivery, occurrence of flare(s), cause of flare(s), persistent course, follow-up duration, side effects, and newborn outcome. Relevant data were not available for every patient; thus, percentages refer to the total number of patients for whom information regarding a specific outcome was available or could be concluded.

The main treatment was defined as the treatment associated with patient clinical outcome at the end of follow-up. In patients who underwent only one line of therapy, main treatment coincided with first-line treatment. Conversely, in patients who underwent more than one line of therapy, treatments which had been given before main treatment were referred to as first-line treatments. The following modalities of treatment were recognized: (i) systemic corticosteroids ± topical corticosteroids and/or antihistamines; (ii) systemic corticosteroids combined

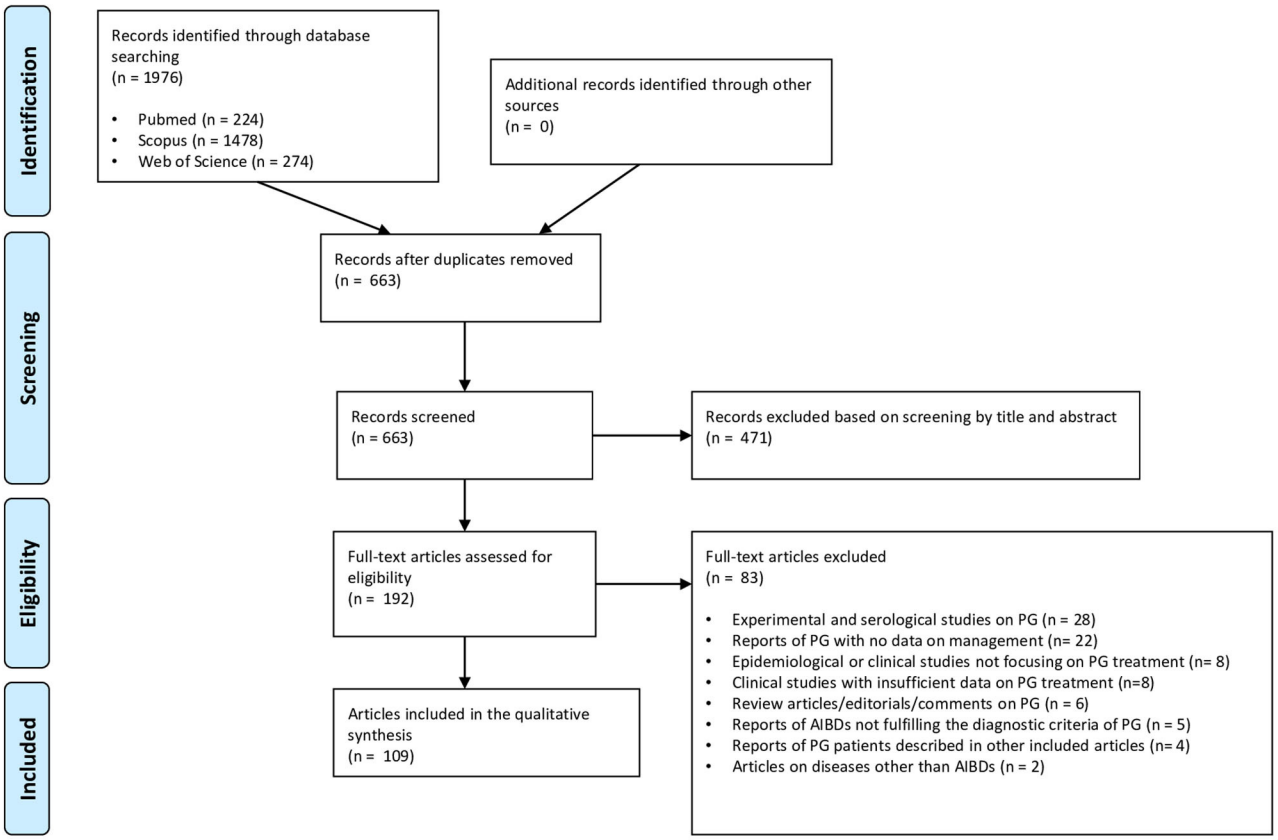

FIGURE 1 | PRISMA flow diagram detailing literature search and study selection process for systematic review.

with steroid-sparing treatments ± topical corticosteroids and/or antihistamines; (iii) steroid-sparing treatments ± topical corticosteroids and/or antihistamines; (iv) topical corticosteroids as monotherapy ± antihistamines. Steroid-sparing treatments included all the treatments that were given in addition to or instead of systemic corticosteroids to lower the dose of systemic corticosteroids needed or avoid using them. CR, defined as the absence of lesions at the end of follow-up, was subdivided into off-therapy or on-therapy. Simplified definitions of CR on-therapy and CR off-therapy were adapted from the definitions and outcome measures for bullous pemphigoid recommended by an international panel of experts (8) CR off-therapy was defined as an absence of new or established lesions or pruritic symptoms while the patient is off all PG therapy, while CR on-therapy was defined as the absence of new or established lesions or pruritus while the patient is receiving any topical or systemic treatment (8). PR was defined as improvement without complete healing of skin lesions, while active disease as absence of improvement/worsening of disease. A flare was defined as the appearance of new lesions or the extension of established lesions in a patient who had previously achieved CR. Regarding the therapeutic algorithm for PG management, mild, and moderate-to-severe PG were defined according to the classification adopted in the European Dermatology Forum/European Academy of Dermatology and Venereology guidelines on bullous pemphigoid management (9). In patients with onset during pregnancy or GTD, the course was defined as persistent if the disease was still active ≥6 months after delivery/abortion/GTD remission. In patients with post-partum onset, the course was regarded as persistent in case of disease activity lasting ≥ 6months from disease onset (10). Categorical variables were reported as frequencies and percentages while continuous variables were reported medians and interquartile range (IQR) or means and standard deviation (SD). Percentages refer to the number of patients for whom information about a specific parameter was available or inferable. The statistical software SAS (release 9.4, SAS Institute, Inc., Cary, North Carolina) was used to perform all the statistical analyses.

Quality and Risk of Bias

Two authors (GG and FD) assessed the methodological quality of the evidence and risk of bias of the included studies independently. Any disagreement was resolved through discussion with a third author (AVM).

RESULTS

Identification of Eligible Articles

As shown in the PRISMA flow diagram (**Figure 1**), the literature search identified 1976 references. After exclusion of duplicates

TABLE 1 | Demographic and clinical features of the 140 patients with pemphigoid gestationis included in the study.

			Reported patients
Age at onset, years, median (IQR)		28 (25-33)	123
Gestational age at pemphigoid gestationis onset, months, median (IQR)		7 (5-8)	108
Condition associated with pemphigoid gestationis onset, *n* (%)	Pregnancy*	119 (85)	140
	Post-partum	15 (10.7)	140
	Hydatiform mole	3 (2.1)	140
	Choriocarcinoma	3 (2.1)	140
Age at main treatment initiation, years, median (IQR)		30 (26-34)	129
Gestational age at first treatment initiation, months, median (IQR)**		7 (5-8)	89
Multigravida patients, *n* (%)		69 (52.3)	132
Primigravida patients, *n* (%)		63 (47.7)	132
Median number of pregnancies, median (IQR)		2 (1-3)	128
Recurrence in different pregnancies in multigravida patients***	Recurrent, *n* (%)	29 (41.4)	70
	Not recurrent, *n* (%)	41 (58.6)	70

IQR, interquartile range.
**One pregnancy was ectopic (tubal), while two pregnancies were achieved after ovodonation.*
***In 24 patients, first treatment was started after delivery/abortion; furthermore, patients with gestational trophoblastic disease or ectopic pregnancy (n = 7) and patients who received no treatment (n = 3) were not included in the analysis.*
****Primigravida patients (n = 63) were not included in the analysis.*

($n = 1313$) and manuscripts deemed irrelevant based on title and/or abstract screening or written in languages other than English ($n = 471$), the remaining 192 articles were reviewed in full text. Eventually, 109 articles, (96 individual case reports and 13 case series), met the eligibility criteria and were included in the qualitative synthesis (**Supplementary Table 2**). A final sample of 140 PG patients from 29 different countries was analyzed. Geographical areas of the reports were subdivided as follows: Europe ($n = 48$); North America ($n = 25$); East Asia ($n = 15$); West Asia ($n = 7$); South Asia ($n = 6$); South America ($n = 3$); Australia ($n = 3$); North Africa ($n = 1$); Southeastern Asia ($n = 1$).

Demographic and Clinical Features

Demographic and clinical data are summarized in **Table 1**. Median age at onset was 28 (IQR: 25-33) years, while the median age at main treatment initiation was 30 (IQR: 26-34) years. PG was associated at onset with pregnancy in 85% ($n = 119$) of cases, whereas it occurred during the post-partum period in 15 (10.7%) patients and in association with GTD in six (4.2%) patients. In particular, two pregnancies followed egg donation and one pregnancy was ectopic (tubal). Median gestational age at PG onset was 7 (5-8) months. Twenty-nine (41.4%) out of 70 multigravida patients with available data experienced PG recurrence in different pregnancies, while in the remaining 41 (58.6%) PG did not recur. On the other hand, 63/132 (45.7%) patients with available data were primigravida.

Treatment Strategies

Systemic corticosteroids ± topical corticosteroids and/or antihistamines were the most frequent main treatment modality ($n = 74/137$; 54%), being followed by systemic corticosteroids combined with steroid-sparing treatments ± topical corticosteroids and/or antihistamines ($n = 35/137$; 25.6%), steroid-sparing treatments ± topical corticosteroids and/or antihistamines ($n = 18/137$; 13.1%) and topical corticosteroids as monotherapy ± antihistamines ($n = 19/137$;7.3%) (**Table 2**). Three patients received no treatment. Among systemic corticosteroids, the most frequently administered was prednisone ($n = 47/109$; 43.1%), followed by prednisolone ($n = 39/109$; 35.8%), betamethasone ($n = 8/109$; 7.3%), methylprednisolone ($n = 6/109$; 5.5%), dexamethasone ($n = 3/109$; 2.8%), and fluocortolone ($n = 2$; 2.8%). The mean initial daily prednisone-equivalent dosage of systemic corticosteroids—specified in 96/115 patients treated with these agents—corresponded to 52.8 mg/day, while the mean maximum prednisone-equivalent dosage of systemic corticosteroids—specified in 93/114 patients—was 71.9 mg/day. Intravenous immunoglobulin therapy was the most commonly used steroid-sparing treatment ($n = 12/54$; 22.2%), followed by azathioprine ($n = 8/54$; 14.8%), dapsone ($n = 7/54$; 13%), cyclosporine ($n = 6/54$; 11.1%), and pyridoxine ($n = 5/54$; 9.3%). Treatments given to ≤ 3 patients included plasmapheresis/plasma exchange, minocycline, nicotinamide, immunoadsorption, surgery ± chemotherapy ± radiotherapy for choriocarcinoma, rituximab, ritodrine, doxycycline, erythromycin, cyclophosphamide, methotrexate, sulfapyridine, chemical oophorectomy with goserelin, surgical oophorectomy + hysterectomy, mycophenolate mofetil, roxithromycin. First-line treatment was initiated during pregnancy in 99/125 (79.2%) patients and after delivery/abortion in 26/125 (20.8%) patients (**Table 3**). Main treatment was started during pregnancy in 76/128 (59.4%) cases and after delivery/abortion in 52/128 (40.6%) cases. Spontaneous CR or PR was achieved without/regardless of treatment in five (3.6%) patients.

Maternal Clinical Outcomes

In the 74 patients with available data, the median follow-up period lasted 9 (IQR: 5-18.3) months (**Table 4**). Clinical outcome was available in 136 patients: among them, CR was achieved by 114 (83.8%) patients, of whom 70 (51.5%) had CR off-therapy, 14 (10.3%) had CR on-therapy and four (2.9%) had spontaneous CR. For the remaining 26 (19.1%) patients with CR, it could not be assessed whether treatment was still ongoing at the end of follow-up period. At the end of follow-up, PR was achieved by 14 (10.3%) patients, while eight (5.9%) still had active disease. Of 68/140 (48.6%) patients treated with only one treatment, 66 (97.1%) achieved CR or PR; of 64/140 (45.7%) patients treated with more than one treatment modality, 58 (90.6%) experienced CR or PR (**Table 3**). As shown in **Table 4**, a persistent course was observed in 32/135 (23.7%) cases and flares were observed in 83/137 (60.6%) patients. The latter ones were mainly linked to the post-partum/post-abortion period ($n = 42/83$; 50.6%) but were also linked to poor response to therapy, corticosteroid tapering, menstruation, hormonal

TABLE 2 | Modalities of main treatment and specific therapy of the included patients with pemphigoid gestationis.

			Reported patients
Modalities of main treatment, *n* (%)*	Systemic corticosteroids ± topical corticosteroids and/or antihistamines	74 (54)	137
	Systemic corticosteroids combined with steroid-sparing treatments ± topical corticosteroids and/or antihistamines	35 (25.6)	137
	Steroid-sparing treatments ± topical corticosteroids and/or antihistamines	18 (13.1)	137
	Topical corticosteroids as monotherapy ± antihistamines	10 (7.3)	137
Systemic corticosteroids, *n* (%)	Prednisone	47 (43.1)	109
	Prednisolone	39 (35.8)	109
	Betamethasone	8 (7.3)	109
	Methylprednisolone	6 (5.5)	109
	Dexamethasone	3 (2.8)	109
	Fluocortolone	3 (2.8)	109
	Systemic corticosteroids not specified	5 (4.6)	109
Prednisone-equivalent dose of systemic corticosteroids, mg/day (SD)	Mean initial dose	52.8 (125)	96
	Mean maximum dose	71.9 (139.6)	93
Steroid-sparing treatments, *n* (%)	Intravenous immunoglobulin therapy	12 (22.2)	54
	Azathioprine	8 (14.8)	54
	Dapsone	7 (13)	54
	Cyclosporine	6 (11.1)	54
	Pyridoxine	5 (9.3)	54
	Plasmapheresis/plasma exchange	3 (5.6)	54
	Minocycline	3 (5.6)	54
	Nicotinamide	3 (5.6)	54
	Immunoadsorption	3 (5.6)	54
	Treatment of choriocarcinoma (surgery ± chemotherapy ± radiotherapy)	2 (3.7)	54
	Rituximab	2 (3.7)	54
	Ritodrine	2 (3.7)	54
	Doxycycline	2 (3.7)	54
	Erythromycin	2 (3.7)	54
	Cyclophosphamide	2 (3.7)	54
	Methotrexate	2 (3.7)	54
	Sulfapyridine	1 (1.9)	54
	Chemical oophorectomy (goserelin)	1 (1.9)	54
	Surgical oophorectomy + hysterectomy	1 (1.9)	54
	Mycophenolate mofetil	1 (1.9)	54
	Roxithromycin	1 (1.9)	54

SD, standard deviation.

**Three patients out of 140 received no treatment.*

contraception/hormonal therapy, treatment withdrawal. Median gestational age at delivery—specified in 67 patients—was 37.5 (IQR: 35-39) weeks. Mode of delivery, reported in 60/133 pregnant women, was elective cesarean section in 25 (41.7%) patients, spontaneous vaginal delivery in 21 (35%), emergency cesarean section in eight (6%), and vaginal delivery after labor induction in seven (5.3%). Intrauterine spontaneous fetal death was observed in 8/133 (5.3%) cases, while voluntary abortion and stillbirth were observed in one patient each.

Newborn Clinical Outcomes

Of 100 live-born children with available data, 83 (83%) were healthy without skin lesions, while 13 (13%) had skin lesions (urticarial and/or bullous). Two babies born from pregnancies complicated by anhydramnios died of sepsis few days after delivery and two had severe growth retardation (**Table 4**).

Maternal Disease Outcomes Stratified by Treatment Modality

As shown in **Table 5**, CR was achieved by 59/74 (79.7%) patients who received systemic corticosteroids ± topical corticosteroids and/or antihistamines, by 30/35 (85.7%) patients who received systemic corticosteroids combined with steroid-sparing agents ± topical corticosteroids and/or antihistamines, by 12/18 (66.7%) patients who received steroid-sparing treatments ± topical corticosteroids and/or antihistamines and by 9/10 (90%) patients who received topical corticosteroids as monotherapy ± antihistamines. Flares were observed in 46/74 (62.2%) patients

TABLE 3 | Lines of therapy stratified by clinical outcome and treatment starting of the included patients with pemphigoid gestationis.

		n (%)	Reported patients
Spontaneous achievement of CR/PR without/regardless of treatment		5 (3.6)	140
Only one treatment modality	CR or PR*	66 (47.1)	140
	Active disease*	2 (1.4)	140
More than one treatment modality	CR or PR	58 (41.4)	140
	Active disease	6 (4.3)	140
First-line treatment initiation	During pregnancy**	99 (79.2)	125
	After delivery/abortion **	26 (20.8)	125
Main treatment initiation	During pregnancy**	76 (59.4)	128
	After delivery/abortion **	52 (40.6)	128

CR, complete remission; PR, partial response.
**Clinical outcome was not available for three patients who were treated with only one treatment modality.*
***Patients with gestational trophoblastic disease or ectopic pregnancy (n = 7) and patients who received no treatment (n = 3) were not included in the analysis.*

treated with systemic corticosteroids ± topical corticosteroids and/or antihistamines, in 25/35 (71.4%) patients treated with systemic corticosteroids combined with steroid-sparing treatments ± topical corticosteroids and/or antihistamines, in 10/18 (55.5%) patients treated with steroid-sparing treatments ± topical corticosteroids and/or antihistamines and in 2/10 (20%) patients treated with topical corticosteroids as monotherapy ± antihistamines.

Newborn Clinical Outcomes Stratified by Treatment Modality

Newborn outcome clinical data were available for 61 deliveries that had main treatment initiation during pregnancy. Among the 48 pregnant patients that had healthy newborns without skin lesions, main treatment consisted of systemic corticosteroids ± topical corticosteroids and/or antihistamines in 30 (62.5%) cases, topical corticosteroids as monotherapy ± antihistamines in 3 (6.25%) cases, steroid-sparing treatments ± topical corticosteroids and/or antihistamines in 6 (12.5%) cases and systemic corticosteroids combined with steroid-sparing agents ± topical corticosteroids and/or antihistamines in 9 (18.75%) cases. Among the 9 pregnant patients that had healthy newborns with skin lesions, main treatment consisted of systemic corticosteroids ± topical corticosteroids and/or antihistamines in 6 (66.7%) cases, topical corticosteroids as monotherapy ± antihistamines in 1 (11.1%) cases, steroid-sparing treatments ± topical corticosteroids and/or antihistamines in 2 (22.2%) cases. All 3 patients whose newborns developed complications such oligohydramnios or growth retardation and the only patient that experienced intrauterine fetal death had been treated with systemic corticosteroids ± topical corticosteroids and/or antihistamines.

Side Effects

Most frequently reported side effects were attributed to corticosteroids (iatrogenic Cushing syndrome [$n = 9$], steroidal diabetes [$n = 5$], arterial hypertension [$n = 3$], steroid myopathy [$n = 1$], osteoporosis [$n = 1$], vaginal bleeding [$n = 1$], mood changes [$n = 1$], striae distensae [$n = 1$], fetal macrosomia and polyhydramnios [$n = 1$]). Other infrequent side effects were ascribed to minocycline (dizziness [$n = 1$]), goserelin (flushing [$n = 1$]), intramuscular gold (proteinuria [$n = 1$]), azathioprine (elevated liver enzymes [$n = 1$]), dapsone (skin toxicity [$n = 1$], malaise [$n = 1$]), sulfapyridine (skin toxicity [$n = 1$]), cyclosporine (elevated creatinine levels [$n = 1$]), intravenous immunoglobulin (headache [$n = 1$]).

DISCUSSION

This systematic review, the first one focusing on PG, analyzed the current literature on PG therapeutic strategies and clinical outcomes, intending to provide accurate insights into its characteristics and help cope with the choice of the best treatment during pregnancy or lactation. Due to the lack of randomized or controlled trials, we collected 140 cases of PG extracted from case reports and small case series.

Bias and Quality Assessment of the Literature on Pemphigoid Gestationis Treatment

The review has been drafted based on single case reports and small case series, part of which sometimes describing clinical outcomes and treatments only succinctly and omitting drug dosages, particularly dosage per body weight of systemic corticosteroids. The limited number of studies available about this rare disease is another weakness of our systematic review. Patients with mild to moderate PG might have been missed either because they have not been reported or not even been diagnosed. Moreover, follow-up was partial or lacking in most cases. The eight studies excluded from the qualitative synthesis due to lack of detailed information about treatments and clinical outcomes have been reported in **Supplementary Table 3** and show results, albeit fragmentary, in line with those highlighted by our analysis. In the reviewed literature, neonatal outcome was almost always mentioned, even though with only occasional reports of neonatal weight or systemic complications bound to maternal treatments. Validated objective scores for measuring severity of illness and outcomes were not used in any study, thus hampering a precise comparison. Assessments on precision cannot be done due to the absence of confidence intervals. Eventually, our systematic review may be affected by publication bias: cases that did not show a good response to treatment are less likely to be published and over-reporting of severe cases may have led to an overestimation of the clinical burden of the disease.

TABLE 4 | Maternal and newborn clinical outcomes of included cases with pemphigoid gestationis.

			Reported patients
Follow-up period, months, median (IQR)		9 (5-18.3)	74
Maternal disease outcome, *n* (%)	Complete remission*	26 (19.1)	136
	Complete remission on-therapy	14 (10.3)	136
	Complete remission off-therapy	70 (51.5)	136
	Complete remission (spontaneous)	4 (2.9)	136
	Partial remission	14 (10.3)	136
	Active disease at the end of follow-up	8 (5.9)	136
Persistent course, *n* (%)		32 (23.7)	135
Flares, *n* (%)		83 (60.6)	137
Cause of flares**	After delivery/abortion/gestational trophoblastic disease remission	42 (50.6)	83
	Poor response to therapy	20 (24.1)	83
	Corticosteroid tapering	18 (21.7)	83
	Menstruation	13 (15.7)	83
	Hormonal contraception/hormonal therapy	7 (8.4)	83
	Treatment withdrawal	5 (6)	83
Gestational age at delivery, weeks, median (IQR)		37.8 (35-39)	66
Pre-term births (< 37 gestational weeks), *n* (%)		23 (34.8)	66
Mode of delivery, *n* (%)***	Elective cesarean section	25 (41.7)	60
	Spontaneous vaginal delivery	21 (35)	60
	Emergency cesarean section	8 (13.3)	60
	Vaginal delivery after labor induction	6 (10)	60
Intrauterine fetal death/ stillbirth, *n* (%)	Spontaneous abortion	7 (77.8)	9
	Voluntary abortion	1 (11.1)	9
	Stillbirth	1 (11.1)	9
Live-born children, *n* (%)***	Healthy without skin lesions****	83 (83)	100
	Healthy with skin lesions	13 (13)	100
	Postnatal death due to sepsis	2 (2)	100
	Severe growth retardation	2 (2)	100

**The concomitant status of treatment could not be concluded in 26 patients who achieved complete remission.*

***More than one cause of flare was scored in 20 patients.*

****Patients with gestational trophoblastic disease or ectopic pregnancy (n = 7) as well as intrauterine fetal deaths or stillbirths (n = 9) were not included in the analysis.*

*****One patient had a twin pregnancy.*

TABLE 5 | Clinical maternal outcomes of included patients with pemphigoid gestationis divided according to treatment modality.

Treatment modality	Complete remission, *n* (%)	Complete remission on-therapy, *n* (%)	Complete remission off-therapy, *n* (%)	Complete remission (spontaneous), *n* (%)	Partial response, *n* (%)	Active disease, *n* (%)	At least one flare during follow-up, *n* (%)
Systemic corticosteroids ± topical corticosteroids and/or antihistamines (*n* = 74)*	15 (21.1)	4 (5.6)	40 (56.3)	0	9 (12.7)	3 (4.2)	46 (65.8)
Systemic corticosteroids combined with steroid-sparing agents ± topical corticosteroids and/or antihistamines, (*n* = 35)	7 (20)	8 (22.9)	15 (42.9)	0	1 (2.9)	3 (8.6)	25 (71.4)
Steroid-sparing treatments ± topical corticosteroids and/or antihistamines, (*n* = 18)	1 (5.6)	2 (11.1)	9 (50)	1 (5.6)	3 (16.7)	2 (11.1)	10 (55.5)
Topical corticosteroids as monotherapy ± antihistamines, (*n* = 10)	3 (30)	0	6 (60)	1 (10)	0	0	2 (20)

**Data on clinical outcome of three out of 74 patients treated with systemic corticosteroids +/− topical corticosteroids and/or oral antihistamines were not available.*

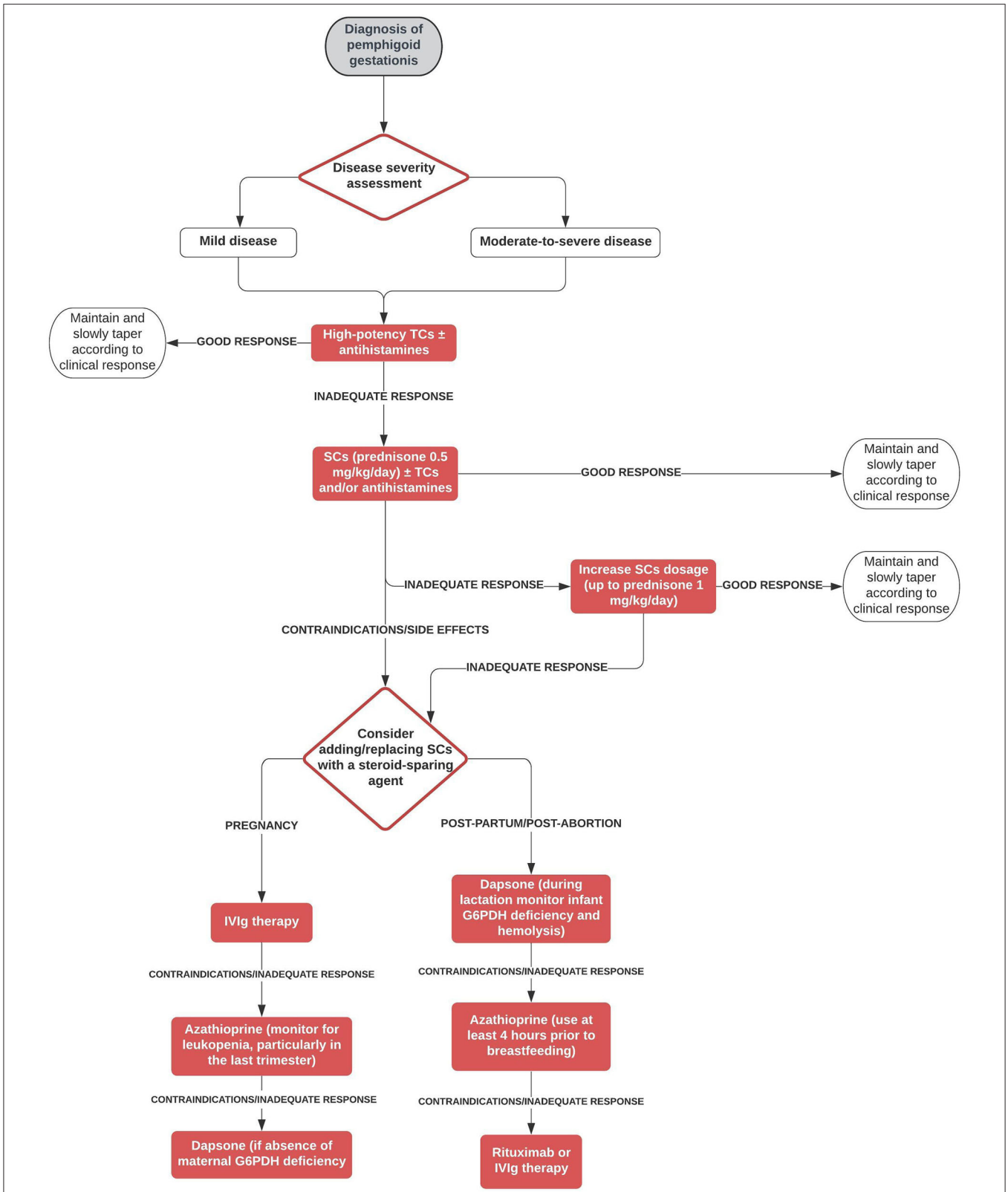

FIGURE 2 | Therapeutic algorithm for pemphigoid gestationis. G6PDH, glucose-6-phosphate dehydrogenase; IVIg, intravenous immunoglobulin; SCs, systemic corticosteroids; TCs, topical corticosteroids.

Evidence About Pemphigoid Gestationis Treatment Emerged From the Systematic Review

The most commonly used treatment modality in our cohort was a combination of systemic corticosteroids ± topical corticosteroids and/or antihistamines, with prednisone being the most frequent type of systemic corticosteroid. Mean initial prednisone-equivalent dosage of systemic corticosteroids was nearly 50 mg/day, with mean maximum dosage being approximately 70 mg/day. These clinical data reflect those by Chi et al. (10), who failed to find significant associations between adverse pregnancy outcomes and systemic corticosteroids in their retrospective study on 61 pregnancies, even after stratifying the systemic corticosteroid treatment by the length of treatment or the trimester of beginning treatment. Based on their findings, Chi et al. suggested that the benefit from systemic corticosteroids in patients with PG likely outweighs their potential harm (11). Most patients (83.8%) of our cohort achieved CR. However, a significant proportion (60.6%) of patients had at least one relapse, mainly due to post-partum exacerbation of the disease, and almost 25% of patients showed a persistent course. Interestingly, ∼40% of multigravida patients showed a recurrent course in different pregnancies. In our cohort, PG required more than one treatment modality in about 50% of patients and the main treatment was represented by a steroid-sparing agent in addition to systemic corticosteroids in about 25% of patients, thus suggesting a refractory nature of the disease in a great proportion of patients. On the other hand, only 5/140 patients achieved spontaneous remission without/regardless of treatment. Main treatment obviously tended to be started after delivery/abortion in a higher proportion of patients compared with first treatment (40.6 vs. 20.8%, respectively). Topical corticosteroids as monotherapy were prescribed only to a minority (7.3%) of patients and led to a 100% CR rate and low (20%) relapse rate. This favorable course of disease in patients treated with topical corticosteroid monotherapy may be explained by the fact that these patients presented usually with mild disease. Setting aside topical corticosteroids as monotherapy ± antihistamines, systemic corticosteroids ± topical corticosteroids and/or antihistamines was the treatment modality that led to CR in the highest proportion of patients (83%), while steroid-sparing treatments ± topical corticosteroids and/or antihistamines were associated with the highest proportion of absence of flares (45.5%). Intravenous immunoglobulin therapy was the most frequently administered steroid-sparing agent, accounting for 22.2% of the group of steroid-sparing treatments. It was successfully used also as monotherapy (12). The effectiveness and safety of intravenous immunoglobulin therapy in treating pregnant patients with autoimmune bullous diseases have already been demonstrated by Ahmed et al. (13). in a group of patients with pemphigus vulgaris. Azathioprine and dapsone were the second and third most commonly prescribed steroid-sparing medications, always given after delivery/abortion. On the other hand, cyclosporine, which was the fourth most frequently administered steroid-sparing drug, was successfully used also during pregnancy (14, 15). Interestingly, rituximab was used in two persistent cases either during pregnancy to prevent disease flare (16) and after delivery (17). As previously observed in pemphigus, rituximab administered 6–12 months before conception may help prevent disease worsening and achieve stable disease during pregnancy (18, 19). The frequent reports of refractory/persistent cases make the definition of therapeutic approaches for PG an unmet clinical need. Therefore, we propose a decisional algorithm on the basis of both the evidence derived from this systematic review merged with current knowledge available of bullous pemphigoid (9), a subepidermal autoimmune blistering diseases that shares some clinical features with PG (**Figure 2**).

Safety concerns during pregnancy and lactation have also taken into consideration. In analogy to bullous pemphigoid, high potency topical corticosteroids are proposed as first-choice treatment both in mild disease and in moderate-to-severe PG. Non-fluorinated steroids such as hydrocortisone and prednisolone should be preferred respect to fluorinated steroids such as dexamethasone, methylprednisolone, and betamethasone that are metabolized less extensively by 11β-hydroxysteroid dehydrogenase and easily cross the placental barrier (5). In refractory disease, systemic corticosteroids may be attempted. Even there is proven evidence, oral antihistamines can be used for itch management. In case of failure/contraindication/side effects of systemic corticosteroids, we suggest to consider during pregnancy intravenous immunoglobulin therapy and, as second step, conventional immunosuppressants/immunomodulating agents such as dapsone or azathioprine, which are the last choice, which can be used safely during pregnancy as inferred from many studies involving patients with other diseases treated with these agents (20–23). In the post-partum/post-abortion period, we suggest as first step treatment immunosuppressant/immunomodulating agents, including dapsone and azathioprine, leaving as second choices IVIg and rituximab due to pharmacoeconomic reasons. As far as concerns pregnancy/newborn outcomes, almost 35% of patients experienced a pre-term delivery and elective cesarean section was the most common mode of delivery, being performed in 43% of patients with available data. Fetal distress, placental insufficiency or poor control of the disease may be possible explanations of the latter finding. Among live-born children, only 13 (13%) had skin lesions suggestive of neonatal pemphigoid. Eventually, intrauterine fetal death was observed only in eight cases.

In conclusion, high-potency topical corticosteroids may be recommended as first-line agents either in mild and in moderate-to-severe PG. Systemic corticosteroids may be a useful approach for recalcitrant disease. Intravenous immunoglobulin therapy alone or in combination with systemic corticosteroids may be considered as further line of treatment. Although most patients achieve CR, a considerable proportion of them experience refractory/persistent disease requiring multiple lines of therapy. Randomized controlled trials are needed to better define the effectiveness of different treatments in PG.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GG: study design, database management, search strategies, and writing of the manuscript. FD: database management, search strategies, and writing of the manuscript. CM: search strategies. EB, SM, and AC: critical review of the manuscript. AM: study design and critical review of the manuscript. All authors listed have made a substantial contribution to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.604945/full#supplementary-material

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Genetic Susceptibility to Antisynthetase Syndrome Associated With Single-Nucleotide Variants in the *IL1B* Gene That Lead Variation in IL-1β Serum Levels

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The antisynthetase syndrome (ASSD) is an autoimmune disorder characterized by myositis, arthritis, mechanic's hands, fever, Raynaud phenomenon, and interstitial lung disease (ILD). We aimed to evaluate single-nucleotide polymorphisms in the *interleukin 1B* (*IL1B*) gene and their association between ILD with antisynthetase autoantibodies, as well as IL-1β serum levels. The most frequent antisynthetase autoantibody was anti-Jo1. The most frequent tomographic pattern was non-specific interstitial pneumonia, whereas in the anti-Jo1 subjects, it was organized pneumonia. Anti-Jo1 patients tend to have more significant arthritis, and Raynaud phenomenon have higher levels of creatinine phosphokinase. In the *IL1B* gene, the GG genotype and G allele of rs1143634 [odds ratio (OR) = 2.21 and OR = 2.60, respectively, $p < 0.05$] are associated with an increased risk, as well as with the dominant and recessive models ($p < 0.05$). This finding is maintained after logistic regression analysis adjusting for potential confounding variables ($p < 0.05$). Subjects with the rs16944/AG heterozygous genotype had higher serum levels of IL-1β compared to homozygous ($p < 0.05$). In conclusion, rs1143634 is associated with a higher risk of ASSD. Also, the GA genotype is associated with higher levels of IL-1β in ASSD patients.

Keywords: antisynthetase syndrome, IL1B gene, IL1-beta, SNP, anti-Jo1, genetic association

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INTRODUCTION

The antisynthetase syndrome (ASSD) is an autoimmune disorder characterized by diverse clinical manifestations, including myositis, arthritis, mechanic's hands, fever, Raynaud phenomenon, and interstitial lung disease (ILD), as well as the presence of aminoacyl-transfer RNA synthase (ARS) autoantibodies (1, 2). The ASSD was firstly associated with idiopathic inflammatory myopathies (IIMs); however, previously, it has been described that many of these patients

may have only slight myositis clinical manifestations and not fulfill with the Bohan and Peter criteria for an inflammatory myopathy (3–5).

The ARS autoantibodies include anti-Jo1 (anti-histidyl), anti-EJ (anti-glycyl), anti-OJ (anti-isoleucyl), anti-PL7 (anti-threonyl), anti-PL12 (anti-alanyl), anti-SC (anti-lysil), anti-KS (anti-asparaginyl), anti-JS (anti-glutaminyl), anti-Ha or anti-YRS (anti-threonyl), anti-tryptophanyl, and anti-Zo (anti-phenylalanyl), with anti-Jo1 being the most common (6). Differences have been described according to the different ARS autoantibodies. For example, PL7 and PL12 are associated with early and severe ILD (7, 8). Also, anti-Jo1 is associated with more muscle involvement and better prognosis (9, 10).

However, despite the clinical characteristics of ASSD have been widely described and are a topic of intensive research, little is known about this nosological entity's genetic background. Previous reports of single-nucleotide polymorphisms (SNPs) in proinflammatory genes associated with IIM, such as *PTPN22*, *PLCL1*, and *TNF*, have been described (11–13). However, despite that interleukin 1β (IL-1β) has been associated with other autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), the association between SNPs in the *IL1B* gene and IIM has been poorly described (14, 15). There is only one previous report of *IL1RN* VNTR polymorphism associated with dermatomyositis (DM) in a Bulgarian population (16). On the other hand, studies related to ILD with ARS autoantibodies are relatively scarce. We aimed to evaluate SNPs in *IL1B*, IL-1β serum levels, and their association between ILD with ARS autoantibodies.

MATERIALS AND METHODS

Subjects Included

Case Group

One hundred fifty-four patients with ASSD diagnosis were included. All of them were evaluated and managed in the Interstitial Lung Disease and Rheumatology Unit (ILD&RU) at the Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas (INER) in México City. Patients in the ILD&RU are evaluated by a multidisciplinary group (pulmonologists, radiologists, and rheumatologists). Included patients were ≥18 years old, born as Mexican mestizos (MMs), no biological relation among themselves or with the patients or controls, with the diagnosis of ILD confirmed by high-resolution computed tomography (HRCT) and be positive to at least one of the following autoantibodies: anti-Jo1, anti-PL7, anti-PL12, and anti-Ej (subjects positive only for anti-OJ antibody were excluded). According to the manufacturer's instructions, all autoantibodies were measured by the Myositis Profile 3 immunoblot 16 strips EUROLINE panel (EUROIMMUN AG, Lübeck, Germany). Also, we included baseline pulmonary function tests, such as single-breath carbon monoxide diffusing capacity (DLco) and spirometry. Furthermore, baseline serum creatinine phosphokinase (CPK) levels were recorded, as well as the history of Raynaud phenomenon, arthritis, mechanic's hands, fever, and smoking history. Patients were evaluated between January 2008 and January 2019. Besides, the case group was divided into anti-Jo1 and non–anti-Jo1 patients for further analyses.

Control Group

A group of five hundred six healthy volunteer subjects (HS) was also included. These subjects had the following characteristics: clinically healthy (with neither chronic nor acute diseases self-reported), ≥18 years old, men and women, born as MMs (no biological relation among themselves or with the patients or controls), and no history of family pulmonary or inflammatory/autoimmune diseases. All subjects had at least three generations born in Mexico (parents and grandparents) and were considered MMs. We have previously demonstrated that this criterion is a good proxy of Mexican ancestry evaluated by ancestry-informative markers (17). All participants underwent a background questionnaire of inherited pathologies, excluding subjects who reported suffering from some lung and chronic inflammatory disease.

Ethics Approval and Informed Consent

The Institutional Committees for Research, Ethics in Research, and Biosecurity of the INER approved this study (approval code numbers: C08-17, B11-19). All participants were previously invited to participate in the protocol; they signed a written informed consent document and provided with a privacy statement describing personal data protection.

All experiments were performed following the relevant guidelines and regulations. The STREGA (STrengthening the REporting of Genetic Association) guidelines were considered to design this genetic association study (18).

DNA Extraction

The DNA was extracted from peripheral blood cells via venipuncture using EDTA tubes from all 668 subjects' using the commercial BDtract Genomic DNA isolation kit (Maxim Biotech, San Francisco, CA, USA). The DNA was quantified by UV absorption spectrophotometry at the 260-nm wavelength employing a NanoDrop 2000 device (Thermo Scientific, Wilmington, DE, USA). Contamination with organic compounds and proteins was determined by measuring the ratio absorbance at 280 and 260 nm. Samples were considered of good quality when the ratio was ∼1.8.

SNP Selection

SNPs were selected based on a bibliographic search in PubMed (NCBI), identifying polymorphisms in *IL1B* previously associated with IIM and other autoimmune diseases, such as RA and SLE. Additionally, according to the HapMap project, we considered a minor allelic frequency (MAF) higher than 5% in the Mexican population in Los Angeles. **Table 1** summarizes the principal characteristics of the evaluated SNPs.

SNP Genotyping

The SNPs' allele discrimination was performed using commercial TaqMan probes (Applied Biosystems, San Francisco, CA, USA) at a concentration of 20× in total subjects included. SNPs evaluated were rs1143634, rs16944, and rs1143623 in the *IL1B*

TABLE 1 | Characteristics of SNPs evaluated.

SNP	Position (pb)	*HWE*	*p*-value	MAF	Alleles
rs1143634	112832813	2.9855×10^{13}	0.0012	0.09	G:A
rs16944	112837290	0.3876	0.3136	0.36	A:G
rs1143623	112838252	0.6825	0.0893	0.4	G:C

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

gene, using quantitative polymerase chain reaction (qPCR) in a 7300 Real-Time PCR System (Applied Biosystems/Thermo Fisher Scientific Inc., Singapore), and the analysis performed by sequence detection software version 1.4 software (Applied Biosystems, CA, USA). Also, three controls without template (contamination controls) included each genotyping plate, and 5% of the genotyped in duplicate as controls for allele assignment.

Besides, to determine the haplotype structure in the *IL1B* gene associated with ASSD susceptibility, we applied Haploview software version 4.2.

Measurement of IL-1β Serum Levels

Once performing the association analyses, we selected sera samples from 62 ASSD patients, carrying genotypes of the three SNPs evaluated. Sample selection was performed to identify representability for each genotype from all SNPs. For the rs1143634, we selected 58 with GG and 4 with GA genotypes. For the rs16944 group, we included 33 with AA, 25 with AG, and 4 with GG genotypes. For the rs1143623, we chose 5 with CC, 28 with CG, and 29 with GG genotypes. Differences in the patients' number, according to genotypes, are due to serum samples availability and the genotype frequencies. The serum levels were measured by the enzyme-linked immunosorbent assay (ELISA) technique; the detectable range of IL-1β was 14.06–900 pg/mL (human IL-1 beta ELISA Kit, ab214025, Abcam Plc, Cambridge, UK). A total of 50 μL of serum for each sample was centrifuged and prepared for analysis, following the manufacturer's protocol.

Statistical Analysis

The differences between groups were assessed by determining and comparing the allele and genotype frequencies. The statistical significance was assessed using SPSS v20.0 (SPSS Inc., Chicago, IL, USA) and Epi Info 7.1.4.0 statistical software (19). The allele and genotype frequencies between groups were analyzed using the χ^2-test. The results were considered to be significant when $p < 0.05$; similarly, the odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to determine the strength of the association. Comparisons made between ASSD and HS are shown. Also, the ASSD patients are divided into anti-Jo1+ and non–anti-Jo1.

A logistic regression analysis was performed to adjust for potential confounding variables [sex, age, and body mass index (BMI)] using Plink v. 1.07.

RESULTS

Demographic Variables in Case and Control Groups

One hundred fifty-four patients with at least one ARS autoantibody and ILD diagnosed by HRCT were included, and 506 healthy subjects as the control group. The ASSD group was older and predominantly women compared with the HS group ($p < 0.05$). There were no significant differences in BMI.

Furthermore, 37% of the ASSD patients are smokers with 21 years of smoking, 5 cigarettes per day, and 6 pack-years' history. The most frequent clinical manifestation was arthritis (70.13%), followed by mechanic's hands, fever, and Raynaud phenomenon. Also, the most frequent ARS autoantibody was anti-Jo1 (43.51%), and the most frequent HRCT pattern was non-specific interstitial pneumonia (NSIP) (45.70%). **Table 2** shows the complete results.

Demographic Variables in Anti-Jo1 and Non–Anti-Jo1 Groups

Besides, we divided the case group into anti-Jo1 and non–anti-Jo1, comparing them to each other. Sixty-seven patients were included in the anti-Jo1 group, whereas 87 were included in the non–anti-Jo1 group. Patients included in the non–anti-Jo1 group were older than the anti-Jo1 subjects ($p < 0.05$). We did not find statistically significant differences between groups comparing sex, BMI, smoking status, pulmonary function (FVC-Pb and DLco), and clinical manifestations, such as mechanic's hands and fever and Raynaud phenomenon ($p > 0.05$). However, those subjects in the anti-Jo1 group tended to present more arthritis ($p = 0.075$). Also, anti-Jo1 patients present the most significant muscle involvement, represented by higher levels of CPK ($p = 0.001$). Interestingly, the most frequent HRCT pattern in the anti-Jo1 group was organized pneumonia (OP) (42.62 vs. 18.98%, $p = 0.002$), whereas in the non–anti-Jo1 group, it was NSIP (54.43 vs. 34.43%, $p = 0.018$). Complete results are shown in **Table 2**.

Allele and Genotype Frequencies

We evaluated three SNPs (rs1143623, 1143634, and rs16944) in the *IL1B*. **Table 3** shows the full genetic association results.

Case and Control Groups

In the HS group, we genotyped 496 HS for rs1143634, 506 for rs16944, and 501 for rs1143623. We did not find statistically significant differences with allele and genotype frequencies between case and control group comparison for the rs1143623 and rs16944, and neither with dominant nor recessive models ($p > 0.05$). However, for the rs1143634, we found significant association with an increased risk of ASSD with G allele ($p = 0.001$, OR = 2.60, 95% CI = 1.44–4.70), which are maintained after Bonferroni correction ($p = 0.003$); in contrast, the A allele offered a reduced risk ($p = 0.001$, OR = 0.38, 95% CI = 0.21–0.70) for ASSD.

Regarding genotype frequencies, we found a statistically significant association ($p = 0.015$) with a reduced risk of ASSD with AA genotype (OR = 0.13, 95% CI = 0.02–0.96). Conversely, we found a significant association ($p = 0.013$) with

TABLE 2 | Demographic and clinical variables from ASSD and HS groups and among ASSD anti-Jo1 and non–anti-Jo1.

Variables	ASSD	HS	*p*-value	Anti-Jo1	Non–anti-Jo1	*p*-value
	(*n* = 154)	(*n* = 506)		(*n* = 67)	(*n* = 87)	
Age (years)	57 (27–83)	45 (26–81)	<0.001	54 (41–73)	57 (38–75)	0.013
Sex, female (%)	109 (70.78)	216 (42.5)	<0.001	47 (70.15)	62 (71.26)	0.880
BMI	26.9 (13.3–45.5)	27.51 (15.82–52.03)	0.954	27.3 (23–35.9)	26.9 (15.6–39.4)	0.804
Smoking status						
Smoker, yes (%)	57 (37.01)			22 (32.84)	35 (40.23)	0.346
Years of smoking	21 (1–63)			18 (1–45)	23 (2–63)	0.431
Cigarettes per day	5 (1–40)			10 (1–40)	5 (1–20)	0.310
Tobacco index	6 (0.5–56)			7.1 (0.5–56)	4.6 (0.35–44)	0.446
Pulmonary function						
FVC % pre-bd	59 (32–114)			57 (32–114)	59 (33–109)	0.199
DLco	47 (10–110)			48 (10–102)	47 (12–110)	0.342
Clinical manifestations						
Arthritis	108 (70.13)			52 (77.61)	56 (64.37)	0.075
Mechanic's hands	82 (53.25)			40 (59.70)	42 (48.28)	0.159
Fever	76 (49.35)			37 (55.22)	39 (44.83)	0.201
Raynaud phenomenon	69 (44.81)			35 (52.24)	34 (39.08)	0.106
CPK	81 (18–7,210)			196.5 (24–7,210)	71 (18–5,619)	0.001
Autoantibodies						
Anti-Jo1 (%)	67 (43.51)					
Anti-PL12 (%)	55 (35.71)					
Anti-PL7 (%)	35 (22.73)					
Anti-EJ (%)	19 (12.34)					
TAC	*n* = 140			*n* = 61	*n* = 79	
NSIP (%)	64 (45.70)			21 (34.43)	43 (54.43)	0.018
OP (%)	41 (29.29)			26 (42.62)	15 (18.98)	0.002
UIP (%)	23 (16.43)			6 (9.84)	17 (21.52)	0.064
LIP (%)	6 (4.29)			3 (4.92)	3 (3.80)	0.745
No class (%)	4 (2.86)			3 (4.92)	1 (1.27)	0.414
Br-ILD (%)	2 (1.43)			2 (3.28)	0	N/A

ASSD, antisynthetase syndrome; HS, healthy subjects; BMI, body mass index; CPK, creatine phosphokinase; DLco, single-breath carbon monoxide diffusing capacity; FVC, forced vital capacity; ILD, interstitial lung disease; LIP, lymphoid interstitial pneumonia; Br-ILD, bronchiolitis-associated interstitial lung disease. NSIP, non-specific interstitial pneumonia; OP, organized pneumonia; UIP, usual interstitial pneumonia. All values are expressed as median and minimum-maximum values. We used the Mann–Whitney U-test and Fisher exact test to make comparisons between groups. p < 0.05 was considered as significant.

a higher risk of ASSD with GG genotype (OR = 2.21, 95% CI = 1.17–4.17). In addition, in the dominant model, we found significant association ($p = 0.013$) with a reduced risk with AG + AA genotypes (OR = 0.45, 95% CI 0.24–0.70) and higher risk with GG genotype (OR = 2.21, 95% CI = 1.17–4.17). Additionally, in the recessive model, we also found a significant association ($p = 0.015$) with reduced risk with AA genotype (OR = 0.13, 95% CI = 0.02–0.96) and higher risk with GG + GA genotypes (OR = 7.78, 95% CI = 1.04–57.99), these associations are maintained after Bonferroni correction ($p = 0.045$). The complete results' list can be consulted in detail in **Table 3**.

Logistic Regression Analysis

Logistic regression was performed to adjust for possible confounding covariables. For rs1143634/A allele, we found statistically significant differences comparing ASSD vs. HS in additive model, suggesting a reduced risk of ASSD ($p = 0.027$, OR = 0.46, 95% CI = 0.23–0.92) and adjusting for sex ($p = 1.21E-07$) and age ($p = 1.79E-14$). Additionally, we found significant differences in adjusting for sex and age for the same comparison with rs16944/G and rs1143623/C ($p < 0.05$). The results are shown in **Supplementary Table 1**.

Anti-Jo1+ and Non–Anti-Jo1 Groups

Regarding allele frequencies, we did not find statistically significant differences between the three SNPs evaluated. Besides, we did not find significant association when we compared genotype frequencies and neither with dominant and recessive genetic association models ($p > 0.05$). Complete results are shown in **Table 4**.

Haplotype Analysis

The haplotype analysis was carried out to determine its association with ASSD susceptibility. The analysis

TABLE 3 | Allele and genotype frequencies and genetic models of *IL1B* SNPs in case and control groups.

Model	ASSD		HS		*p*-value	*p*-value Bonferroni correction	OR	(95% CI)
	n = 154	*F* (%)	*n* = 496	*F* (%)				
rs1143634								
Genotypes								
GG	142	92.21	418	84.27	0.013	0.039	2.21	1.17–4.17
GA	11	7.14	54	10.89	0.18	NA	0.63	0.32–1.24
AA	1	0.65	24	4.84	0.015	0.045	0.13	0.02–0.96
Alleles								
G	295	95.78	890	89.72	0.001	0.003	2.60	1.44–4.70
A	13	4.22	102	10.28			0.38	0.21–0.70
Dominant								
GG	142	92.21	418	84.27	0.013	0.039	2.21	1.17–4.17
AG + AA	12	7.79	78	15.73			0.45	0.24–0.86
Recessive								
GG + GA	153	99.35	472	95.16	0.015	0.045	7.78	1.04–57.99
AA	1	0.65	24	4.84			0.13	0.02–0.96
rs16944								
Genotypes			*n* = 506					
AA	69	44.81	197	38.93	0.19	NA	1.27	0.88–1.83
AG	68	44.16	248	49.01	0.29		0.82	0.57–1.18
GG	17	11.04	61	12.06	0.73		0.91	0.51–1.60
Alleles								
A	206	66.88	642	63.44	0.27	NA	1.16	0.89–1.52
G	102	33.12	370	36.56			0.86	0.66–1.13
Dominant								
AA	69	44.81	197	38.93	0.19	NA	1.27	0.88–1.83
GA + GG	85	55.19	309	61.07			0.79	0.55–1.13
Recessive								
AA + GA	137	88.96	445	87.94	0.73	NA	1.10	0.62–1.95
GG	17	11.04	61	12.06			0.91	0.51–1.60
rs1143623								
Genotypes			*n* = 501					
GG	62	40.26	171	34.13	0.16	NA	1.30	0.90–1.89
CG	73	47.40	246	49.1	0.71		0.93	0.65–1.34
CC	19	12.34	84	16.77	0.19		0.70	0.41–1.19
Alleles								
G	197	63.96	588	58.68	0.10	NA	1.25	0.96–1.63
C	111	36.04	414	41.32			0.84	0.61–1.04
Dominant								
GG	62	40.26	171	34.13	0.16	NA	1.30	0.90–1.89
CG + CC	92	59.74	330	65.87			0.77	0.53–1.11
Recessive								
GG + CG	135	87.66	417	83.23	0.19	NA	1.43	0.84–2.44
CC	19	12.34	84	16.77			0.70	0.41–1.19

ASSD, antisynthetase syndrome; HS, healthy subjects. NA, not applied. p < 0.05 was considered as significant and was corrected by the Bonferroni test.

included the three SNPs in the *IL1B*, comparing ASSD vs. subjects in the control group. One of these polymorphisms evaluated (rs1143634) did not meet the Hardy–Weinberg equilibrium ($p < 0.05$). Haplotypes and their frequencies are summarized in **Figure 1**. The block shows that the haplotype shaped by rs16944 and rs1143623 is in high linkage disequilibrium (LD, $r^2 = 98$). Moreover, according to the frequencies, GAG (conformed by all common alleles) haplotype is associated with a higher risk of ASSD ($p = 0.022$, OR = 1.37, 95% CI = 1.05–1.75). On the other hand, AGC

TABLE 4 | Allele and genotype frequencies and genetic models of *IL1B* SNPs among the case group.

Model	Anti-Jo1		Non–anti-Jo1		*p*-value	OR	95% CI
	n = 67	*F* (%)	*n* = 87	*F* (%)			
rs1143634							
Genotypes							
GG	64	95.52	78	89.66	0.23	2.46	0.64–9.47
GA	3	4.48	8	9.20	0.35	0.46	0.12–1.82
AA	0	0	1	1.15	NA		
Alleles							
G	131	97.76	164	94.25	0.16	2.66	0.71–9.87
A	3	2.24	10	5.75		0.38	0.10–1.39
Dominant							
GG	64	95.52	78	89.66	0.23	2.46	0.64–9.47
GA + AA	3	4.48	9	10.34		0.41	0.11–1.56
Recessive							
GG + GA	67	100	86	98.85	NA		
AA	0	0	1	1.15			
rs16944							
Genotypes							
AA	32	47.76	37	42.53	0.52	1.24	0.65–2.34
AG	28	41.79	40	45.98	0.60	0.84	0.44–1.60
GG	7	10.45	10	11.49	0.84	0.90	0.32–2.50
Alleles							
A	92	68.66	114	65.52	0.56	1.15	0.71–1.86
G	42	31.34	60	34.48		0.87	0.54–1.40
Dominant							
AA	32	47.76	37	42.53	0.52	1.24	0.65–2.34
AG + GG	35	52.24	50	57.47		0.81	0.43–1.54
Recessive							
AA + AG	60	89.55	77	89	0.84	1.11	0.40–3.10
GG	7	10.45	10	11		0.90	0.32–2.50
rs1143623							
Genotypes							
GG	27	40.30	35	40.23	0.99	1.02	0.63–1.63
GC	32	47.76	41	47.13	0.94	1.03	0.54–1.94
CC	8	11.94	11	12.64	0.90	0.94	0.35–2.48
Alleles							
G	86	64.18	111	63.79	0.94	1.02	0.63–1.63
C	48	35.82	63	36.21		0.98	0.62–1.57
Dominant							
GG	27	40.30	35	40.23	0.99	1	0.52–1.92
GC + CC	40	59.70	52	59.77		1	0.52–1.91
Recessive							
GG + GC	59	88.06	76	87.36	0.90	1.07	0.40–2.82
CC	8	11.94	11	12.64		0.94	0.35–2.48

NA, not applied; SNP, single-nucleotide polymorphism.

(conformed by all minor alleles) and AAG (conformed by one minor allele and two common alleles) haplotypes are associated with reduced risk of ASSD ($p = 0.028$, OR = 0.49, 95% CI = 0.26–0.95; $p = 0.012$, OR = 0.110, 95% CI = 0.01–0.81).

IL-1β Serum Levels

Sixty-two serum samples from the ASSD group were selected, carrying genotypes of the three SNPs evaluated. We did not find significant differences between serum levels of IL-1β in rs1143623 and rs1143634. On the other hand, AG genotype (63.24 pg/mL) from rs16944 showed higher levels of IL-1β than homozygous genotypes [AA (43.61 pg/mL) and GG (35.84 pg/mL)], being statistically significant ($p = 0.049$). **Figure 2** shows these results.

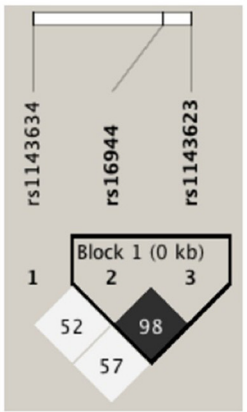
DISCUSSION

The ASSD is a rare systemic connective tissue disease, which usually affects joints, skin, muscles, and lungs. The clinical characteristics of the ASSD group showed that the median age was 57 years, and 70.6% were female. Also, arthritis was the most frequent clinical manifestation, whereas the most frequent ARS autoantibody was anti-Jo1 (41.36%). Also, NSIP was the most frequent tomographic pattern. These findings agree with two previous reports of our research group (1, 5).

Clinical manifestations can differ according to the different ARS autoantibodies. Pinal-Fernández et al. (10) showed that those anti-Jo1+ patients have more arthritis, muscle weakness, and Raynaud phenomenon. Rojas-Serrano et al. (6) described that anti-Jo1 patients had more arthritis, proximal muscle weakness, and higher levels of CPK than those non–anti-Jo1. These findings are similar to our current report and supported by the results shown in the largest ASSD patients cohort confirmed by the American and European NEtwork of Antisynthetase Syndrome collaborative group (20). They included 828 patients from 10 countries and 63 hospitals and found that anti-Jo1 was the most frequent ARS autoantibody, and these subgroups of patients had more muscle and articular involvement. These findings suggest that anti-Jo1 ARS could be associated with multiorgan involvement, whereas non–anti-Jo1 ARSs are mainly lung limited.

Interestingly, the most frequent tomographic pattern in anti-Jo1 patients was OP, whereas in non–anti-Jo1 patients, it was NSIP. These two tomographic patterns have been widely described as the most prevalent in the ASSD (6, 21), with inconsistent frequencies among studied populations. For example, in a previous study of our research group, we reported that in our ASSD cohort, the most frequent HRCT pattern was OP, followed by NSIP (1). Conversely, other studies have shown that NSIP is the most frequent tomographic pattern (7, 9, 22). This finding could be due to differences in the sample size, different inclusion criteria, and distinct population characteristics.

For most of the rheumatic diseases, a genetic susceptibility component has been established. SNPs located in proinflammatory gene cytokines have been associated with an important number of these autoimmune diseases. One of the most studied genes is *IL1B*, which has been associated in previous studies with RA and SLE (23–25). However, only a few studies are trying to find genetic associations between single-nucleotide



Haplotypes	ASSD (%)	HS (%)	*p*-value	OR	95% CI
GAG	**63.2**	**55.8**	**0.022**	**1.37**	**1.05-1.78**
GGC	29.2	29.1	0.955	1.01	0.76-1.33
AGC	**3.5**	**7.0**	**0.028**	**0.49**	**0.26-0.95**
GAC	3	4.5	0.266	0.64	0.31-1.32
AAG	**0.4**	**2.9**	**0.012**	**0.110**	**0.01-0.81**

FIGURE 1 | Haplotype analysis. ASSD, antisynthetase syndrome; HS, healthy subjects.

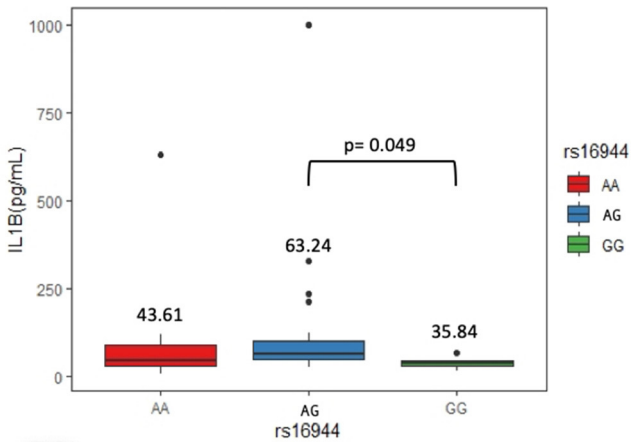


FIGURE 2 | IL-1β serum levels between genotypes of rs61944 in the ASSD group.

variations and ASSD, with controversial results. Our analysis found some associations with rs1143634, being the first time that these associations in ASSD patients in an MM population are described. However, in a previous study by Beretta et al. (26) described that rs1143634 is associated with a more restrictive ILD pattern in patients with systemic sclerosis (SSc) in an Italian population. Although we found an association with a reduced risk of ASSD with the minor allele of rs1143634, this finding could suggest an indirect association with genetic variants that were not considered in this study, as Balding says in his report for candidate polymorphisms (27).

In contrast, Sugiura et al. (28), in a Japanese population, described that *STAT4* rs7574865 was associated with a higher risk of DM/polymyositis (PM) but not with the presence of ILD in this group of patients. This finding is shared with SSc, whereas STAT4 rs7574865 is associated with a higher risk of SSc and is also associated with reduced risk of SSc-related ILD in a Caucasian population (29).

Also, in a Chinese Han population, Chen et al. (30, 31) found two SNPs in *ETS1* (rs7117932 and rs6590330) and one SNP in *CCL21* (rs951005) associated with a higher risk of DM/PM and with ILD related to IIM. Conversely, the same research group found a decreased frequency of minor allele rs7731626-A (*ANKRD55*) in DM-ILD and DM/PM-ILD patients, suggesting that the A variant may be protective against DM/PM-ILD (32). Even though we did not evaluate the same SNP, this finding agrees with our results because we also found that rs1143634 minor allele could play a protective role in ASSD.

IL1B polymorphisms have also been evaluated in other ILDs because of its profibrotic activity inducing fibroblast proliferation via platelet-derived growth factor (33). Volobaev et al. (34) showed in their study that an SNP in *IL1B* (rs16944) was associated with a higher risk of anthracosilicosis in coal miners in Russia. Also, it has been described that WNT/β-catenin signaling induces IL-1β expression by alveolar epithelial cells in a murine model of pulmonary fibrosis and an up-regulation of IL-1β in bronchoalveolar lavage fluid (BALF) in bleomycin-induced lung fibrosis *in vivo* (35). These findings suggest that IL-1β could play an essential role in several ILDs because of its proinflammatory and profibrotic properties.

In our knowledge, this is the first time that ASSD patients are divided into two subgroups (anti-Jo1 and non–anti-Jo1) and compared, taking into consideration the different autoantibodies to make allele and genotype associations from SNPs. These analyses were conducted because of the clinical and prognostic differences between the autoantibodies spectrum reported by different cohorts (6, 8, 20).

To the best of our knowledge, we described for the first time three haplotypes associated with higher and reduced risk of ASSD, even in IIM. However, haplotypes in the *IL1B* gene have previously been described in other inflammatory diseases such as osteoarthritis, RA, and SLE (15, 36, 37). Although one of the three SNPs does not meet the Hardy–Weinberg equilibrium, it has been previously described that for mestizo populations, this criterion is not always necessary to establish genetic associations because of the recombination events. The MM population has been previously described as a rich genetic variability product

of many years of genetic recombinations between ancestral populations (Amerindian), Caucasian, and African descendants (38, 39).

Our study found that the AG genotype of rs16944 is associated with higher levels of IL-1β in ASSD patients. Several studies have identified higher levels of IL-1β in patients with diverse rheumatic and ILDs. For example, serum levels of IL-1β in idiopathic pulmonary fibrosis patients were significantly increased compared to healthy controls, as well as in BALF (40). Also, a higher expression of IL-1α, IL-1β, and transforming growth factor β in muscle tissue has been previously reported in patients with IIM (41). Previous reports have established that genetic variants in the *IL1B* gene may contribute to differences in the expression, translation, and secretion of IL-1β. Hall et al. (42) demonstrated that rs16944 promoter polymorphism could induce a greater affinity of the transcriptional factors, promoting a higher expression of IL-1β, which concurs with our results. Iglesias-Molli et al. (43) showed that the rs16944 heterozygous genotype is associated with changes in mRNA expression of IL-1β in patients with type 2 diabetes, and this is in agreement with previous publications where an IL-1β haplotype in its promoter region was associated with increased IL-1β mRNA (44).

This study is not free of limitations. First, we evaluated only three SNPs in the *IL1B* gene, and one of them did not meet the Hardy–Weinberg equilibrium. Second, we did not include a control group of subjects with IIM without ILD because our center attends only to those with pulmonary involvement. Additionally, we only were able to measure the IL-1β in only a single part of our cohort. However, we have one of the largest sample sizes for genetic association in ASSD. Also, we made an intracase analysis to evaluate the relationship between the SNPs and the different ARS autoantibodies.

In conclusion, rs1143634/G is associated with a higher risk of ASSD. Also, rs61944/GG genotype has a higher frequency in those patients positive for the anti-Jo1 autoantibody, and the GA genotype is associated with higher levels of IL-1β in ASSD patients. Three haplotypes are associated with ASSD; two of them (AGC and AAG) are associated with reduced risk of ASSD, whereas one is associated with higher risk. More studies are required to elucidate the role of proinflammatory cytokines in the disease and to offer new therapeutic targets for these patients.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: ClinVar system, VCV000869137, VCV000869138, and VCV000869139; https://www.ncbi.nlm.nih.gov/clinvar/?term=%22HLA%20Laboratory%2C%20Instituto%20Nacional%20de%20Enfermedades%20Respiratorias%20Ismael%20Cosio%20Villegas%22[submitter]+AND+%22IL1B%22[gene].

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Committees for Research, Ethics in Research, and Biosecurity of the Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas (INER) approved this study (approval code numbers: C08-17, B11-19). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ER-M, JR-S, and RF-V: conceptualization. KN-Q and MG-P: data curation. MP-G and AG-C: formal analysis. IB-R: funding acquisition. ER-M, AG-C, KN-Q, EA-O, and MG-P: investigation. ER-M, AG-C, KN-Q, and EA-O: methodology. JR-S: project administration. MM, MG-P, IB-R, and JR-S: resources. MM, EA-O, and IB-R: software. MM, GP-R, IB-R, JR-S, and RF-V: supervision. MM and GP-R: validation. MM, GP-R, and JR-S: visualization. MP-G and RF-V: writing—original draft and writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.547186/full#supplementary-material

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Secondary Membranous Nephropathy. A Narrative Review

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Membranous nephropathy (MN) is a common cause of proteinuria and nephrotic syndrome all over the world. It can be subdivided into primary and secondary forms. Primary form is an autoimmune disease clinically characterized by nephrotic syndrome and slow progression. It accounts for ∼70% cases of MN. In the remaining cases MN may be secondary to well-defined causes, including infections, drugs, cancer, or autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), urticarial vasculitis, sarcoidosis, thyroiditis, Sjogren syndrome, systemic sclerosis, or ankylosing spondylitis. The clinical presentation is similar in primary and secondary MN. However, the outcome may be different, being often related to that of the original disease in secondary MN. Also, the treatment may be different, being targeted to the etiologic cause in secondary MN. Thus, the differential diagnosis between primary and secondary MN is critical and should be based not only on history and clinical features of the patient but also on immunofluorescence and electron microscopy analysis of renal biopsy as well as on the research of circulating antibodies. The identification of the pathologic events underlying a secondary MN is of paramount importance, since the eradication of the etiologic factors may be followed by remission or definitive cure of MN. In this review we report the main diseases and drugs responsible of secondary MN, the outcome and the pathogenesis of renal disease in different settings and the possible treatments.

Keywords: NSAIDs, HBV infections, cancer, membranous lupus nephropathy, secondary membranous nephropathy, primary membranous nephropathy

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INTRODUCTION

The term membranous nephropathy (MN) indicates a pathological condition characterized, at light microscopy, by thickening of the glomerular basement membrane (GBM), which is diffuse to all glomeruli and involves the whole glomerulus. In most cases MN is an autoimmune disease caused by autoantibodies directed against phospholipase A2 receptor (PLA2R) or, more rarely, thrombospondin type-1 domain-containing 7A (THSD7A) (1, 2). However, the antigen THSD7A is not specific for primary MN; it can also be detected in MN patients with cancer. When secondary causes are excluded, the disease is called primary MN (**Figure 1**). Immunofluorescence analysis shows granular sub-epithelial deposits of immunoglobulin G (mainly IgG4) and C3, with lesser amounts of IgM or IgA and uncommonly C1q, suggesting that there is not complement activation by the classical pathway (3). Electron microscopy can detect deposits of varying electron density and shape confined to the subepithelial space of glomeruli or incorporated into irregular projections of GBM-like material ("spikes and domes"). Mesangial electron deposits are absent or scanty in primary MN (**Table 1**).

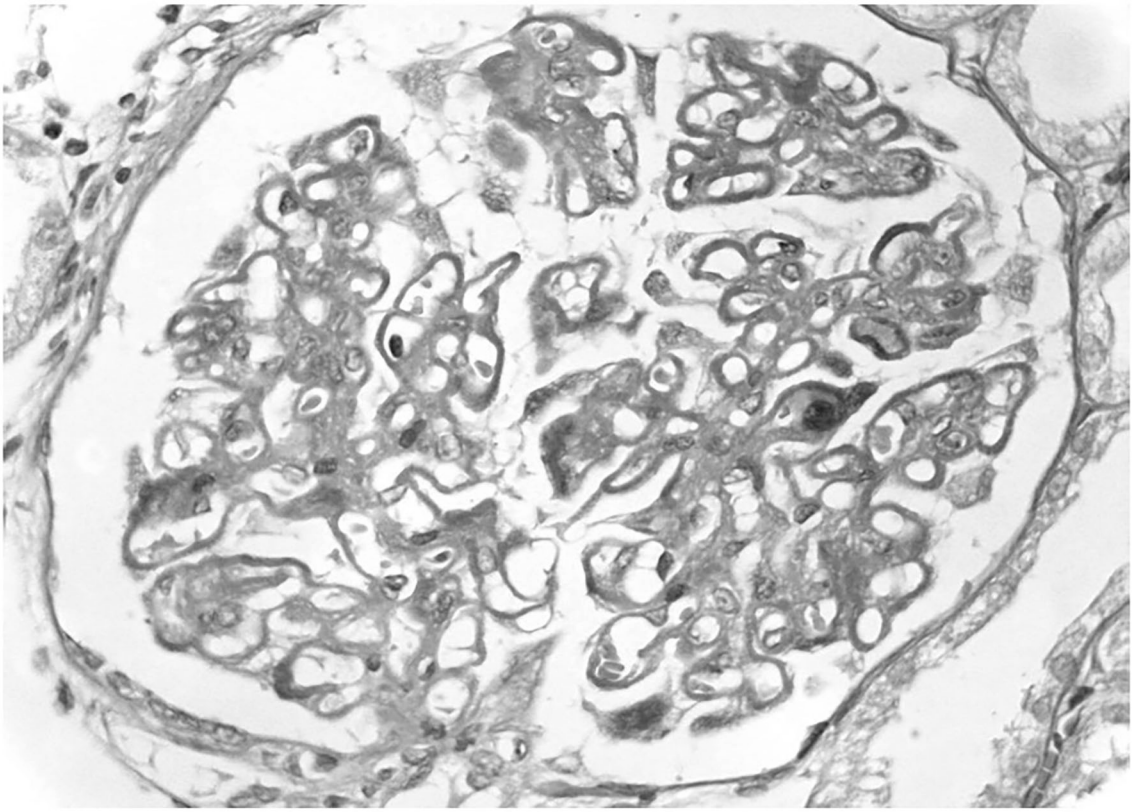

FIGURE 1 | Primary membranous nephropathy. A light microscopy there is diffuse thickening of glomerular capillary walls due to the presence of many immunedeposits in subepithelial position.

However, in some cases a picture of MN at light microscopy may be associated with infection, drug exposure, cancer, or other autoimmune diseases (**Table 1**). These secondary MNs may show peculiar aspects at immunofluorescence and electron microscopy and may have different clinical presentation and natural course. Any effort should be made to distinguish primary from secondary MN, since their treatment may be different and sometimes opposed with devastating consequences in case of wrong.

A narrative review was performed to identify cases of MN associated with different types of infections or developed during or after the use of drugs, or secondary to malignancy, or autoimmune diseases. We reviewed the literature by searching for the following terms on Pubmed.gov: Membranous nephropathy, Secondary Membranous Nephropathy, Infection and Glomerulonephritis, Drugs and Glomerulonephritis, Autoimmune disease and Glomerulonephritis, Cancer and Glomerulonephritis, Lupus membranous nephropathy, Nephrotic Syndrome, Rheumatoid Arthritis, Sarcoidosis, IgG4 disease, Urticarial vasculitis, Hematopoietic Stem Cell Transplant, Thyroiditis, Systemic Sclerosis, Sjogren Syndrome, Ankylosing spondylitis. We selected the papers reporting cases of secondary membranous nephropathy.

POST-INFECTIVE MEMBRANOUS NEPHROPATHY

Viral Infections

Membranous nephropathy is the most common extrahepatic manifestation of hepatitis B virus (HBV) infection. It is generally associated with active viral replication, as indicated by the presence of B-viral DNA and hepatitis B antigen. At time of diagnosis of MN, liver enzymes may be normal or only mildly elevated. The prevalence of HBV-associated MN is strictly correlated with the geographic prevalence of HBV infections. In recent years, the use of HBV vaccine allowed to minimize the diffusion of HBV infection in developed countries. The few cases, reported nowadays in the Western areas, occur in high-risk subjects such as in intravenous drug addicts (4), but in tropical countries HBV-associated MN remains a frequent cause of nephrotic syndrome, particularly in children.

The light microscopic histological appearances are similar to idiopathic MN, but mild mesangial proliferation may be seen in HBV-MN; on electron microscopy there are typical subepithelial deposits and a few subendothelial deposits. The demonstration by immunofluorescence of the presence HBV antigens, mainly HBe, in form of granular deposits along the GBM, may support the pathogenetic role of HBV infections in the development of

TABLE 1 | Differential diagnosis from primary to secondary membranous nephropathy at renal biopsy.

	Primary membranous nephropathy	Secondary membranous nephropathy
Light microscopy	Uniform thickening of GBM diffuse to all glomeruli. No proliferation.	Endocapillary hypercellularity may be seen in MN secondary to SLE or cancer. Mesangial proliferation in MN secondary to SLE, cancer, or Sjogren syndrome.
Immuofluorescence	Subepithelial deposits of IgG (usually IgG4) and C3. Staining with PLA2R (70% of cases).	Subepithelial deposits of IgG (usually IgG1, IgG2, or -IgG3), C1q, IgA, IgM in SLE, cancer, and in some cases of drug-induced MN. Staining with PLA2R in some cases of HBV infection, S.mansoni, SLE, cancer, sarcoidosis.
Electron microscopy	Subepithelial electron-dense deposits.	Subepithelial and subendothelial deposits in HBV, SLE, Tubulo-interstitium and vessels deposits in SLE.

glomerular diseases (5). Theoretically, the small size and the cationic charge of this molecular weight antigen might pass through the GBM and localize in the subepithelial area eliciting the formation of antibodies (6). However, there is currently little evidence to support this hypothesis. In a Chinese study, 25 of 39 (64%) patients with HBV-associated MN showed PLA2R overlapped with HBsAg along the capillary loop, suggesting that HBV antigen may colocalize with anti-PLA2R antibody (7). As in idiopathic forms, the clinical presentation of HBV-related MN is characterized both in children and in adults, by variable degree of proteinuria from mild to nephrotic extent, often associated with microscopic hematuria. Hypocomplementemia has been reported in some cases in the initial phases of the disease. The clinical course seems to be different in children and in adults. The diagnosis of MN in children was generally done during a screening campaign. Most children with HBV-associated MN had a spontaneous remission of proteinuria within 1 year after the diagnosis. In a minority of children proteinuria persisted and chronic renal insufficiency or end stage renal failure (ESRD) developed later (8). In adults, spontaneous remission of proteinuria is infrequent, and the clinical course is more frequently progressive. In a cohort of 21 adults with HBV related MN, after a mean follow-up of 60 months, 29% developed chronic renal insufficiency and 10% entered ESRD (9). In patients with abnormal liver function tests and nephrotic syndrome the progression to ESRD was more rapid (10).

Few cases of MN have been diagnosed in HCV-positive patients (11). The clinical presentation is characterized by nephrotic proteinuria. Hypocomplementemia have been reported rarely, while cryoglobulin and rheumatoid factors were absent. The demonstration by indirect immunofluorescence of presence of HCV core RNA in the glomerular deposits in two patients with MN supports the role of HCV infections in the pathogenesis of these forms of MN (12). Glucocorticoids or rituximab in both hepatitis B/C viral-associated MN resulted ineffective and contraindicated as these drugs can increase viral replication. However, rituximab may obtain reduction of HCV in cryoglobulinemic glomerulonephritis (13) Antiviral therapy may obtain remission of MN (14). Tenofovir, entecavir, telbivudine, and lamivudine may obtain HBeAg clearance and remission of proteinuria (15, 16). Tacrolimus combined with entecavir rapidly and effectively induced partial or complete remission of HBV-MN in a series of Chinese adults (17).

Cases of MN in patients affected by HIV infection have also been reported (18). It is likely that other viruses, including influenza vaccination (19), may be involved in the pathogenesis of secondary MN. The diagnostic criteria for virus-related nephropathy include detailed clinical and laboratory data, and tissue molecular analysis. Several mechanisms are involved in the pathogenesis of virus-related nephropathy, including tropism of the virus in the kidney, induction of abnormal immune complexes, direct cytopathogenic effects, and multiorgan failure (20).

Parasitic and Bacterial Infections

Schistosomiasis is a parasitic disease caused by organisms from the genus Schistosoma. The incidence of glomerular involvement in the various forms of schistosomiasis is estimated in 5–6% and increases to 15% in the hepatosplenic form (21). The association of MN with schistosomiasis is infrequent (22, 23). In old studies, antigens from Schistosoma mansoni have been found in the sera of humans and animals infected with the parasite, suggesting that MN was a secondary form (24, 25). However, recent reports challenged this interpretation. The analysis of renal biopsy demonstrated that at light microscopy there was thickening of the GBM often associated with granulomatous reaction in the renal interstitium. Immunofluorescence revealed granular deposits of IgG1 and IgG4 and electron microscopy indicated subepithelial electron-dense deposits. Surprisingly, a diffuse staining with PLA2R antibodies was observed (26, 27), suggesting the possibility of the coincidental presence of schistosomiasis and MN. The few available studies reported that MN was refractory both to the standard treatment used for primary MN and to specific antiparasitic treatment with praziquantel alone or in combination with artemether or artesunate.

In Plasmodium malaria, Filariasis and Mycobacterium leprosy (28, 29) membranoproliferative and mesangioproliferative glomerulonephritis are prevalent, while MN is infrequent.

Infection of syphilis can also be associated with MN. Treatment with penicillin for secondary syphilis can obtain normalization of renal function and resolution of the nephrotic syndrome (30).

DRUG INDUCED MEMBRANOUS NEPHROPATHY

Membranous nephropathy secondary to drug exposure is not infrequent. In a single center experience, in out of 129 patients

with MN, an underlying cause was identified in 40 cases (31%). In 18 of them (45%) MN was secondary to drugs (31).

The pathogenetic mechanism of drug-induced MN is probably due to an immune response to the drug or to a by-product that acts as planted antigen on the subepithelial position of the GBM. The most plausible mechanism is that cationic drug–derived antigens traverse the GBM, are planted in the subepithelial space, and are targeted *in situ* by circulating antibodies directed against these antigens (32), leading to alterations of the GBM and glomerular filtration barrier eventually resulting in proteinuria. Patients with drug-induced nephrotic syndrome frequently have the HLA-B8 and DR3 antigens (33).

At renal biopsy, drug-induced MN is not different from primary forms. In the past, the most frequent drugs that caused MN were gold salts, penicillamine, and bucillamine that contained a sulfhydryl group, also called Thiol group. However, the use of these drugs has progressively reduced after the introduction of biological agents in the treatment of rheumatoid arthritis. Nevertheless, cases of MN secondary to the use of the monoclonal antibody adalimumab (34). A patient with rheumatoid arthritis and osteoporosis developed MN after treatment with denosumab (35). Some cases of MN have been reported with the use of Captopril. The development of MN can be attributed to a sulfhydryl group which is present in captopril but is absent in other ACE inhibitors (36). As a matter of fact, no other drugs of ACE family have been reported to induce MN. A rare case of lithium associated MN has been reported in an adolescent (37). Another rare cause of MN was chronic mercury exposure secondary to occupational exposures, contaminated fish, dental amalgams, but also cosmetics such as skin-lightening creams (38). Eleven patients were described by Li et al., all had normal function at presentation and proteinuria was in nephrotic range in 3 cases only. At light microscopy mild mesangial proliferation, and some leukocytes were present in the capillary lumen. At immunofluorescence IgG1 was predominant with C3, but other immunoglobulins and C1q were present (39). In most cases of drug-induced MN the disease remitted after withdrawal of the offending drug, sometimes years later.

Membranous nephropathy may also develop during the exposure to non-steroidal anti-inflammatory drugs (NSAIDs). MN was reported with all NSAIDs including selective cyclooxygenase-2 inhibitors, suggesting that the possible mechanism of action on renal damage could be mediated through their common action on cyclooxygenase inhibition (40). The exact rate of MN in patients in treatment with NSAIDs is not known. A retrospective study at the Mayo Clinic reported that 13 out of 125 cases (10.4%) of MN stage I or II diagnosed between 1975 and 1995 met the criteria for NSAIDs-associated MN (41). Patients with NSAIDs associated MN were generally older than those with idiopathic forms, probably due to the widely use of these drugs in old people. The duration of NSAIDs treatment before the development of MN is extremely variable from few weeks too years. Another characteristic is the very rapid development of nephrotic proteinuria, the hallmark of the disease. This rapid development of proteinuria explains the early stage of MN (class I or II) at renal biopsy. In most cases the withdrawal of the offending drug allows the remission of proteinuria without the need of immunosuppressive therapy. However, proteinuria may take some months before disappearing. Immune deposits can also resolve completely at repeated renal biopsy (42). The disease does not recur even after a long-term follow-up.

COW'S MILK

A particular and rare form of secondary MN is caused by cow's milk. High levels of cationic circulating anti-bovine serum albumin (BSA) antibodies of IgG1 and IgG4 subclasses may be detected in these cases, and BSA may be recognized in subepithelial immune deposits. These data suggest that in a few children with MN, cationic BSA introduced with cow's milk may result in pathogenic MN if it passes the intestinal barrier. Once in the blood, BSA may bind to the anionic glomerular capillary wall, be reached by antibodies, and cause *in situ* formation of immune complexes (43). However, it is still unknown if removing cow's milk from the diet of an affected patient with anti–bovine serum albumin antibodies can modulate MN (44).

MEMBRANOUS NEPHROPATHY SECONDARY TO CANCER

The association of increased malignancy risk with glomerulonephritis is well-known (45–47), Lefaucheur et al. (48) found that 24 of 240 patients with MN developed a malignancy at the time of renal biopsy or within a year later. Compared with the general population, the incidence of cancer was 9.8 times higher for men and 12.3 higher for women, independently of age. Patients with MN and cancer were more frequently heavy smoker than controls. The risk of cancer associated MN increased with age, being around 2% in patients with <55 years and reaching 20–25% after 60 years. In a Norwegian study based on registry data of both cancer and MN, cancer was present at time of diagnosis of MN in 11 out of 166 patients with MN, and it was diagnosed in a median time of 60 months later in other 24 patients (49). A systematic review and meta-analysis of 6 observational studies that included 785 MN patients reported that the prevalence of cancer in patients with MN was 10%. The mean age of patients with cancer was 67 years and 2/3 of patients were males. In 20% of patients, cancer was diagnosed before the development of MN, while the other cases were diagnosed at time of renal biopsy or during the follow-up (50). The message from these studies is that the search for malignancy is warranted in patients with MN over the age of 55–60 years (51).

Which criteria should be used to define the causal relationship between MN and malignancy? This remains an open problem. The simultaneous or close diagnosis of both MN and cancer, the remission of proteinuria in cases of healing of neoplasia and its recurrence if neoplasia recurs should be the best criteria for defining this association and for minimizing detection bias. However, many patients with malignancies and proteinuria are not submitted to renal biopsy in consideration of the limited

possibility of treating renal diseases. On the other hand, the risk of development of cancer may persist for a long time. In a study, the mean annual incidence ratio of cancer was 2.1/100 person-years in the 0–5-year period and 2.8/100 person-years for the 5–15 years after kidney biopsy (49).

Proteinuria is the main clinical manifestation and can be associated with renal failure and arterial hypertension in several cases. Although the histological presentation is similar to that of primary MN, some characteristics may identify the forms associated to cancer. At renal biopsy, glomeruli may be almost normal at optic microscopy, but mesangial hypercellularity together with infiltration of leukocytes in glomerular capillaries lumen have been reported in cases with underlying neoplasia. In particular, the presence of at least eight inflammatory cells per glomerulus was able to identify the forms associated with cancer with a specificity of 75% and a sensitivity of 92% in the study of Lefaucheur et al. (48) (**Figure 2**). At immunofluorescence subepithelial deposits of IgG1 and IgG2 are frequently detected in cancer associated MN while IgG4 dominates in idiopathic MN (52). This difference may represent one of the criteria for the differential diagnosis with primary MN (53) (**Table 1**). Compared with PLA2R- and THSD7A-positive forms of MN, there was a greater proportion of cases with malignancies in the nerve epidermal growth factor-like 1(NELL1)-associated group. Thus, NELL1-associated MN has a unique histopathology characterized by incomplete capillary loop staining, IgG1-predominance, and is more often associated with malignancy than other known types of MN (54).

Rare cases of detection of tumor antigens in subepithelial immune deposits of GBM together with antitumor antibodies have been reported (55). Beck et al. (56) proposed different mechanistic interpretations to explain the role of cancer in the development of MN; they include (i) the expression of planted tumor antigens triggering the production of circulation antibodies and the *in situ* formation of immune complexes composed by tumor antigens and antibodies; (ii) the formation of antibodies against a tumor antigen immunologically similar to a podocyte antigen; (iii) an abnormal immune response activated by an extrinsic process such as a viral infection. However, these hypotheses have not been confirmed because of the lack of a reliable experimental model. A simultaneous expression of THSD7A in gallbladder carcinoma and kidney has been described in a woman with MN. Moreover, out of 25 patients with MN and circulating anti- THSD7A antibodies, 7 had a malignant tumor (57). It has been outlined that patients with anti-PLA2R-negative MN are at higher risk of having a cancer-related form of MN compared to patients with anti-PLA2R positive MN (58). However, in a study, anti-PLA2R antibodies have been detected in 3 out of 10 patients with solid tumors. In these 3 patients there was a moderate subepithelial deposition of IgG4 (59). The cancers most frequently associated with MN are solid tumors such as lung, gastrointestinal, and prostate and uterus carcinoma, although cases associated with hematologic neoplasia and less frequently to melanoma have been reported (60, 61).

In view of the potential risks of underestimating a possible diagnosis of malignancy associated with MN a work-up to search for malignancies is suggested not only at the time of diagnosis of MN, but also during the follow-up in patients with negative anti-PLA2R1 antibodies and after the exclusion of other secondary forms of MN. The presence of anemia may also raise the suspicion of malignancy. Particularly in old patients, the work-up should include colonoscopy, prostate specific antigen search, mammography, and chest imaging in smokers (62). Once the diagnosis is done, the treatment should be directed to the associated cancer (63, 64). For patients with nephrotic syndrome, symptomatic treatment with ACE inhibitors and diuretics is indicated. Prevention of thromboses with anticoagulation in severe nephrotic syndrome is suggested. As expected, the prognosis of patients with cancer associated MN is worse than that of idiopathic forms.

HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT)

MN is the most frequent glomerular diseases associated with HSCT. In a study, out of 14 patients who developed a nephrotic syndrome after HSCT, 10 had a biopsy-proven MN (65). Clinical presentation is characterized by proteinuria usually in a nephrotic range that develops months after HSCT and is often associated with graft vs. host disease (GVHD).

The pathogenesis of MN is still incompletely elucidated. Some investigators feel that MN may represent the renal manifestation of GVHD (66). Other data support an autoimmune hypothesis (67). T cells are key players in GVHD (68, 69), but B cells can also contribute through both antibody-dependent and antibody-independent mechanisms (70), suggesting that GVHD may trigger an autoimmune response. Indeed, development of autoantibodies in association with a chronic GVHD has been reported (71). These data and the absence of PLA2R antibodies (72) support the hypothesis that HSCT-associated MN is a disease secondary to an autoimmune reaction to an allogeneic transplant (73).

Treatment depends on the amount of proteinuria. ACE inhibitors may be used in case of asymptomatic proteinuria. Most patients with nephrotic syndrome can respond to glucocorticoids, cyclosporine, or rituximab (74–76). However, a few patients are refractory to therapy and progress to ESRD.

MEMBRANOUS NEPHROPATHY SECONDARY TO IMMUNOLOGICAL AND RHEUMATOLOGICAL DISEASES

Lupus Membranous Nephropathy (LMN)

LMN is a rare subtype of lupus nephritis. It accounts for ∼15–20% of cases of lupus nephritis and mainly affects females (77). The mean age at presentation ranges around 30–35 years. In the current classification of lupus nephritis, MN is categorized as class V and includes cases of global or segmental subepithelial immune deposits with or without mesangial alterations (78). At light microscopy, LMN shares the similar characteristics of primary MN (**Table 1**). Mesangial proliferation is minimal or absent in primary MN while it may be present in LMN. At immunofluorescent study, the most important features in LMN

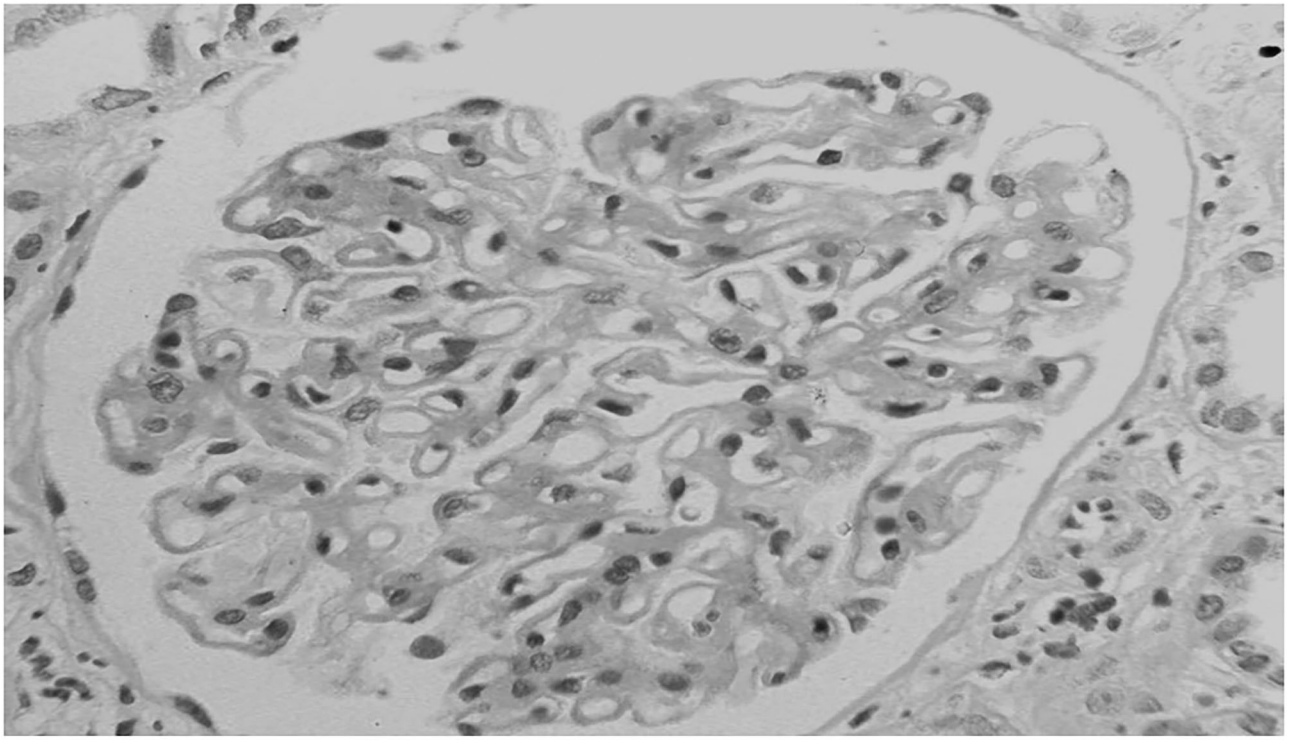

FIGURE 2 | Membranous nephropathy in a patient with lung cancer. At light microscopy together with the diffuse thickening of glomerular capillary walls, some infiltration of leukocytes is present in capillary lumens.

are "full-house" deposits of IgG, IgM, IgA, and intense C1q and C4 staining in subepithelial and occasionally mesangial position. Deposits of IgG 1-3 may also distinguish lupus from primary MN, in which deposits of IgG4 are preponderant (79). The electron microscopy may show subepithelial and subendothelial deposits together with tubulo-reticular structures ("Interferon-fingerprints") in endothelial cells (**Figure 3**). A recent study detected exostosin 1 (EXT1) and exostosin 2 (EXT2) staining in 80% of 26 patients with PLA2R negative MN and clinical features of autoimmune disease including lupus. Although serum EXT antibodies were not detected, these proteins may represent putative antigens in patients with this distinct subtype of secondary MN (80).

The pathogenesis of lupus MN is still incompletely elucidated. Some studies showed that insufficient apoptosis and neutrophil extracellular traps (NET) favor the exposure of nucleic acids and their binding proteins that are recognized as autoantigens (81–83). The loss of self-tolerance (84) favors the production of circulating antibodies that bind to autoantigen in podocyte membrane with consequent *in situ* formation of subepithelial immune deposits (85), which activate the lytic late components of complement (C5b-C9), release T cells and inflammatory cells, and produce reactive oxygen species. This sequence of events leads to glomerular injury and dysfunction of the glomerular barrier resulting in proteinuria.

The clinical presentation of LMN is variable. Some patients do not have extrarenal signs or symptoms of lupus and present with proteinuria and abnormal urinary sediment as the sole renal manifestations. In symptomatic patients, proteinuria may exceed 3.5 g per day and may be associated with hypoalbuminemia, dyslipidemia and variable degrees of edema. Hematuria and hypertension are common. Renal function is usually normal or subnormal.

Only few studies reported the long-term outcome of LMN. Progression to ESRD is slow and 72–97% of patients are still alive with kidney functioning at 10 years (86–90). Renal prognosis is largely influenced by the development of renal flares and transformation to proliferative disorders. Renal flares may be subdivided into proteinuric flares, characterized by increase in proteinuria with stable kidney function, and nephritic flares, characterized by a substantial increase in serum creatinine (91). Nephritic flares are difficult to manage and may lead to irreversible lesions, while proteinuric flares usually respond to treatment although remission may occur after weeks or months (92), Flares in patients with LMN are frequently associated with conversion to proliferative glomerulonephritis, as shown by repeat renal biopsy (93–95). Persistent nephrotic syndrome and prolonged use of corticosteroids can be responsible of dyslipidemia, diabetes, arterial hypertension, and hypercoagulability (96–98).

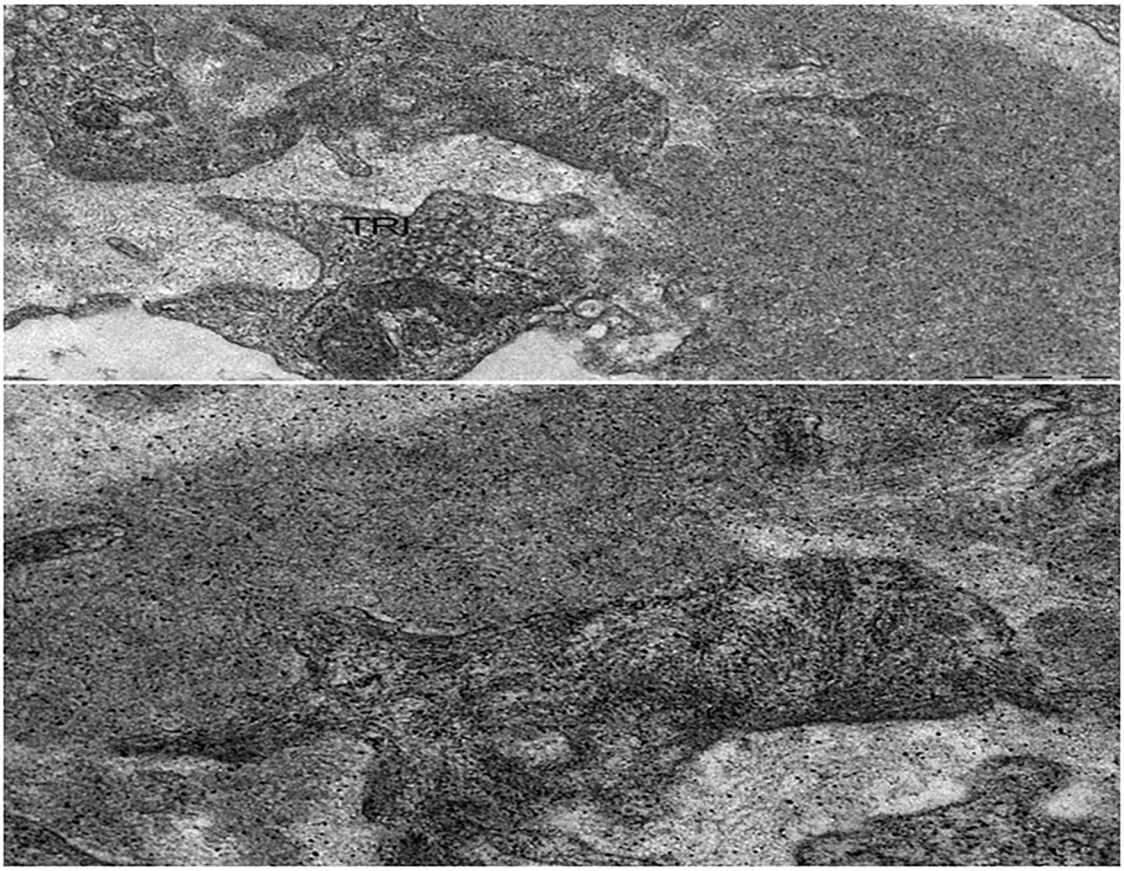

FIGURE 3 | Lupus membranous nephropathy. At electron microscopy, sub-endothelial immune complexes deposits, structured in aggregates of concentric lamellae to form "finger prints" images; a tubulo-reticular inclusion (TRI) is observed in the endothelial cell. Bars: 500 nm.

There is agreement that patients with persistent nephrotic syndrome despite the use of RAS inhibitors should receive immunosuppressive therapy, while there is controversy about the use of immunosuppression in patients with subnephrotic proteinuria. The Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) and several authorities recommended the use of corticosteroids and immunosuppressive drugs in pure class V nephritis, when the ratio urine protein/creatinine exceeds 1,000 mg/g despite the optimal use of renin–angiotensin–aldosterone system blockers (99–101). Corticosteroids alone are poorly effective, but they are largely used in combination with other immunosuppressive drugs. In a randomized controlled trial 42 patients were randomly assigned to prednisone alone or prednisone combined with cyclosporine for 11 months or alternate-month intravenous pulse cyclophosphamide for six doses. Both cyclophosphamide and cyclosporine were more effective than prednisone alone in inducing remissions of proteinuria, but relapses were more frequent after withdrawal with cyclosporine than cyclophosphamide (102). In the Aspreva trial (103) 370 patients with lupus nephritis were randomized to mycophenolate mofetil (MMF) or intravenous cyclophosphamide. In a pooled analysis of two randomized studies that included a subset of 65 participants with LMN, no difference was seen between response to MMF and to intravenous cyclophosphamide (104). Another randomized controlled study compared 6 months therapy of tacrolimus vs. MMF followed by azathioprine; all patients were also given high-dose prednisolone. The subgroup of patients with LMN treated with tacrolimus had significant more improvement of proteinuria and achieved more frequent complete and partial remission at 6 months in comparison to MMF (105). Years ago, we treated 11 LMN patients with a 6-month cyclical regimen based on alternating corticosteroids and cyclophosphamide every other month. After a mean follow-up of 83 months, 7 patients were in complete remission, and 3 patients were in partial remission (106). In the Rituxilup study, 22 patients with LMN were given 2 doses of rituximab (1 g) and methylprednisolone (500 mg) on days 1 and 15, and maintenance treatment with MMF. At 1 year, complete or partial remissions were achieved in >80% of patients (107). A systematic analysis of the use of rituximab in refractory lupus nephritis reported that this drug achieved complete or partial response in 67% of patients with refractory LMN (108). Thus, different treatments

proved to be effective, but the choice depends on personal experience and previous treatments.

Whatever is the chosen immunosuppression, it is important not to neglect the treatment of complications, including hypertension, dyslipidemia, diabetes, thrombotic events, infections, and osteoskeletal disease.

Rheumatoid Arthritis (RA)

As pointed out above, MN in RA was mainly secondary to the use of gold salts, penicillamine, or bucillamine. Today, these drugs are rarely used, and the frequency of MN decreased (109, 110). The lesions of RA-related MN are similar those of primary MN on examination by light microscopy, electron microscopy, and immunofluorescence (111). The pathogenetic mechanisms of RA-related MN are unclear. One may speculate that circulating autoantibodies in RA may target some podocyte proteins and eventually cause MN. Rituximab may probably represent the elective treatment of RA-related MN, being active both on RA and MN (112, 113).

Urticarial Vasculitis (UV)

UV is an urticarial eruption that is often painful or has a burning sensation. The skin lesions consist of inflamed and reddened patches or weals that can persist of more than 24 h. There are two variants of UV: the normocomplementemic UV with a less severe clinical course, and the hypocomplementemic form which is considered as an immune complex-mediated disorder characterized by low serum levels of C1q, C2, C3, and C4 and the presence of circulating anti-C1q antibodies (114, 115). The skin lesions result from a cutaneous leukocytoclastic vasculitis of small vessels that mainly involves the skin but can also extend to joints, eye, lungs, gastrointestinal tract, and other organs (115). Hypocomplementemic UV is frequently associated with SLE, RA, drug reactions, infections, or malignancy (116, 117).

Cases of MN associated with UV have been reported (118, 119). A systematic review outlined that the most frequent glomerular disease in UV was membranoproliferative glomerulonephritis, 35% of cases, followed by mesangioproliferative glomerulonephritis, 21%, and MN, 19% (120). As for other systemic vasculitis, renal involvement carries a poorer prognosis, but the outcome can be improved by aggressive immunosuppressive treatment. Biologic agents, including omalizumab, corticosteroids, cyclophosphamide, MMF, cyclosporine, and hydroxychloroquine proved to be effective for both skin and systemic symptoms (121).

IgG4 Membranous Nephropathy

IgG4-related disease is a fibroinflammatory disorder that can involve nearly any organ, including the kidney. Tubulointerstitial nephritis is the most common renal manifestation, but MN may also occur (122). PLA2R antibodies are negative in IgG4-MN (123). In contrast to primary MGN, granular C1q deposits are sometimes prominent, and concurrent tubulointerstitial nephritis is often seen. Elevation of serum IgG4 often accompanies IgG4-related disease; however, it is not specific in reaching the diagnosis. The pathogenesis of IgG4-related disease is not clarified. It responds promptly to steroids, although there is a high relapse rate on discontinuation of immunosuppression. In the case of steroid resistance, rituximab represents the second-line treatment (124).

Sarcoidosis

Renal manifestations of sarcoidosis are rare but may occur at any age including childhood (125). Granulomatous interstitial nephritis and glomerulonephritis can occur. Sarcoidosis-associated glomerulonephritis includes a variety of histological forms, the most frequent being MN (126). In rare cases, both MN and granulomatous interstitial nephritis can be seen at renal biopsy (127). Glomerular disease may appear before, simultaneously or after other manifestations of sarcoidosis. There may be a long latency period between the development of active sarcoidosis and glomerular involvement and inversely (128).

Whether the presence of MN in patients with sarcoidosis is a mere coincidence or is due to a causal relationship is unclear (129). Anti-PLA2R antibodies in serum or PLA2R antigen in biopsy can be detected in patients with sarcoidosis and MN. The high prevalence of PLA2R antigen in patients with MN associated with active sarcoidosis should suggest a causal link between the two diseases (130). On the other hand, primary MN is rare in children, and anti-PLA2R antibodies may also been detected in a few cases of MN secondary to cancer or hepatitis B.

Corticosteroids have been largely used in patients with associated MN and granulomatous interstitial nephritis. In these cases, the rapid administration of high-dose corticosteroids may prevent irreversible interstitial fibrosis and tubular atrophy. In case of isolated MN with asymptomatic proteinuria, RAS inhibitors are used. In the presence of nephrotic syndrome, the treatment is similar to that adopted for primary MN.

Autoimmune Thyroiditis

Autoimmune thyroiditis (Hashimoto or Graves' disease) is caused by autoantibodies directed against thyroid proteins, such as thyroglobulin, thyroid peroxidase, or thyroid stimulating hormone receptor. It is often associated with asymptomatic proteinuria and sometimes nephrotic syndrome. Different renal diseases may be detected in those instances, MN representing one of the most frequent underlying glomerular disease (131). Only rarely, MN caused by autoimmune thyroiditis is associated with profound hypothyroidism. In many patients with Hashimoto disease the free thyroxine level may be normal while thyrotropic stimulating hormone (TSH) is elevated. At least initially, symptoms of hypothyroidism may be absent or mild; in addition, ∼37% of patients with primary MN show a decrease in serum triiodothyronine (132), so that the diagnosis may be difficult and may be done with delay.

The mechanisms linking autoimmune thyroiditis and MN are still poorly defined. However, both in children (133) and adults (134) the development of MN is associated with deposition of immune complexes mediated by anti- thyroid-peroxidase antibodies, suggesting that renal disease may be caused by the production of autoantibodies against podocyte antigens. Immunofluorescence examination demonstrates bright granular staining of IgG along the GBM, corresponding to glomerular granular staining of thyroid-peroxidase while no thyroglobulin

deposits are present. Electron microscopy shows subepithelial electron-dense deposits.

The treatment of Hashimoto thyroiditis is thyroid hormone replacement with levothyroxine sodium. It is often enough to obtain remission of proteinuria. In case of severe nephrotic syndrome or superimposed crescentic glomerulonephritis (135), prednisone, and oral cyclophosphamide may be used.

Sjögren Syndrome

Renal involvement is rare in Sjogren syndrome, affecting <10% of patients (136). Tubulointerstitial nephritis and tubular acidosis are prevalent but different glomerular diseases can also be seen (137, 138). A Chinese review of patients with Sjögren syndrome and biopsy-proven renal diseases reported that 36% had a MN (139). Light microscopy, immunofluorescence, and electron microscopy are similar to primary MN, but interstitial infiltrates are often present in Sjogren syndrome. The prognosis appears to be worse in patients with glomerular involvement, with lower survival rates and higher incidence of lymphoma compared to patients with predominantly tubulointerstitial involvement (140, 141). Transformations from MN to membranoproliferative glomerulonephritis or crescentic glomerulonephritis have been observed (142, 143). Little information is available about the effectiveness of corticosteroids or other immunosuppressive agents to slow progression of renal disease.

Systemic Sclerosis

Renal involvement is common in systemic sclerosis. Some individuals are initially asymptomatic or show only mild proteinuria, microscopic haematuria, and occasional casts. These patients may follow an indolent course until hypertension and progressive deterioration of kidney function develop (144–146). Cases of MN have been reported. In most cases they were related to the use of D-penicillamine (147), but in a few patients no cause but scleroderma was identified (148, 149). Subepithelial deposits were seen on electron microscopy, suggesting that autoantibodies directed against S-cl70, centromere or polymerase III (150) may cause formation *in situ* of immune complexes.

Ankylosing Spondylitis

Amyloidosis is the most frequent glomerular disease in ankylosing spondylitis, but exceptional cases of MN have also been reported (151). Apart from few cases secondary to treatment with gold salts, at light microscopy, immunofluorescence, and electron microscopy the findings are similar to those of primary MN, but the negative PLA_2R may suggest a diagnosis of secondary MN (152, 153). This would be confirmed by resolution of pain and rapid decrease in proteinuria after administration of adalimumab (153).

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

CP conceived the study. CP and GM contributed in reviewing the literature and in writing the paper. All authors contributed to the article and approved the submitted version.

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Pregnancy Outcomes in Patients With Adult-Onset Still's Disease: A Cohort Study From China

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Objective: Adult-onset Still's disease (AOSD) is an autoinflammatory disease with a higher prevalence rate in young females. The purpose of this study is to investigate whether AOSD has an adverse impact on pregnancy outcomes, or conversely exacerbated by pregnancy.

Methods: The outcomes of 191 pregnancies were evaluated in 86 female patients with AOSD. The generalized linear mixed model and propensity score matching method were conducted to evaluate the influence of AOSD on pregnancy outcomes. A dependent sample sign test was applied to assess the impact of pregnancy on the relapse of AOSD.

Results: The results showed that the post-AOSD group had a lower proportion of normal delivery (25.0 vs. 52.4%, $p = 0.036$) and a higher proportion of spontaneous abortion (STA) (18.8 vs. 0.6%, $p = 0.002$) compared with the pre-AOSD group. Moreover, pregnancy after being diagnosed with AOSD was a significant high risk factor of STA (adjusted OR = 4.577, 95% CI: 4.166–845.119; $p = 0.003$). Disease flare upon conception was observed in one of 16 post-AOSD pregnancies ($p = 1.000$). There were 11 patients with new-onset AOSD during gestation or postpartum, among which five (45.4%) evolved into the polycyclic course.

Conclusions: AOSD patients might suffer from a higher risk of STA, however, pregnancy might not be related with the exacerbation of diagnosed AOSD. New-onset AOSD during gestation or postpartum tend to evolve into the polycyclic course.

Keywords: pregnancy, outcome, generalized linear mixed effect model, adverse impact, adult-onset still's disease

INTRODUCTION

Adult-onset Still's disease (AOSD) is a systemic disorder of unknown etiology characterized by spiking fever, arthralgia or arthritis, and evanescent rash (1, 2). AOSD is a rare autoinflammatory disease with an estimated prevalence of one to 34 cases per million people (3), and predominantly affects female at a young age (4, 5). The etiology and pathogenesis of AOSD is unknown.

Our previous studies showed that CMV infections, increased neutrophil extracellular traps, and multiple other factors were involved in the development of AOSD (6–10).

In clinical practice, one of the major problems that make young female patients worried is the reproductive health condition after being diagnosed with AOSD. Currently, the relationship between pregnancy and AOSD, including disease onset and relapse (pregnancy outcomes in patients with AOSD), still remains unknown. Only limited numbers of studies are published in the forms of case reports or short series of literature reviews. Hence, conclusive interpretations are not available (11–14). We performed a cohort study to explore the probable interaction between AOSD and pregnancy.

MATERIALS AND METHODS

Patients

This cohort study was conducted in the Department of Rheumatology and Immunology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. Inclusion criteria were: (i) AOSD diagnosis fulfilling the Yamaguchi criteria; (ii) exclusion diagnosis including infections, malignancies, and other systemic immune diseases; (iii) inpatients and outpatients of Rheumatology and Immunology, Ruijin Hospital from September 2015 to March 2019; and (iv) female AOSD patients reported at least one pregnancy. This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University (ID: 2016-61). According to the Declaration of Helsinki, informed consent was obtained from each patient.

Data Collection

Data on gestation history included age at pregnancy, gravidity, pregnancy outcomes, comorbidities, and obstetric complications. Clinical characteristics of AOSD and medication usage during pregnancy were collected from medical records and questionnaires interviewed by a research team member. Pregnancies were categorized as pre-AOSD (defined as delivery at least 12 months before AOSD onset), post-AOSD (pregnancy after AOSD diagnosis), gestational AOSD (defined as AOSD onset during pregnancy), and postpartum AOSD (defined as AOSD onset within 1 year after delivery). Exposure variables included temporal relationship with AOSD, maternal age, gravidity (defined as the total number of previous pregnancies), disease activity (determined by the presence of fever and/or any suggestive cutaneous and/or inflammatory arthralgia/arthritis and/or sore throat), and conception-disease interval (defined as the interval time between disease onset and conception). The pregnancy outcomes included normal delivery, induced abortion, induced labor, preterm birth (PTB, defined as the pregnancy ended between 28 and 37 weeks), full-term cesarean section (CS, defined as the use of surgery for delivery after 37 weeks), and spontaneous abortion (STA, defined as spontaneous embryonic/fetal loss prior to 20 weeks). The disease pattern of AOSD patients was divided into three distinct types: monocyclic, polycyclic, and chronic courses over a 12-month follow-up period (1).

Statistical Analysis

We performed the Mann-Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables. A generalized random mixed-effect model was constructed with individuals as the random effect; temporal relation (categorical), maternal age (continuous), and gravidity (continuous) as the fixed effects. To remove the effects of confounding factors, we performed the propensity score matching method to compare the pregnancy outcome between pre-AOSD and post-AOSD groups. Besides, we also performed a dependent sample sign test to evaluate whether disease relapse increased during pregnancy.

A two-sided $p < 0.05$ was considered statistically significant. Quantitative variables were expressed as median (IQR) and categorical variables were presented as frequency (percentage). Statistical analysis was performed with the IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA) and R statistical software version 3.5.2.

RESULTS

A total of 86 patients were enrolled in this study. The clinical manifestations of 86 patients at the time of AOSD diagnosis are presented in **Supplementary Table 1**, the median age at AOSD onset was 37 years, and the median of disease duration was 18 months. The most common clinical manifestations were fever (100%), skin rash (91.9%), and arthralgia (88.4%) at disease onset. Among the 191 pregnancies, 164 (85.9%) occurred before AOSD, 16 (8.4%) after AOSD, three (1.6%) coincided with the onset of AOSD, and eight (4.2%) were postpartum AOSD (**Table 1**). All the conceptions were natural. The median age at pregnancy onset was 24 years, which is similar to the nationwide sample survey (15). No patients had the history of smoking or drinking. The comorbidities of pregnancy included hypertension, diabetes mellitus, and thyroid dysfunction. There was no previous record of intrauterine growth restriction or premature rupture of membranes during pregnancy in our study. Conception-disease interval was 234.0 months in pre-AOSD, 30.0 months in post-AOSD, 2.0 months in gestational AOSD, and 13.0 months in postpartum AOSD. Therapeutic strategies before and during pregnancy were also shown in **Table 1**. Hydroxychloroquine (31.3%) and glucocorticoids (18.8%) were most frequently selected in post-AOSD group. The detailed treatment information was shown in **Supplementary Table 2**.

Regarding the two major groups: pre-AOSD and post-AOSD group, the post-AOSD group had significantly older maternal age (29 years [26–32 years] vs. 25 years [23–28 years], respectively; $p = 0.005$), a lower proportion of normal delivery (25.0 vs. 52.4%, $p = 0.036$) and a higher proportion of STA (18.8 vs. 0.6%, $p = 0.002$) compared with the pre-AOSD group. Obstetric complications and neonatal situation showed no significant difference between the two major groups (**Table 2**). In order to reduce the interference of confounding factors and potential biases, we performed the propensity score matching method stratified by maternal age and gravidity (**Supplementary Figure 1**). Following propensity score matching, the post-AOSD group still had a significantly lower

TABLE 1 | Characteristics of 191 pregnancies grouped by temporal relationship between disease onset and pregnancy.

Variable	Total	Pre-AOSD	Post- AOSD	Gestational AOSD	Postpartum AOSD
Patients, *n* (%)	86 (100.0)	79 (91.9)	13 (15.1)	3 (3.5)	8 (9.3)
Pregnancy episodes, *n* (%)	191 (100.0)	164 (85.9)	16 (8.4)	3 (1.6)	8 (4.2)
Gravidity, median (IQR)	2 (1–2)	2 (1–2)	2 (1–4)	2 [1–3]*	2 (1–2)
Comorbidities during pregnancy, *n* (%)					
Hypertension	1 (0.5)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)
Diabetes mellitus	1 (0.5)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Thyroid dysfunction	1 (0.5)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)
Conception-disease interval#, months, median (IQR)	168.0 (48.0–336.0)	234 (84.0–348.0)	30.0 (22.8–45.6)	2.0 [1.8–3.0]*	13.0 (11.2–15.5)
Treatment before pregnancy△					
No medication	185 (96.9)	164 (100.0)	10 (62.5)	3 (100.0)	8 (100.0)
Glucocorticoids	3 (1.6)	–	3 (18.8)	–	–
Methotrexate	1 (0.5)	–	1 (6.3)	–	–
Cyclosporine	2 (1.0)	–	2 (12.5)	–	–
Hydroxychloroquine	5 (2.6)	–	5 (31.3)	–	–
Treatment during pregnancy					
No medication	183 (95.8)	164 (100.0)	10 (62.5)	1 (33.3)	8 (100.0)
Glucocorticoids	5 (2.6)	–	3 (18.8)	2 (66.7)	–
Methotrexate	1 (0.5)	–	1 (6.3)	0 (0.0)	–
Cyclosporine	2 (1.0)	–	2 (12.5)	0 (0.0)	–
Hydroxychloroquine	5 (2.6)	–	5 (31.3)	0 (0.0)	–

Qualitative variables were expressed as n (%); continuous variables were expressed as median (IQR); AOSD, Adult-onset Still's disease.

#*Conception-disease interval was defined as the interval between disease onset and conception.*

**Presented as [min, max].*

△*The medication within three months before pregnancy was recorded.*

proportion of normal delivery (20.0 vs. 50.0%, $p = 0.045$) and a higher proportion of STA (20.0 vs. 0.0%, $p = 0.017$) compared with the pre-AOSD group (**Supplementary Table 3**).

In order to demonstrate whether AOSD could have any impact on pregnancy outcome independent of known risk factors including maternal age and gravidity, we constructed a generalized linear mixed effect model, with the binomial variable (pre- or post-AOSD) as fixed effect, and individual female patient as random effect. Pregnancy after AOSD was significantly associated with STA (adjusted OR = 4.577, 95% CI: 4.166–845.119; $p = 0.003$) (**Table 3**).

In the post-AOSD group, the disease activity of 13 patients (16 pregnancies) at the time of conception was inactive. Eight patients didn't take any medication during gestation. The outcomes were one induced abortion, two STA, three full-term CS, and four normal delivery. Five patients (six pregnancies) were taking medications during gestation: low-dose glucocorticoids (3/6), hydroxychloroquine (5/6), cyclosporine (2/6), and methotrexate (1/6). Except one pregnancy ended up with induced abortion because of methotrexate treatment, the outcomes of the other five pregnancies were one STA, three induced abortion, and one full-term CS. Disease flare upon conception was observed in one out of 16 post-AOSD pregnancies. A dependent sample sign test deduced that disease activity was not exacerbated by pregnancy ($p = 1.000$) (**Table 2**) for the post-AOSD group.

Among the 86 patients, 3.5% (3/86) patients had disease onset during gestational period and 9.3% (8/86) during postpartum period. The detailed characteristics of pregnancies in gestational and postpartum groups were shown in **Supplementary Table 2**. In the gestational AOSD group ($n = 3$), two onsets of AOSD occurred in the first trimester of pregnancy, both achieved disease remission after induced abortion. Another onset of AOSD occurred in the 12th week of gestation, and the initial therapy of prednisone 40 mg/day failed to relieve the symptoms. Due to oligohydramnios, she underwent cesarean section and the symptoms were relieved. However, the disease flared 3 months after delivery and complicated with macrophage activation syndrome (MAS). She was treated with dexamethasone and etoposide (VP16), followed by cyclosporine. The postpartum group included eight patients (8 pregnancies), the medications used including median-dose glucocorticoids (3/8), high-dose glucocorticoids (5/8), cyclosporine (4/8), methotrexate (8/8), etanercept (1/8), and hydroxychloroquine (7/8). Over a 12-month follow-up period, the disease courses of gestational and postpartum groups ($n = 11$) evolved into monocyclic course (27.3%), polycyclic course (45.4%), and chronic course (27.3%).

DISCUSSION

Akin to lots of autoimmune diseases with a strong female preponderance, relationships between pregnancy and

TABLE 2 | Maternal characteristics of pregnancies in women that occurred before and after AOSD diagnosis.

	Pre-AOSD	Post-AOSD	*p*-value
Pregnancy episodes, *n*	164	16	
Age at pregnancy onset, median (IQR), years	25 (23–28)	29 (26–32)	0.005
Duration of pregnancy, median (IQR), weeks	40 (39–40)	40 (39–40)	0.944
Gravidity, median (IQR)	2 (1–2)	2 (1–4)	0.057
Pregnancy outcomes, *n* (%)			
Normal delivery	86 (52.4)	4 (25.0)	0.036
Induced abortion	50 (30.5)	5 (31.2)	1.000
STA	1 (0.6)	3 (18.8)	0.002
Full-term CS	23 (14.0)	4 (25.0)	0.268
Induced labor, *n* (%)	3 (1.8)	0 (0.0)	0.497
PTB	1 (0.6)	0 (0.0)	1.000
Obstetric complications, *n* (%)			
Oligohydramnios	1 (0.6)	0 (0.0)	1.000
Antepartum hemorrhage	5 (3.0)	0 (0.0)	0.246
Length of hospital stay, median (IQR), days	4 (4–5)	4 (3–7)	0.869
Neonatal situation			
Birth weight, median (IQR), kg	3.4 (3.0–3.8)	3.6 (2.9–3.9)	0.655
Neonatal death, *n* (%)	1 (0.6)	0 (0.0)	1.000
AOSD features			1.000*
Flare		1 (6.3)	
Remission		15 (93.7)	

Pre-AOSD, delivery at least 12 months before AOSD diagnosis; Post-AOSD, pregnancy after AOSD diagnosis; PTB, preterm birth; STA, spontaneous abortion; CS, cesarean section; AOSD, Adult-onset Still's disease; IQR, interquartile range.
Mann-Whitney U-test was used for continuous variables and Fisher's exact test was used for categorical variables.
**A dependent sample sign test.*

TABLE 3 | The ORs of pregnancy outcomes in post- AOSD pregnancy.

Pregnancy outcomes	Adjusted OR[a]	95% CI	*p*-value
Normal delivery	0.239	0.043–1.317	0.100
Induced abortion	0.670	0.198–2.267	0.519
STA	4.577	4.166–845.119	0.003
Full term CS	2.894	0.485–17.278	0.244
Induced labor	Model undetectable		
PTB	Model undetectable		

[a]Adjusted for maternal age and gravidity; OR, odds ratio; PTB, preterm birth; STA, spontaneous abortion; CS, cesarean section; AOSD, Adult-onset Still's disease.
A generalized random mixed-effect model was constructed with individuals as the random effect; temporal relation (categorical), maternal age (continuous), and gravidity (continuous) as the fixed effects.

consequent development of AOSD have been the source of studies, even though they usually showed conflicting results (16). Here, we conducted a cohort study, which might provide the largest amount of information on pregnancy and AOSD. Our data showed that AOSD increased more than 4-fold the odds of STA during pregnancy, while pregnancy might not trigger the relapse of AOSD. Besides, new-onset AOSD during gestation and postpartum tended to evolve into the polycyclic course.

The reoccurrence of AOSD during pregnancy was first reported in 1980 (17). Le Loët et al. reported five pregnancies in four AOSD patients including two diagnosed AOSD. They found that pregnancy had no adverse effect on AOSD, and AOSD had no influence on pregnancy outcomes (11). On the contrary, adverse influence of pregnancy on AOSD has also been reported, especially during the first, second trimester, and postpartum period. In 2004, Mok et al. reported the maternal and fetal outcomes of five pregnancies in three AOSD patients (12). Even though all of them were in medicine-free remission at conception, an exacerbation occurred in the fourth and fifth months of gestation and during the postpartum period. In 2012, Yamamoto et al. reviewed 23 pregnancies in 19 AOSD women (18). There were nine patients who developed the onset of AOSD during pregnancy, and most occurred in the second trimester until puerperium. In our study, 3.5% (3/86) AOSD patients had their first AOSD-related manifestations during the gestational period and 9.3% (8/86) during the postpartum period. The high percent of AOSD diagnosis during the gestational and postpartum periods suggest that sex hormones may increase the risk of new-onset AOSD. In 1971, Bywaters noticed the female predominance in AOSD indicating that sex hormones might influence the disease susceptibility (19). The underlying biological mechanism is not clear. Previous study reported that estrogens could activate macrophages to produce tumor necrosis factor (TNF-α), IL-6, and IL-1 (20). It could also boost the expression of IL-1 mRNA through monocytes as well as increase several aspects of endothelial-cell biological functions, such as adhesion to matrix proteins, migration and cell differentiation, and promoting inflammation (21). Besides, a recent research speculated that increased IL-18 during pregnancy may participate in the pathogenesis of the onset of AOSD (18, 22), which requires further studies to confirm.

However, only one diagnosed AOSD patient had disease relapse during pregnancy, which indicated that AOSD will not be commonly exacerbated by pregnancy if it is well-controlled. The impacts of pregnancy on the disease onset are diverse with regard to different diseases. The flare rate of SLE increased during pregnancy and postpartum, ranging from 25 to 60% of pregnancies (23). Nevertheless, studies showed that 54–95% of patients with RA improved during pregnancy, with nearly 40% patients achieving a state of remission (24, 25). After delivery, there is an increased risk of a flare in disease activity of RA, postpartum exacerbations varied from 62 to 90% (26). These different flare rates between different rheumatic diseases are probably due to (1) sex hormones such as estrogen might play different role in different diseases (27). And, (2) maternal shift from a Th1 to Th2 immune response during pregnancy. What is more, Léo Plaçais et al. reviewed 19 AOSD cases during pregnancy. They found that none of the cases presented as a chronic articular form and no obvious difference was found between monocyclic and polycyclic patterns. Our results showed that new-onset AOSD during pregnancy and postpartum had a higher percent to evolve

into the polycyclic course (45.4%), which is different from the previous study.

It is still unknown whether AOSD can lead to poor pregnancy outcomes. Previously, Leo reported a case-based review gathering data about 19 cases of AOSD revealed during pregnancy (28). The obstetrical complications occurred in nearly 50% of AOSD patients including prematurity (10/20), pre-term premature rupture of membranes (3/20), intrauterine growth restriction (3/20), oligohydramnios (2/20), or neonatal death (1/20). In our study, 18.8% (3/16) of diagnosed AOSD had STA, and the generalized linear mixed model and propensity score matching method demonstrated that AOSD contributed to an increased risk of STA. In addition, one patient in gestational group underwent cesarean section due to oligohydramnios, however, the patient was not exposed to any NSAID, which was reported to be associated with low amniotic fluid levels (29). Besides, treatment during pregnancy may increase the incidence of adverse outcome. Some studies indicated that gestational exposure to corticosteroids led to a slightly increased risk of premature birth (30). In our study, one patient underwent induced abortion for fear of abnormal fetal development resulting from methotrexate exposure during pregnancy. As a result, drug factors should be taken into account cautiously.

The treatment of AOSD during pregnancy is challenging. Glucocorticoids are a mainstay of treatment for patients with AOSD (31, 32), despite the potential increased risk of gestational diabetes, arterial hypertension, intrauterine growth restriction, or pre-term premature rupture of membranes (33–36). Intravenous immunoglobulin (IVIG) has been reported for the management of AOSD during pregnancy (37), especially for the life-threatening complications. Recently, Smith reported Anakinra was successfully used in five patients during pregnancy with no serious complications or adverse pregnancy outcomes (38), which provided alternative therapies for pregnancy-related AOSD.

Currently, our study provided the largest sample of AOSD patients to clarify the relationship between pregnancy and AOSD. The enrolled patients had a similar gestational age to the reported distribution of the nationwide survey, which makes our sample more representative. What is more, through a mixed effect logistic regression model, we found post-AOSD is robust associated with STA. We also performed the propensity score matching method to reduce the effects of confounding in our observational study. However, there are still some limitations. The fact that results only from single tertiary center give the generalizability many limitations, so multi-centered studies should be conducted in order to further confirm the interaction between pregnancy and AOSD. Moreover, the follow-up sample about gestational and postpartum groups is too small to reach a definitive conclusion of disease evolution; as a result, prospective studies with a larger sample size are needed in the future.

CONCLUSIONS

In summary, we found that pregnancy in patients diagnosed with AOSD was associated with an increased risk of complicated pregnancies, which should be anticipated by both rheumatologists and obstetricians. However, the disease activity of AOSD was not exacerbated by pregnancy if it was well-controlled, which emphasizes the importance of disease evaluation before pregnancy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University (ID: 2016-61). According to the Declaration of Helsinki, informed consent was obtained from each patient.

AUTHOR CONTRIBUTIONS

ZW and HC performed statistical analysis and drafted the manuscript. QD, TF, LW, JT, QH, JJ, TL, XW, and ZZ collected the data. TZ, HL, XC, JY, HS, and YSun extracted the data. CY and YSu conceived the study and contributed to discussion. All authors reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.566738/full#supplementary-material

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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.

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A Cohort Study of Liver Involvement in Patients With Adult-Onset Still's Disease: Prevalence, Characteristics and Impact on Prognosis

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Objective: Adult-onset Still's disease (AOSD) is a systemic disorder commonly accompanied by liver involvement. This study aims to illustrate the detailed information of liver abnormalities in patients with AOSD and evaluate the impact on the prognosis.

Methods: A total number of 128 hospitalized patients, who met the Yamaguchi criteria of AOSD in the Department of Rheumatology and Immunology, Ruijin Hospital from July 2016 to August 2019 were consecutively enrolled and followed up. The demographic characteristics, clinical features, laboratory tests, treatments and prognosis were recorded. Correlations of liver function tests (LFTs) with disease activity and laboratory parameters were analyzed by the Spearman test. Risk factors of the refractory AOSD were evaluated by multivariate logistic regression analysis.

Results: Liver involvement was presented in 104 (81.3%) patients with AOSD. We observed that 34 (32.7%) patients were with mild elevation, 32 (30.8%) patients were with moderate elevation, and 38 (36.5%) patients were with severe elevation. The majority of elevated ALT, AST and ALP decreased to normal within the range of 2 months, except for GGT. Furthermore, the LFTs were found significantly correlated with disease activity. Besides, we found patients with higher levels of LFTs tended to require more intensive treatments and suffered from poorer prognosis. Multivariate logistic regression analysis showed ALP $\geq$ 141 IU/L and GGT $\geq$ 132 IU/L are independent risk factors of refractory AOSD.

Conclusion: Liver involvement is common in patients with AOSD, the levels of LFTs are associated with disease activity and related to the treatment strategies and prognosis.

Keywords: adult-onset Still's disease, liver involvement, refractory, treatment, prognosis

INTRODUCTION

Adult-onset Still's disease (AOSD) is a rare systemic autoinflammatory disorder. The etiology and pathogenesis of AOSD still mostly undetermined (1, 2). Patients with AOSD often presented with high-spiking fevers, evanescent skin rash, arthralgia/arthritis, neutrophilic leukocytosis and hyperferritinemia. In addition to these major manifestations, liver involvement is common but very heterogeneous, ranging from minimal liver enzyme elevation to life-threatening fulminant hepatic failure (3–7). The prevalence of elevated transaminases varies from 23 to 94%, according to different studies. However, a majority of them reported that more than half of the patients had abnormal liver function tests (LFTs) (3, 8–13). Although only limited data revealed the characteristics and outcomes of liver involvement in patients with AOSD, the detailed features of liver involvement remain rather scarce. For example, the time needed for recovery of the abnormal LFTs was ambiguous, and the relationship of LFTs with treatment and prognosis is undermined (3, 11).

The present study aims to illustrate the detailed information, treatment strategies and outcomes of liver involvement in patients with AOSD, to analyze the correlations of LFTs with other laboratory values and to further explore the prognostic importance.

MATERIALS AND METHODS

Patients

A total of 128 AOSD patients admitted to the Department of Rheumatology and Immunology, Ruijin Hospital from July 2016 to August 2019 were consecutively enrolled and followed up. All patients met the Yamaguchi diagnostic criteria (14). Besides, patients with a history of alcohol abuse, evidence of other chronic hepatobiliary or pancreatic diseases were excluded. Informed consent was obtained from all patients, and the clinical records were anonymized before analysis. This survey was approved by the Institutional Research Ethics Committee of Ruijin Hospital (ID: 2016–62) and was conducted following the Principles of the Declaration of Helsinki.

Data Collection

The demographic characteristics, comorbidities, clinical features, laboratory values, and treatment strategies were collected. The following clinical features were recorded: fever, typical rash, arthralgia, arthritis, myalgia, lymphadenopathy, sore throat, splenomegaly, hepatomegaly, abdominal pain, and sore throat. The splenomegaly, hepatomegaly and lymphadenopathy were evaluated by ultrasound or computed tomography (CT) scans. Pleural effusion or pleuritis and pneumonia were assessed by CT scans. Pericarditis was confirmed by echocardiography or CT scans.

The laboratory parameters recorded including complete blood counts, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), prealbumin (preAlb), albumin (Alb), total bilirubin (TBil), prothrombin time (PT), lactate dehydrogenase (LDH), ferritin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), levels of interleukin(IL)-1β, the soluble receptor of IL-2 (sIL-2R), IL-6, IL-8, IL-10, IL-18 and tumor necrosis factor (TNF)-α, and profiles of T-cell subsets ($CD3^+$, $CD4^+$, and $CD8^+$) and B-cell subsets ($CD19^+$ and $CD20^+$). All laboratory tests were performed in a core laboratory.

The liver involvement was defined as hepatomegaly and/or elevation of any LFT throughout the disease course. The LFTs including ALT, AST, ALP, and GGT. The upper limits of normal (ULN) are 40 IU/L in ALT and AST, 126 IU/L in ALP, and 64 IU/L in GGT. For this study, the peak values of LFTs were recorded and the levels of LFTs elevation were categorized into normal, mild (higher than ULN but $\leq$2 ULN), moderate (higher than 2 ULN but $\leq$5 ULN) and severe (>5 ULN) according to times to ULN. Patients were grouped as normal, mild, moderate or severe LFT abnormality according to the highest level of elevation among the four LFTs. The LFTs were followed at various points during hospitalization and follow-up clinic visits to count the recovery time. However, the recovery time of three patients who died in the hospital was obtained.

The systemic score proposed by Pouchot et al. was calculated to evaluate the disease activity and severity (3). This scoring system counts the total number of the following 12 manifestations: fever, typical skin rash, sore throat, myalgia, abdominal pain, pneumonia, pleuritis, pericarditis, splenomegaly, lymphadenopathy, hepatomegaly or abnormal liver function tests, and leukocytes > 15,000/mm^3. The diagnosis of hemophagocytic lymphohistiocytosis (HLH) was based on 2004-HLH criteria (15).

The treatment strategies were also recorded. The dosages of glucocorticoid were calculated equivalent to prednisolone, and a dose equivalent to prednisolone more than 100 mg was classified as a very high dose (16). Besides, refractory AOSD was defined as active disease status despite prednisolone over 1 mg/kg/day for more than 1 week with or without disease-modifying antirheumatic drugs (DMARDs). This was further confirmed by two experienced rheumatologists. The disease course of patients with AOSD was divided into three distinct types: monocyclic, polycyclic and chronic courses over more than 1 year of follow-up (1). The joint radiographs were obtained from patients with joint involvement. The disease pattern was considered a "chronic articular pattern" when patients had radiographic joint space narrowing, erosion, or ankylosis, otherwise, it was a systemic pattern (17).

Statistics

Variables were presented as frequency counts (%) for categorical variables and median (interquartile range, IQR) for continuous data, while the values of LFTs were presented as median [range]. The continuous data were compared using Mann-Whitney *U*-test for two groups or Kruskal–Wallis tests for multiple groups. Proportions were analyzed using χ^2 test or Fisher's exact test, as appropriate. Spearman correlation test was used to assess the correlations between LFTs with different variables. Receiver-operating characteristic (ROC) analyses were calculated to determine values at the maximum Youden index as cut-off points for continuous variables. Variables (including socio-demographic

TABLE 1 | Demographic and selected clinical features of AOSD patients.

Variables	All patients $n = 128$	Patients with liver involvement $n = 104$	Patients without liver involvement $n = 24$	*p*-values
Age (years)	35 (27, 47)	35 (27, 47)	35 (28, 49)	0.903
Female	102 (79.7)	79 (76)	23 (95.8)	**0.027**
Body mass index (kg/m^2)	21.47 (19.48, 23.31)	21.47 (19.40, 23.04)	21.59 (19.66, 23.57)	0.696
Diabetes mellitus	9 (7)	8 (7.7)	1 (4.2)	1.000
Hypertension	8 (6.3)	6 (5.8)	2 (8.3)	0.643
Disease duration (month)	2.3 (1.03, 10.04)	1.88 (0.92, 8.17)	4.46 (1.43, 18.37)	**0.035**
Fever > 39°C	121 (94.5)	98 (94.2)	23 (95.8)	1.000
Typical skin rash	57 (44.5)	44 (42.3)	13 (54.2)	0.292
Pleuritis	60 (46.9)	54 (51.9)	6 (25)	**0.017**
Pneumonia	44 (34.4)	41 (39.4)	3 (12.5)	**0.016**
Pericarditis	30 (23.4)	30 (28.8)	0 (0)	**0.001**
Myalgia	65 (50.8)	54 (51.9)	11 (45.8)	0.591
Splenomegaly	68 (53.1)	59 (56.7)	9 (37.5)	0.089
Hepatomegaly	18 (14.1)	18 (17.3)	0 (0)	**0.024**
Lymphadenopathy	115 (89.8)	92 (88.5)	23 (95.8)	0.460
Sore throat	103 (80.5)	82 (78.8)	21 (87.5)	0.406
Abdominal pain	12 (9.4)	11 (10.6)	1 (4.2)	0.462
Arthralgia	120 (93.8)	96 (92.3)	24 (100)	0.350
Arthritis	56 (43.8)	45 (43.3)	11 (45.8)	0.819
Leukocytes > 15,000/mm^3	79 (61.7)	63 (60.6)	16 (66.7)	0.580
Ferritin > 5 ULN	99 (77.3)	85 (81.7)	14 (58.3)	**0.014**
Systemic score	7 (5, 8)	7 (6, 8)	5 (4.25, 6)	**<0.001**

Data are presented as median (IQR) for continuous variables, and as frequency counts (%) for categorical variables. ULN, upper limit of normal. A $p < 0.05$ is shown in bold type.

variables, clinical features, disease activity score and laboratory values) were further assessed by logistic regression analyses to estimate the risk for refractory AOSD. Variables identified in univariate analyses ($p < 0.05$) were then entered into a forward stepwise multivariable logistic regression model. All *p*-values were two-sided, and a $p < 0.05$ was considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics for Mac, version 26.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Demographic Characteristics and Clinical Features of 128 Patients With AOSD

A total of 128 cases with AOSD were enrolled in the present study, and the characteristics of the participants are summarized in **Table 1**. The median age of the patients was 35 (27, 47) years old with a female predominance (79.7%). The most common clinical presentations were high fever (94.5%), arthralgia (93.8%), lymphadenopathy (89.8%), liver involvement (81.3%), and sore throat (80.5%). 77.3% of patients with AOSD had ferritin > 5 ULN and 61.7% had leukocytes > 15,000/mm^3. The median systemic score was 7 (5, 8). We identified 104 (81.3%) patients with AOSD who had liver involvement, of which 18 (17.3%) had hepatomegaly. Besides, we found male patients tend to have liver involvement ($p = 0.027$), while no significant differences were observed in age, body mass index, or comorbidities between patients with and without liver involvement. Of note, patients with liver involvement had significantly higher systemic score ($p < 0.001$) and increased possibility of pleuritis ($p = 0.023$), pneumonia ($p = 0.016$), pericarditis ($p = 0.001$), and ferritin > 5 ULN ($p = 0.027$). Patients with liver involvement tended to exhibit a severer clinical picture with raised disease activity.

The Liver Abnormalities and Recovery Time of LFTs in AOSD Patients With Liver Involvement

The features of liver abnormalities in patients with liver involvement were shown in **Table 2**. We found all patients with hepatomegaly had elevated LFTs. Among patients with liver involvement, 81.7% had elevated ALT, 87.5% had elevated AST, 51.0% had elevated ALP, and 72.1% had elevated GGT throughout the disease course. Furthermore, 78 (75.0%) patients had decreased preAlb, 88 (84.6%) patients had decreased Alb, 6 (5.8%) patients had elevated TBil, and 3 (2.9%) patients had prolonged PT. The median of peak ALT was 99 [11, 3,436] IU/L; the peak AST was 87 [16, 3,237] IU/L; the peak ALP was 132.5 [38, 491] IU/L; and the peak GGT was 117 [14, 696] IU/L. To evaluate the levels of elevation in patients with AOSD, we grouped the patients according to the highest levels of elevation among four LFTs and identified 34 (32.7%) patients with mild

TABLE 2 | LFTs and associated laboratory values of patients with liver involvement.

Variables	$n = 104$
Elevation of ALT	85 (81.7)
Elevation of AST	91 (87.5)
Elevation of ALP	53 (51.0)
Elevation of GGT	75 (72.1)
Peak ALT (IU/L)	99 [11, 3436]
Peak AST (IU/L)	87 [16, 3237]
Peak ALP (IU/L)	132.5 [38, 491]
Peak GGT (IU/L)	117 [14, 696]
Levels of elevation	
Mild (ULN < LFTs ≤ 2 ULN)	34 (32.7)
Moderate (2 ULN < LFTs ≤ 5 ULN)	32 (30.8)
Severe (LFTs > 5 ULN)	38 (36.5)
PreAlb < 180 mg/L	78 (75.0)
Alb < 35 g/L	88 (84.6)
TBil > 24 μmol/L	6 (5.8)
PT > 16 s	3 (2.9)
Hepatomegaly	18 (17.3)

Data are presented as median [range] for the peak values of LFTs and as frequency counts (%) for categorical variables. LFTs, liver function tests; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ULN, upper limits of normal; preAlb, prealbumin; Alb, albumin; TBil, total bilirubin; PT, prothrombin time.

elevation, 32 (30.8%) patients with moderate elevation, and 38 (36.5%) patients with severe elevation.

In addition, the distribution of LFTs recovery time was described in **Figure 1** according to the levels of elevation, respectively. In general, the majority of elevated ALT, AST, and ALP decreased to normal within the range of 2 months, except for GGT. The mild elevated LFTs dropped to normal in a month were 63.0% in ALT, 70.3% in AST, 80.5% in ALP and 60.0% in GGT. Besides, 66.7% of the moderate elevated AST and 55.6% of moderate elevated ALP could recover in a month. The elevated AST seems to recover faster regardless of the levels of elevation. Only 16.7% of severe elevated AST recovered after 2 months. Conversely, it took more than 2 months for over one-third of moderate (37.0%), severe (39.3%) elevated ALT, and moderate (33.3%) elevated ALP, and at least half of moderate (50.0%) and severe (68.8%) elevated GGT took more than 2 months to recover. It appears that the recovery time was related to the levels of elevation. Typically, higher levels of elevated GGT had significantly longer recovery period ($p = 0.009$).

The Correlations Between Baseline LFTs, Prealbumin and Albumin With Disease Activity Score and Laboratory Values

To investigate the relationship between liver function and disease activity, we conducted a correlation matrix based on Spearman r values between LFTs, preAlb and Alb with disease activity score as well as laboratory values (**Figure 2**). All LFTs were significantly positively correlated with the adjusted systemic score, LDH, and ferritin, while the preAlb and Alb were negatively correlated with the systemic score, leukocytes, N%, ESR, CRP, LDH, and ferritin. The AST had the highest correlation with the LDH ($r = 0.781$, $p < 0.0001$). Moreover, ALP was correlated with leukocytes, N%, CRP. Collectively, LFTs were associated with disease activity score and relevant laboratory tests.

In addition, we further explored the associations of LFTs, preAlb and Alb with IL-1β, sIL-2R, IL-6, IL-8, IL-10, and TNF-α (**Figure 2**). The AST was very highly correlated with sIL-2R ($r = 0.599$, $p < 0.0001$), followed by IL-18 ($r = 0.443$, $p < 0.0001$), IL-10 ($r = 0.368$, $p = 0.0001$), and TNF-α ($r = 0.347$, $p = 0.0004$). The ALT correlated with sIL-2R ($r = 0.238$, $p = 0.015$) and IL-18 ($r = 0.266$, $p = 0.007$). The preAlb significantly correlated with multiple cytokines including sIL-2R ($r = -0.465$, $p < 0.0001$), IL-6 ($r = -0.522$, $p < 0.0001$), IL-10 ($r = -0.373$, $p = 0.0001$), and TNF-α ($r = -0.297$, $p = 0.0027$), and the Alb was negatively correlated with sIL-2R ($r = -0.438$, $p < 0.0001$), IL-10 ($r = -0.316$, $p = 0.0012$) and TNF-α ($r = -0.280$, $p = 0.0046$).

To date, the expression of peripheral T and B cell subsets and the associations with LFTs were not elucidated. Interestingly, we found AST was positively correlated with the proportion of CD8+ T cells ($r = 0.292$, $p = 0.004$), and negatively correlated with CD4+ Tcells ($r = -0.254$, $p = 0.013$), and the percentage of CD19+ ($r = -0.403$, $p = 0.0002$) and CD20+ ($r = -0.426$, $p = 0.0005$) B cells.

The Treatments and Outcomes of Patients According to the Levels of LFTs

To investigate the influence of LFTs on the treatments, the therapy strategies were compared among patients with different levels of LFTs (**Table 3**). Our analysis showed that patients with higher levels of elevation were ultimately prescribed with higher dosages of glucocorticoid ($p < 0.001$); moreover, 63.2% of patients with severe elevation required a very high dose of glucocorticoid ($p < 0.001$). With regards to DMARDs, patients with higher levels of LFTs were more often treated with cyclosporine A (CsA) ($p = 0.015$), intravenous immunoglobulin (IVIG) ($p < 0.001$) and etoposide ($p < 0.001$), while less likely to be treated with methotrexate (MTX) ($p < 0.001$) and hydroxychloroquine (HCQ) ($p = 0.014$). There was no significant difference in the application of biologics. Among all receiving biologic agents, one patient with a severe elevation of LFTs was refractory to multiple therapeutic options and was finally treated with glucocorticoid combined with tofacitinib plus anakinra during follow-up. As a result, patients with high levels of LFTs tend to require more intensive treatments.

Besides, our results showed that the incidence of HLH ($p < 0.001$) was significantly higher in patients with higher levels of LFTs elevation, the same as the incidence of refractory AOSD ($p = 0.001$). Furthermore, three in-hospital died patients had significantly elevated LFTs with peak ALT 787 [740, 1932] IU/L, AST 3102 [1435, 3237] IU/L, ALP 318 [218, 341] IU/L, and GGT 201 [133, 273] IU/L, and two of them suffered from acute hepatic failure. However, the disease courses and patterns were similar among different levels of LFTs elevation over more than 1-year follow-up.

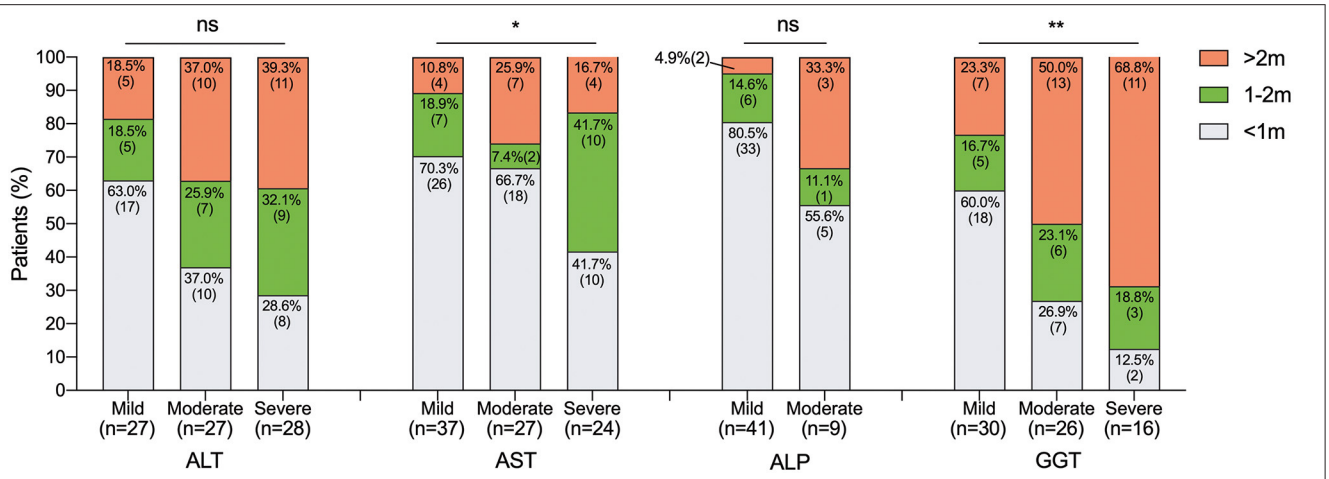

FIGURE 1 | Recovery time of LFTs according to the levels of elevation. Data are expressed as % (*n*). Ns, not significant, $^{*}p < 0.05$, and $^{**}p < 0.01$. LFTs, liver function tests; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; m, month.

The Predictive Values of the LFTs in Refractory AOSD

The response to therapy varied in patients with AOSD and was difficult to predict. As shown in **Table 3**, we found patients with significant liver involvement tend to be refractory AOSD. To evaluate the predictive values of the clinical and laboratory variables in refractory AOSD, we further performed logistic regression analyses (**Table 4**). The univariate analyses indicated that these factors significantly associated with refractory AOSD: fever, skin rash, arthritis, splenomegaly, hepatomegaly, pleuritis, pneumonia, ferritin $\geq$ 3,427 ng/ml, ESR $\geq$ 69 mm/h, CRP $\geq$ 127.5 mg/L, N% $\geq$ 86.3%, Hb $\leq$ 109.5 g/L, LDH $\geq$ 450 IU/L, ALT $\geq$ 87 IU/L, AST $\geq$ 111 IU/L, ALP $\geq$ 141 IU/L, GGT $\geq$ 132 IU/L, preAlb $\leq$ 163 mg/L, Alb $\leq$ 31.5 g/L or PT $\geq$ 13.55s. Given the association between many of the covariates, multiple logistic regression analysis was then performed, simultaneously including all variables with statistical significance. Multivariable logistic regression analysis by stepwise forward selection identified the skin rash (OR: 5.66; 95%CI: 1.06, 30.11), splenomegaly (OR: 5.27; 95%CI: 1.77, 15.67), ESR $\geq$ 69 mm/h (OR: 6.95; 95%CI: 2.17, 22.21), ALP $\geq$ 141 IU/L (OR: 5.48; 95%CI: 1.40, 21.55) and GGT $\geq$ 132 IU/L (OR: 5.13; 95%CI: 1.30, 20.22) as independent predictors of refractory AOSD. Thus, the LFTs may be predictors for refractory AOSD, especially ALP and GGT, which are independent risk factors.

DISCUSSION

AOSD is a systemic inflammatory disease, and liver involvement is frequently observed but quite heterogeneous with non-specific histopathologic changes (3). However, the characteristics and prognosis of liver involvement remain not well-elucidated. Herein, we first described the detailed data of LFTs in patients with AOSD, analyzed the associations with disease activity score and laboratory values, represented the distribution of recovery time, and demonstrated the prognostic importance of LFTs.

The presence of liver involvement varies in different countries and races (3, 8–11, 13). Zhu et al. found that 62.3% of the patients with AOSD had abnormal transaminases, 32.9% had elevated ALP, and 48.1% had elevated GGT (11). The higher incidence of abnormal LFTs in our cohort may related to the longer observation period and the enrolment of only hospitalized patients. Besides, the liver abnormalities in patients with AOSD were considered mainly a mild to moderate increase in aminotransferase activity (1). Zhu et al. revealed one-quarter of the patients with abnormal transaminases were five times higher than ULN (11). Consistently, the elevation of LFTs was mild to moderate in more than half of the patients, while more than one-third of the patients had severe LFTs elevation. The abnormal liver enzymes are usually mild cytolysis, but severe cytolysis and cholestasis may occur (3, 11). Formerly, the elevation of liver enzymes was reported mostly transient (3, 11, 18). Our study, for the first time, described the distribution of recovery time of abnormal LFTs. However, the recovery time was not as quick as expected, especially in GGT. In general, liver involvement is very common and, in most cases, not severe. But high levels of LFTs could occur in some patients so that close monitoring would be needed.

The correlation analyses of LFTs with disease biomarkers showed a significant correlation with LDH, an indicator of cell death and tissue damage (19). Besides, strong associations were found with ferritin, high levels of which act as a sign of macrophage activation and have been hypothesized as a pathogenic protein contributing to the development of a self-perpetuating cytokine storm (20, 21). The cytokine cascade plays an important role in the pathogenesis of AOSD (1). Regarding the cytokines, we found that AST had the strongest correlation with sIL-2R, a truncated protein cleaved from the IL-2Rα protein when T cells are activated, acting as a surrogate indicator of T cell activation as well as an important diagnostic marker of

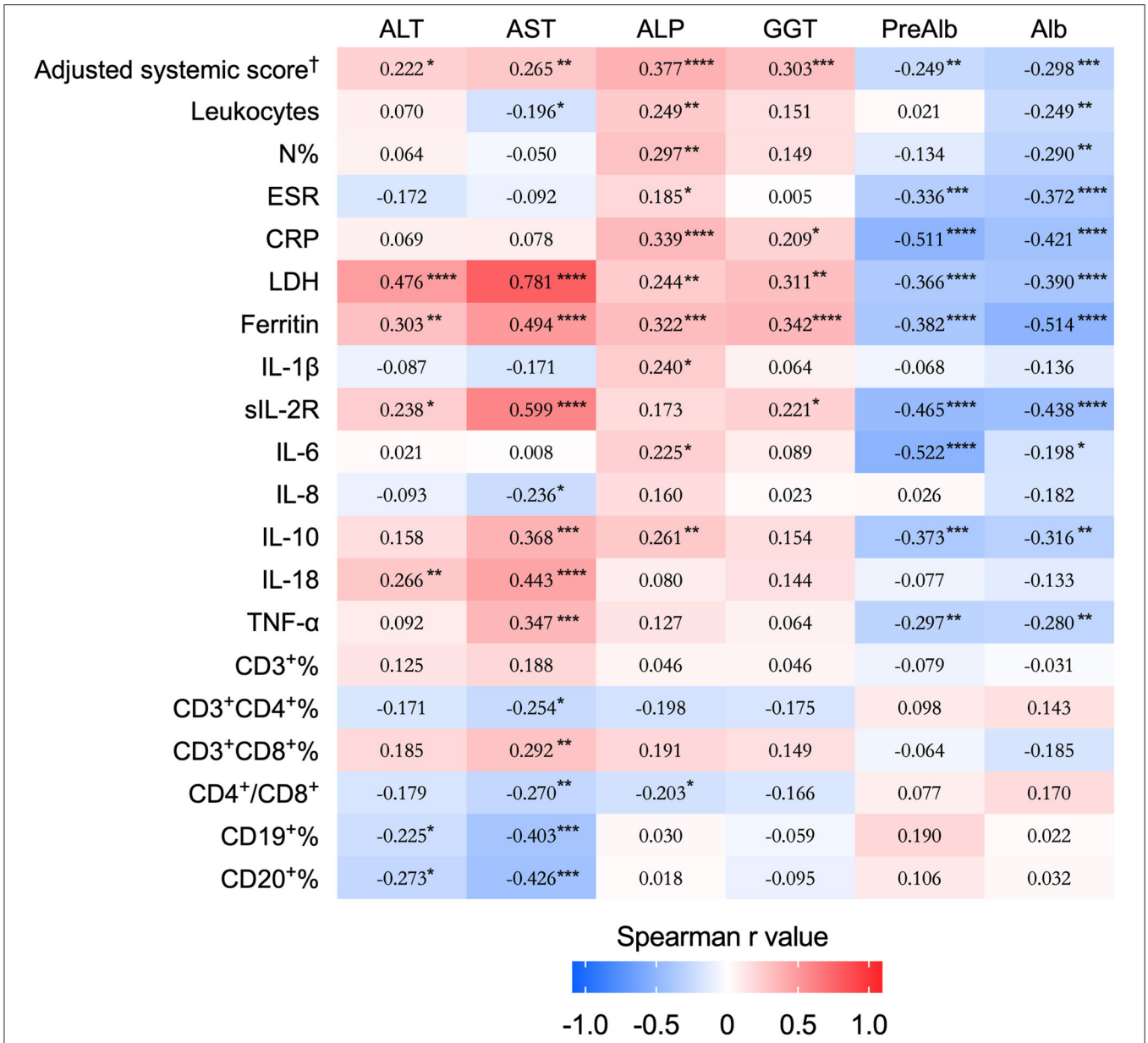

FIGURE 2 | Correlation matrix of LFTs and prealbumin and albumin with disease activity score and laboratory parameters. The heat-map displays the Spearman's rank coefficient to show the correlation strength, and the significances are presented as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. †The adjusted systemic score excluded the item "hepatomegaly or abnormal liver function" from the calculation of the score. LFTs, liver function tests; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; preAlb, prealbumin; Alb, albumin; N%, the percentage of neutrophils; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactate dehydrogenase; IL, interleukin; sIL-2R, soluble receptor of IL-2; TNF, tumor necrosis factor.

HLH, followed by IL-18, IL-10, and TNF-α, cytokines reflecting the diseases activity of AOSD (22–24). Previously study showed that IL-18 markedly increased in AOSD patients, and Priori et al. revealed intensive expression of macrophage-derived IL-18 in liver parenchyma in an AOSD patient with hepatitis, which indicated that IL-18 may contribute to liver damage (6, 25, 26).

The adaptive immunity is also considered involved in the pathogenesis of AOSD including deficiency in regulatory T cells (26). We found the percentage of $CD8^+$ T cells positively associated with AST, while the $CD4^+/CD8^+$ ratio, the rates of $CD19^+$ and $CD20^+$ subsets of B cells correlated negatively. Jung et al. further explored the T cell differentiation in 14 patients with AOSD. They demonstrated that ALT and AST positively correlated with $CD4^+$ naïve T cells and $CD4^+$ central memory T cells, and negatively correlated with $CD4^+$ effector memory T cells, and ALT positively correlated with $CD8^+$ central memory T cells (27). However, the data of the B-cell subsets were scarce, although a successful treatment of refractory AOSD with B cell

TABLE 3 | The treatments and outcomes of patients according to the levels of LFTs elevation.

	Normal (LFTs ≤ ULN) *n* = 24	Mild (ULN < LFTs ≤ 2 ULN) *n* = 34	Moderate (2 ULN < LFTs ≤ 5 ULN) *n* = 32	Severe (LFTs > 5 ULN) *n* = 38	*p*-values
Treatments					
Application of glucocorticoid	22 (91.7)	33 (97.1)	32 (100)	37 (97.4)	0.401
Dosage (mg)[†]	75 (26.25, 100)	100 (50, 108.13)	100 (56.25, 145.81)	175 (100, 200)	**<0.001**
Very high dose of glucocorticoid[‡]	2 (8.3)	8 (23.5)	9 (28.1)	24 (63.2)	**<0.001**
Application of MTX	19 (79.2)	26 (76.5)	21 (65.6)	12 (31.6)	**<0.001**
Application of CsA	5 (20.8)	7 (20.6)	5 (15.6)	18 (47.4)	**0.015**
Application of HCQ	18 (75)	23 (67.6)	15 (46.9)	15 (39.5)	**0.014**
Application of biologic agents	5 (20.8)	4 (11.8)	6 (18.8)	3 (7.9)[§]	0.404
Tocilizumab	1 (4.2)	1 (2.9)	1 (3.1)	1 (2.6)	
Etanercept	1 (4.2)	1 (2.9)	1 (3.1)	0	
Tofacitinib	3 (12.5)	2 (5.9)	4 (12.5)	2 (5.3)	
Anakinra	0	0	0	1 (2.6)	
Application of IVIG	0	1 (2.9)	3 (9.4)	15 (39.5)	**<0.001**
Application of etoposide	0	0	2 (6.3)	10 (26.3)	**<0.001**
Outcomes					
Disease pattern					
Systemic pattern	22 (91.7)	30 (88.2)	27 (84.4)	35 (92.1)	0.776
Chronic articular pattern	2 (8.3)	4 (11.8)	5 (15.6)	3 (7.9)	
Disease course					
Monocyclic course	12 (50)	24 (70.6)	15 (46.9)	22 (57.9)	0.164
Polycyclic course	7 (29.2)	6 (17.6)	5 (15.6)	8 (21.1)	
Chronic course	5 (20.8)	4 (11.8)	12 (37.5)	5 (13.2)	
Refractory AOSD	6 (25)	11 (32.4)	13 (40.6)	26 (68.4)	**0.001**
HLH	0	2 (5.9)	4 (12.5)	17 (44.7)	**<0.001**
Death	0	0	0	3 (7.9)	0.063

[†] Equivalent to prednisolone; [‡] Dosage equivalent to prednisolone > 100 mg was considered very high dose of glucocorticoid; [§] One patient was prescribed with tofacitinib plus anakinra. Data are presented as median (IQR) for continuous variables, and as frequency counts (%) for categorical variables. A p < 0.05 is shown in bold type. LFTs, liver function tests; ULN, upper limits of normal; MTX, methotrexate; CsA, cyclosporine A; HCQ, hydroxychloroquine; IVIG, intravenous immunoglobulin; HLH, hemophagocytic lymphohistiocytosis.

depletion was reported (28). A thorough profile of peripheral immunophenotyping in patients with AOSD is required to investigate further.

In addition, we first discovered that the levels of preAlb and Alb in patients with AOSD were negatively associated with multiple well-known disease activity biomarkers, which reflect disease activity. Although the reduced levels of preAlb and Alb might result from malnutrition, the systemic inflammation can also suppress the production of preAlb and Alb, as part of the acute phase response (29–32). The decreased levels of these two proteins were also described in rheumatoid arthritis (RA), and the Alb levels were found to reflect disease activity (33, 34). Besides, the serum preAlb or Alb levels were previously revealed associated with poor prognosis in systemic sclerosis, cardiovascular diseases and renal diseases (35–38). In conclusion, the levels of preAlb and Alb are also disease activity markers in patients with AOSD.

Besides, we revealed that patients with higher levels of LFTs had a higher possibility of developing HLH, which is a life-threatening complication of AOSD (39). According to Ruscitti et al., patients with liver involvement had an almost 6-fold higher risk of HLH than those without (39). Néel et al. observed the association of bone marrow hemophagocytosis with AST in AOSD (40). Patients with high levels of LFTs might indicate exaggerated inflammatory responses requiring a high dose of glucocorticoids and/or more powerful immunosuppressants. As secondary HLH is commonly triggered by infections, malignancies, or autoinflammatory/autoimmune disorders, we think that liver function abnormalities should weight more for the diagnose of HLH secondary to AOSD, as it is already included in the recent criteria for MAS in systemic juvenile idiopathic arthritis (sJIA), a continuum of a single disease entity of AOSD (41).

However, it's quite a management dilemma that patients with active AOSD with a severe elevation of LFTs required more intensive therapies, but hepatic toxicity was reported in some drugs (42–44). Although a previous study showed that the presence of LFTs abnormalities does not contraindicate methotrexate prescription, the administration of MTX and HCQ declined with the elevation of LFTs in our cohort (45). Néel et al. showed that the efficacy rate of IVIGs, cyclosporine, and anakinra were 11/27 (41%), 13/18 (72%), and 8/9 (89%), respectively in patients with AOSD admitted to intensive care medicine (ICU) (40). From our results, the application rate of CsA, etoposide,

TABLE 4 | Logistic regression of risk factors of refractory AOSD.

Parameter[†]	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Fever	4.35 (1.91, 9.93)	**<0.001**		
Skin rash	3.43 (1.28, 9.21)	**0.015**	5.66 (1.06, 30.11)	**0.042**
Arthritis	2.44 (1.13, 5.27)	**0.033**		
Splenomegaly	5.00 (2.34, 10.68)	**<0.001**	5.27 (1.77, 15.67)	**0.003**
Hepatomegaly	9.55 (2.04, 44.69)	**0.001**		
Pleuritis	4.02 (1.91, 8.46)	**<0.001**		
Pneumonia	2.65 (1.22, 5.77)	**0.019**		
Ferritin ≥ 3,427 (ng/mL)	4.58 (1.95, 10.73)	**<0.001**		
ESR ≥ 69 (mm/h)	3.31 (1.54, 7.13)	**0.002**	6.95 (2.17, 22.21)	**0.001**
CRP ≥ 127.5 (mg/L)	3.05 (1.24, 7.51)	**0.015**		
N% ≥ 86.3%	2.70 (1.15, 6.34)	**0.032**		
Hb ≤ 109.5 (g/L)	3.40 (1.62, 7.12)	**0.001**		
LDH ≥ 450 (IU/L)	3.80 (1.77, 8.17)	**0.001**		
ALT ≥ 87 (IU/L)	4.88 (2.20, 10.82)	**<0.001**		
AST ≥ 111 (IU/L)	6.00 (2.43, 14.84)	**<0.001**		
ALP ≥ 141 (IU/L)	8.27 (3.53, 19.38)	**<0.001**	5.48 (1.40, 21.55)	**0.015**
GGT ≥ 132 (IU/L)	8.00 (3.24, 19.73)	**<0.001**	5.13 (1.30, 20.22)	**0.020**
PreAlb ≤ 163 (mg/L)	2.50 (1.13, 5.55)	**0.034**		
Alb ≤ 31.5 (g/L)	2.96 (1.37, 6.42)	**0.006**		
PT ≥ 13.55 (s)	3.50 (1.43, 8.58)	**0.005**		

[†] *ROC analyses were applied to determine values at the maximum Youden index as cut-off points for continuous variables. Data are presented as OR (95%CI). A p < 0.05 is shown in bold type. ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; N%, the percentage of neutrophils; Hb, hemoglobin; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; preAlb, prealbumin; Alb, albumin; PT, prothrombin time; OR, odds ratio; CI, confidence interval.*

IVIGs and a high dose of glucocorticoids was higher in patients with severe elevation of LFTs. To sum up, the patients with higher levels of LFTs may be more severe and ultimately required more intensive treatments in clinical practice.

The combination of heterogeneous symptoms, complex laboratory results and polymorphic prognosis predicts responses to treatment extremely difficult in patients with AOSD. Néel et al. found that the overall response rate of glucocorticoids was only 50% (40). A similar response rate was identified in our cohort; in addition, we found that refractory AOSD was more common in patients with a higher level of LFTs. However, a predictive tool for refractory AOSD was not available yet. Our analyses showed that multiple factors could reflect the risk of refractoriness; more importantly, two of the LFTs (ALP ≥ 141 IU/L and GGT ≥ 132 IU/L) were independent predictive factors for refractory AOSD. Several newly biologic agents, such as anakinra, tocilizumab and tofacitinib, showed strong efficacy and steroid-sparing effects in AOSD patients (46–49). As a result, the prediction of refractory AOSD may be helpful in timely tailoring therapy to improve the prognosis.

Our study provided details of liver involvement in AOSD patients with relatively large sample size. We revealed a high proportion of patients with elevated LFTs and recorded the recovery time of liver enzymes. Also, we analyzed the association of LFTs with detail clinical features and various cytokines, treatment strategies and prognosis. Furthermore, we explored the risk factors that contributed to refractory AOSD and proposed potential predictive factors for the first time. However, there are still some limitations. Firstly, due to the retrospective design setting, it's hard to completely exclude the effects of confounding factors such as treatments. Besides, this study was based on hospitalized patients in a tertiary hospital which might lead to selection bias. As a result, multi-center well-designed studies are needed to verify the results of our cohort.

CONCLUSION

To sum up, our study confirmed that the liver involvement was common; meanwhile, the elevated liver enzymes correlated with disease activity in patients with AOSD and the recovery time of abnormal LFTs was not always as quick as we expected. Besides, patients with higher levels of LFTs tend to receive more intensive treatments and suffer from poorer prognosis. Lastly, several biochemical biomarkers could be predictors of refractory AOSD, especially elevated ALP and GGT, which are independent risk factors.

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 human participants were reviewed and approved by The Institutional Research Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YS (25th author), CY, HC, and ZW: study conception and design. HC, JM, PH, LZ, YS (8th author), QH, HZ, LW, HL, XC (12th author), JY, HS, XW, JJ, TL, ZZ, XQ, MW, FW, and XC (23rd author): acquisition of data. HC, YS (25th author), and CY: drafting and revising the article. HC, TF, and JT: analysis and interpretation of data. All authors reviewed this article and approved the final manuscript.

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The Clinical Characteristics of Other HLA-B Types in Chinese Ankylosing Spondylitis Patients

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HLA-B27 has an established relationship with the development of ankylosing spondylitis (AS). After reviewing the HLA-B genotype from 407 Chinese subjects (318 patients and 89 sex-matched controls), we found that 252 patients and 32 controls were HLA-B27(+) and that HLA-B*27:04 was the dominant HLA-B27 subtype ($N = 224$). In all participants, HLA*27:04 homozygous were only detected in two patients. In the HLA-B27(+) group, HLA-B40 was observed in 51 cases and one control ($p < 0.05$, OR = 7.87, 95% CI 1.05–59.0); of these, the most genotype was HLA-B*27:04/B*40:01($N = 38$). Two hundred thirty-nine patients' clinical information was recorded. Cases with HLA-B27/B46 had more peripheral joint involvement (OR = 3.95, 95% CI 1.77–8.79) in HLA-B27(+) AS. HLA-B*15:02 may be a significant risk element to peripheral joint involvement ($p < 0.05$) in HLA-B27(−) patients. Therefore, we believe HLA-B*40:01, HLA-B*46:01, and HLA-B*15:02 can be the test indicators for AS diagnostic value.

Keywords: ankylosing spondylitis, HLA-B40, HLA-B46, HLA-B genotype, peripheral joint involvement

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INTRODUCTION

Human leukocyte antigen (HLA)-B27 is the most critical gene in ankylosing spondylitis (AS). About 90–95% of AS cases were HLA-B27 positive, while only 1–2% of HLA-B27 positive persons can develop to AS (1, 2). Results showed that the occurrence of AS with HLA-B27 appeared in family aggregation. Among the first-degree relatives of HLA-B27 positive AS, the prevalence is 10–30% (3). Above 45 HLA-B27 subtypes, like B*27:02, B*27:10, and B*27:15, were found to be associated with AS, and their distribution varied in different populations (4, 5). B*27:04 is the primary subtype in the Chinese Han population (6), whereas the Caucasian people are dominated by the B*27:05 (4). On the contrary, B*27:06 and B*27:09 are unrelated to AS. Previous research found homozygous B*27:04 can affect AS susceptibility but not its clinical manifestations and functional disability (7, 8). How about HLA-B27 heterozygote with other HLA-B alleles in AS? Our studies aimed to evaluate the influence of heterozygous HLA-B27 on the clinical manifestations of AS patients.

METHODS

Study Subjects

Three hundred eighteen Chinese Han patients and 89 sex-matched controls were recruited from the hospitals in Guangdong Province of China. All patients were older than 18 years old and met the 1984 modified New York criteria for AS (9). Two hundred thirty-nine patients had their clinical information collected by two trained rheumatologists during a face-to-face interview at the study visit. Clinical information included peripheral manifestations (uveitis, peripheral joint involvement, dactylitis, and enthesitis), onset age, body mass index (BMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Bath Ankylosing Spondylitis Functional Index (BASFI). We also collected past and current medications, including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs), and biologic agents. The general information included age, gender, and smoking (current) and drinking history (current). According to the 2009 ASAS classification criteria (either axial or peripheral) (10), patients without any peripheral manifestations (uveitis, peripheral joint involvement, dactylitis, and enthesitis) were classified as the axial AS (axAS). Controls were free of any history of rheumatic disease. Written informed consent was obtained from all the subjects. The ethics committee of the Third Affiliated Hospital of Sun Yat-Sen University approved our study. All participants gave written informed consent before enrollment.

HLA-B Genotype

Genomic DNA was extracted from peripheral blood using a standard salting-out method. All of the individuals were genotyped for HLA-B loci using the polymerase chain reaction sequence-based typing (PCRS-BT) method. Briefly, we performed locus-specific PCR amplification and bidirectional Sanger sequencing of HLA-B exons 2, 3, and 4. Amplification and sequencing of relevant exons was performed using "in-house" primers. Sequencing was performed on a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). The typing results were accomplished using uTYPE v6.0 software (One Lambda, Canoga Park, CA, USA) against the IMGT/HLA database. When encountering ambiguous genotyping results (several genotypic combinations perform identically on sequencing results), alleles were assigned by referring to the most common alleles in the Chinese population (11).

Statistical Analysis

We analyzed the data in two steps. In step 1, we analyzed the HLA-B types in all participants. Then we researched the relationship with HLA-B types and clinical phenotype (**Figure 1**). For continuous variables, we calculated mean ± standard deviation (SD) and percentage for categorical variables. We performed Student's *t*-test or rank-sum test to make group

TABLE 1 | The basic information of all AS and controls (step 1).

	AS (*N* = 318)	HC (*N* = 89)	*p*	OR (95% CI)
Sex			0.483[a]	
Male, *n* (%)	220 (69.2)	65 (73.0)		
Female, *n* (%)	98 (30.8)	24 (27.0)		
Age (years), mean ± SD	29.55 ± 8.83	39.86 ± 18.03	<0.001[b]	
<20	33	5		
20–40	224	41		
40–60	31	18		
60–80	1	9		
B27(+), *n* (%)	252 (79.2)	32 (35.96)	<0.001[a]	6.801 (4.081–11.335)

AS, ankylosing spondylitis; HC, health controls; B27 HLA-B27; OR, odds ratio; CI, confidence interval; [a]Chi-squared test; [b]Student's t-test.

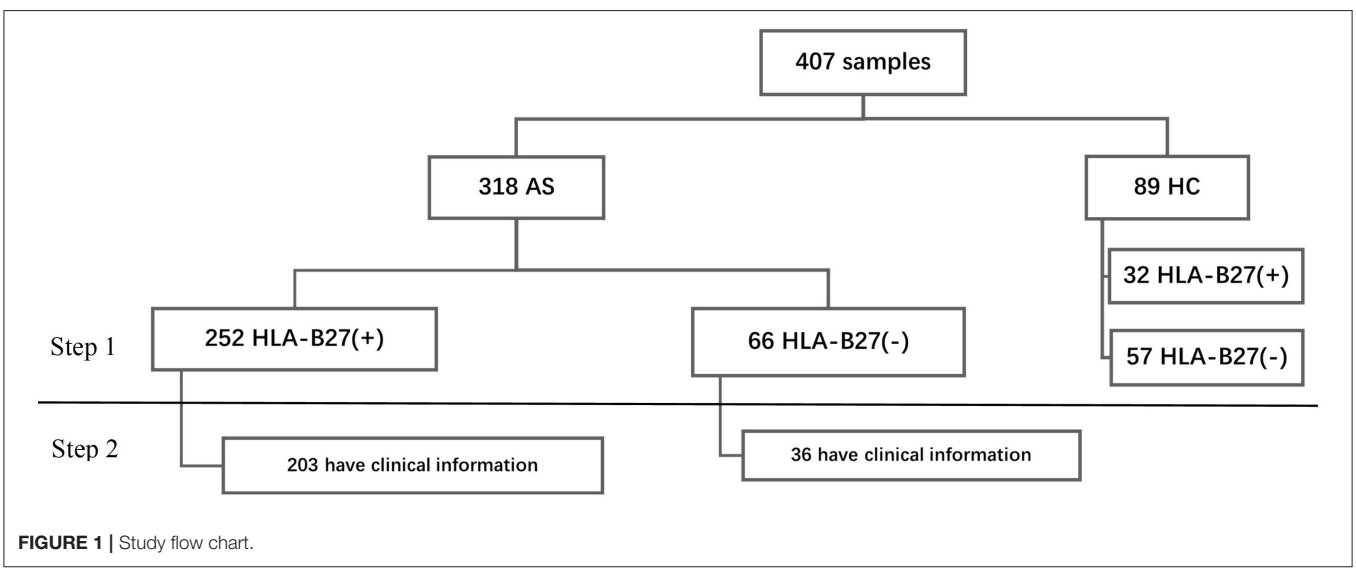

FIGURE 1 | Study flow chart.

comparisons for continuous data and chi-squared tests for categorical variables (Fisher's exact test where appropriate). All contrasts were bilateral and considered significant when $p < 0.05$. Data were collected, processed, and analyzed using the Statistical Package for the Social Sciences (SPSS) software v.19. The heatmaps were drawn by R software v3.6.1.

RESULTS

Step 1

The HLA-B Genotypes Distribution in all Samples

A total of 407 subjects were analyzed using HLA-B typing including 318 AS and 89 sex-matched controls (**Figure 1**) from January 2016 to September 2020. As shown in **Table 1**, the mean age of AS patients was 29.55 ± 8.83 years old and controls was 39.86 ± 18.03 years old. The AS patients were younger than controls ($p < 0.05$). The main age group of patients was under 40 years old. After HLA-B typing, we found 24 low-resolution HLA-B types and 55 high-resolution HLA-B subtypes in all participants, including eight homozygous and 399 heterozygous. The major HLA-B type was HLA-B27. Two hundred fifty-two (79.25%) cases and 32 (35.96%) controls ($p < 0.05$) were HLA-B27(+). Other HLA-B-type distributions are shown in **Figure 2**.

In 252 B27(+) patients and 32 B27(+) controls, HLA-B*27:04 was found in 224 cases (88.89%) and in all 32 controls (100%),

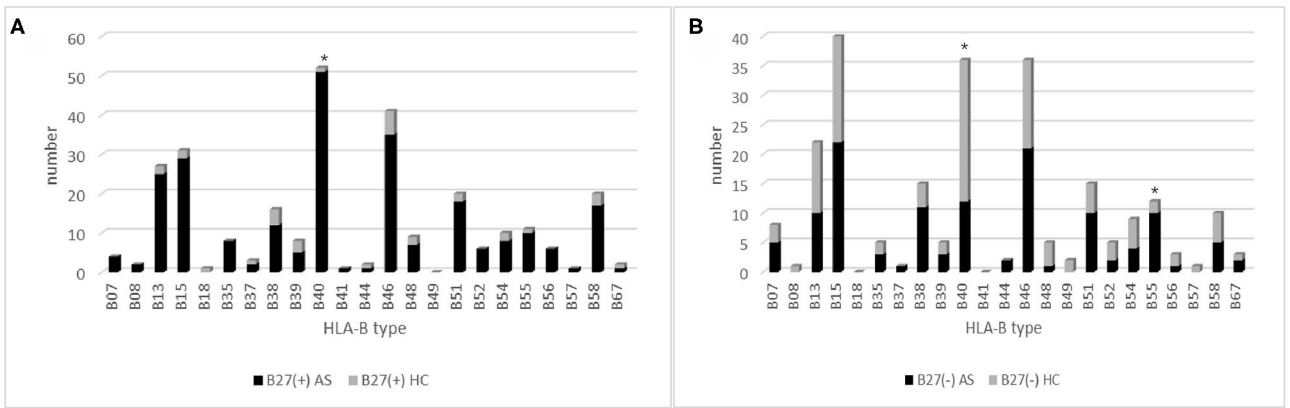

FIGURE 2 | The HLA-B type distribution in HLA-B27(+) and HLA-B27(−) group: **(A)** HLA-B type distribution in HLA-B27(+) patients and HLA-B27(+) controls, and **(B)** HLA-B type distribution in HLA-B27(−) patients and HLA-B$^{(-)}$ controls. Chi-squared test were used to determine significance (*$p < 0.05$). AS, ankylosing spondylitis; HC, health control.

TABLE 2 | The HLA-B27 subtypes in all B27(+) groups and the associated HLA-B types in B27(+) and B27(−) groups (step 1).

	B27(+)				B27(−)			
	AS	**HC**	***p***	**OR (95% CI)**	**AS**	**HC**	***p***	**OR (95% CI)**
N	252	32			66	57		
Sex			0.166				0.171	
Male, *n* (%)	184 (73.0)	27 (84.4)			36 (54.55)	38 (66.67)		
Female, *n* (%)	68 (27.0)	5 (15.6)			30 (45.45)	19 (33.33)		
Age (years), mean ± SD	29.3 ± 8.4	41.2 ± 20.4	0.003		30.7 ± 10.2	39.0 ± 16.4	0.003	
Homozygote	2 (0.8)				4 (6.0)	2 (3.5)		
HLA-B27 subtype, *n* (%)								
B*27:04	224 (88.9)	32 (1.00)	0.095					
B*27:02,	3 (1.2)							
B*27:05	21 (8.3)							
B*27:06	1 (0.4)							
B*27:07	1 (0.4)							
B*27:15	2 (0.8)							
Other HLA-B types, *n* (%)								
B40	51 (20.2)	1 (3.1)	0.018	7.87 (1.05-59.0)	12 (18.18)	24 (42.11)	0.004	0.306 (0.135–0.692)
B*40:01	41 (16.3)	0	0.028		9 (13.64)	21 (36.84)	0.003	0.271 (0.112–0.656)
B55	10 (4.0)	1 (3.1)	0.800		10 (15.15)	2 (3.51)	0.03	4.911 (1.029–23.442)
B*55:02	9 (3.6)	1 (3.1)	0.900		9 (13.64)	2 (3.51)	0.05	
B15	29 (11.5)	2 (3.1)	0.550		22 (33.33)	18 (31.58)	0.84	
B*15:17					0	7 (12.28)	0.011	

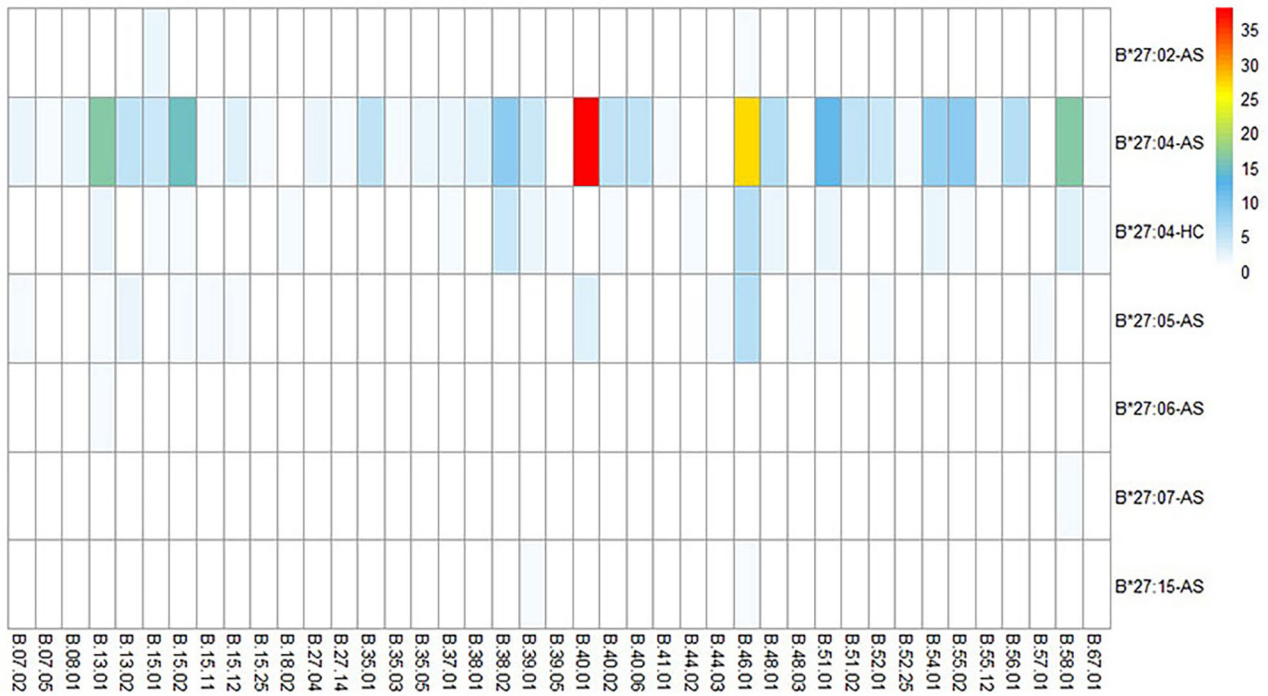

FIGURE 3 | HLA-B alleles heatmap of different HLA-B27 subtypes combined with other HLA-B subtypes. Each square represents a genotype, like HLA-B*27:04/B*40:01. AS, ankylosing spondylitis; HC, health control.

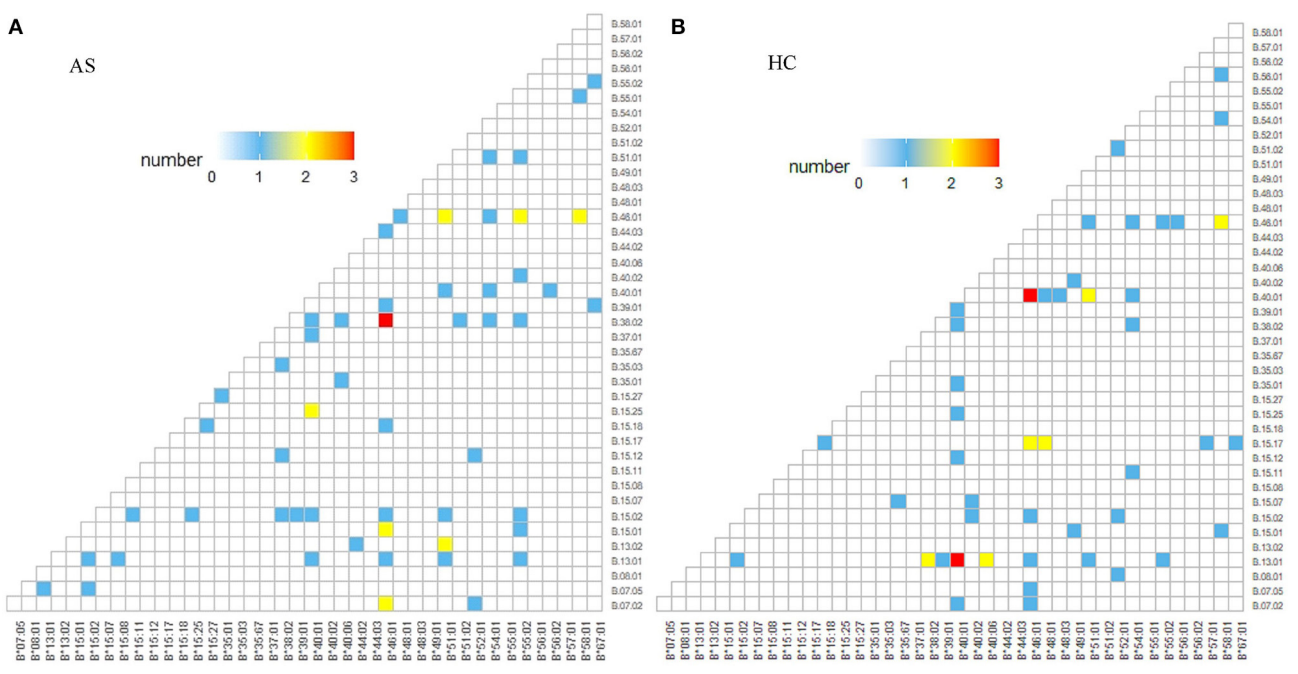

FIGURE 4 | HLA-B alleles heatmap of HLA-B27(−) group. Each square represents a genotype, like HLA-B*40:01/B*46:01: **(A)** HLA-B alleles heatmap of HLA-B27(−) AS patients, and **(B)** HLA-B alleles heatmap of HLA-B27(−) controls. AS, ankylosing spondylitis; HC, health control.

TABLE 3 | Demographic and disease characteristics of the B27(+) and B27(−) AS patients (step 2).

	B27(+)		B27 (−)		*p*
	***N* = 203**	**Mean ± SD or %**	***N* = 36**	**Mean ± SD or %**	
Gender, male	147	72.4	20	55.6	0.042[a]
Age (years)	203	29.66 ± 8.57	36	29.6 ± 11.22	0.990[b]
Onset age (years)	203	22.39 ± 7.58	36	24.39 ± 8.97	0.158[b]
Family history	48	24.1	7	21.9	0.395[a]
Current smoking	30	14.9	3	8.3	0.319[a]
Alcohol consumption	12	5.9	1	2.8	0.715[a]
Body mass index (kg/m^2)	200	21.31 ± 3.01	36	21.37 ± 2.94	0.913[b]
axAS	118	58.1	18	50.0	0.364[a]
Uveitis	34	16.7	5	13.9	0.669[a]
B*27:04	29				
B*27:05	3				
B*27:02	2				
Enthesis	39	19.2	7	19.4	0.974[a]
B*27:04	34				
B*27:05	4				
B*27:02	1				
Peripheral joint involvement	48	23.6	9	25.0	0.860[a]
B*27:04	40				
B*27:05	5				
B*27:15	1				
B*27:02	2				
Dactylitis	9	4.4	1	2.8	0.995[a]
B*27:04	8				
B*27:02	1				
BASDAI	177	2.74 ± 1.86	33	2.82 ± 1.7	0.807[b]
BASFI	178	1.45 ± 2.29	33	1.01 ± 1.74	0.292[b]
NSAIDs use (only)	36	17.7	10	2.8	0.159[a]
DMARDs use					
Methotrexate (ever)	14	6.9	0		0.215[a]
Sulfasalazine (ever)	80	39.4	8	22.2	0.049[a]
Biological therapy (ever)	66	32.5	6	16.7	0.056[a]

axAS, axial ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; NSAIDs, Non-Steroidal Anti-inflammatory Drugs; DMARDs, disease-modifying antirheumatic drugs; [a]Chi-squared test, [b]Student's t-test.

respectively ($p > 0.05$). HLA-B*27:05 was detected in 21 cases (8.3%) but not found in controls. We also observed another HLA-B subtype (one HLA-B*27:07, one HLA-B*27:06, two HLA-B*27:15, three HLA-B*27:02, and one HLA-B*27:14) in patients (**Table 2**). The one with HLA-B*27:14 was a HLA-B*27:04 heterozygote (HLA-B*27:04/B*27:14). Fifty-one cases (15.87%) and one control (3.13%) carried B27/B40 ($p = 0.018$); the majority genotype was HLA-B*27:04/B*40:01 ($N = 38$, **Figure 3**). In HLA-B*27:04 carriers, the HLA-B*40:01 was also associated with AS ($p = 0.024$). In 21 HLA-B*27:05 patients, HLA-B*27:05/B*46:01 was the most HLA-B genotype ($N = 6$, 28.6%) (**Figure 3**). Between HLA-B*27:04 and HLA-B*27:05 patients, the distribution of the HLA-B*40:01 and HLA-B*46:01 had no significant difference. Another HLA-B27 subtype genotype is shown in **Figure 3**.

Between 66 B27(−) patients and 57 B27(−) controls (**Table 2**), HLA-B40 was detected in 12 cases (18.18%) and 24 controls (42.11%) ($p = 0.004$). There was also a significant difference in HLA-B55 between the two groups ($p = 0.03$). At the high-resolution level, we found the number of HLA-B*40:01 and HLA-B*15:17 was significantly higher in the control group as compared to the case group ($p < 0.05$). The number range of every HLA-B heterozygous genotypes was 1 to 3 (**Figure 4**).

Step 2

Comparisons of the Clinical Characteristics Between B27(+) and B27(−) AS Patients

Two hundred thirty-nine patients had detailed clinical information, including 203 B27(+) and 36 B27(−) patients. As observed in **Table 3**, a significant difference was found in sex between two groups ($p = 0.042$), with more male participants in B27(+) patients. However, there was no statistical significance in the current age, age at symptom onset, family aggregation, smoking status, alcohol consumption, BMI, BASDAI, BASFI,

peripheral manifestations (uveitis, peripheral joint involvement, dactylitis, and enthesitis), and medications. In B27(+) patients, we also compared clinical characteristics between HLA-B*27:04 and HLA-B*27:05 cases. We did not find any significant difference (**Table 4**).

TABLE 4 | Disease characteristics of B*27:04 and B*27:05 AS patients (step 2).

	B*27:04	B*27:05	*p*
Number	179	17	
Age, mean ± SD	29.41 ± 8.24	31.00 ± 10.88	>0.05[b]
Age onset, mean ± SD	22.06 ± 7.14	24.53 ± 8.57	>0.05[b]
Sex			>0.05[a]
Male, *n* (%)	128 (71.5)	15 (88.2)	
Female, *n* (%)	51 (28.5)	2 (11.8)	
Peripheral manifestations			
Peripheral joint involvement, *n* (%)	40 (22.3)	5 (29.4)	>0.05[a]
Uveitis, *n* (%)	29 (16.2)	3 (17.6)	>0.05[a]
Enthesis, *n* (%)	34 (19.0)	4 (23.5)	>0.05[a]
Dactylitis, *n* (%)	8 (4.4)		
BASDAI, mean ± SD	2.34j ± 1.73	1.36 ± 2.16	>0.05[b]
BASFI, mean ± SD	1.56 ± 1.40	0.21 ± 0.31	>0.05[b]

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; [a]Chi-squared test; [b]Student's t-test.

The Low-Resolution HLA-B Genotypes Distribution in Different Peripheral Manifestations

We analyzed the low-resolution HLA-B genotypes of B27(+) patients. In 48 peripheral joint involvement patients with B27(+), 15 cases (31.25%) carried HLA-B46. HLA-B40 was observed in seven cases (14.58%). HLA-B58, HLA-B51, and HLA-B13 were detected in four cases (8%), respectively for each type (**Figure 5A**). In 39 B27(+) patients with enthesis (**Figure 5D**), HLA-B46, HLA-B40, and HLA-B15 were found in 11 cases (28.21%), nine cases (23.08%), and four cases (10.26%), respectively. As shown in **Figure 5C**, in 34 B27(+) patients with uveitis, eight cases (23.53%) carried HLA-B46. HLA-B40 and HLA-B15 were detected in seven cases (20.59%) and five cases (14.71%), respectively. There were nine B27(+) patients with dactylitis (**Figure 5B**), and HLA-B46 was observed in three cases (33.33%). Compared with HLA-B40 and other HLA-B types in B27(+) patients, HLA-B46 had relationships with peripheral joint involvement and enthesis, respectively (**Table 5**).

In nine B27(−) patients with peripheral joint involvement patients, seven cases (77.78%) carried HLA-B15. There was a significant difference in HLA-B15 ($p = 0.014$) (**Table 6**). For a small number of HLA-B27(−) patients with other manifestations (uveitis and enthesitis), the HLA-B type distributions are shown in **Figure 6**. One patient with dactylitis has a HLA-B46/B58 in his HLA-B allele.

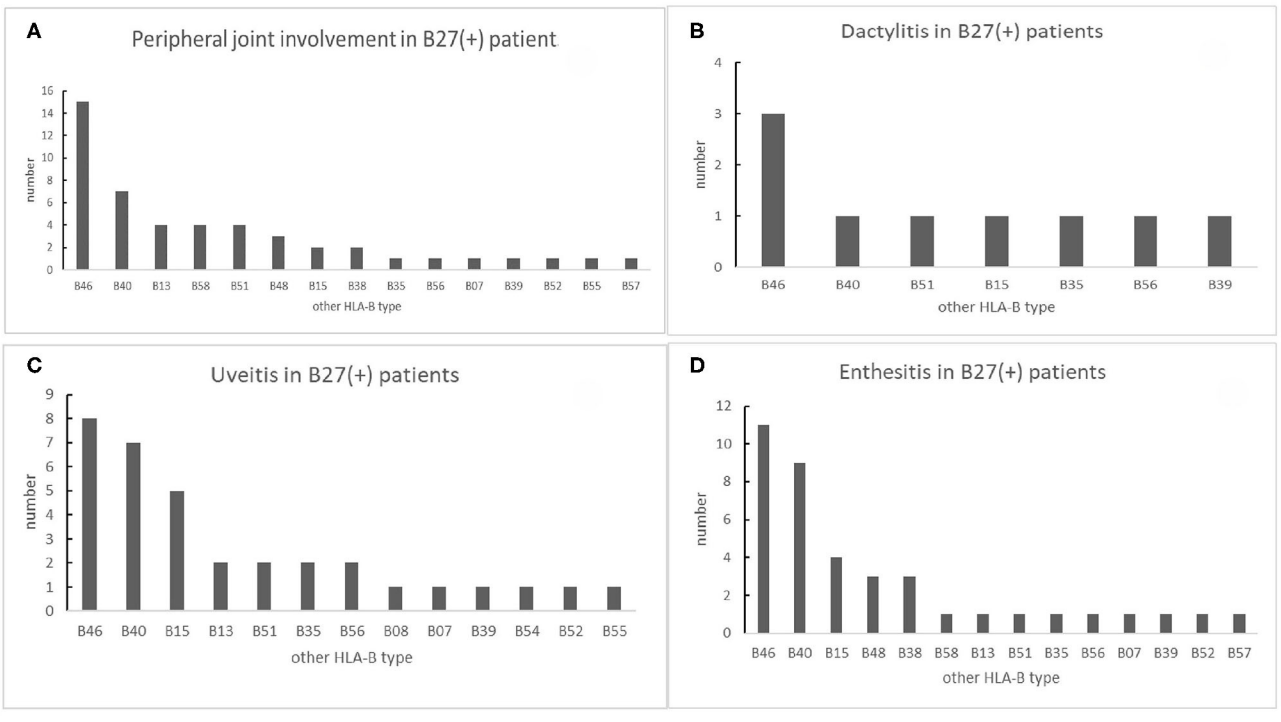

FIGURE 5 | The number of other HLA-B types shown in B27(+) patients with different peripheral manifestations: **(A)** number of other HLA-B types shown in B27(+) patients with peripheral joint involvement, **(B)** number of other HLA-B types shown in B27(+) patients with dactylitis, **(C)** number of other HLA-B types shown in B27(+) patients with uveitis, and **(D)** number of other HLA-B types shown in B27(+) patients with enthesis.

The Clinical Manifestations in HLA-B27 Subtypes Homozygote and Heterozygote AS Patients

The most frequent subtype was HLA-B*27:04 in the B27(+) group, so we further analyzed the clinical manifestations of HLA-B*27:04 homozygous and heterozygous AS patients. One of the HLA-B*27:04/B*27:04 patients was a woman who only had the axial phenotype. An X-ray showed that the front edge of each vertebra of the spine was straight, the facet joints of the thoracolumbar and lumbar vertebra were blurred, that there was bony ankylosing, scoliosis, atlantoaxial subluxation, bilateral sacroiliac joint fusion, narrowed hip space, osteoid destruction, and that the surface of the left ischial tuberosity was rough.

In HLA-B*27:04 heterozygous AS patients, HLA-B*27:04/B*40:01 was the most frequent genotype ($N = 35$), and the second was HLA-B*27:04/B*46:01($N = 24$). But HLA-B*27:04/B*46:01 was significantly more frequent in patients with peripheral joint involvement compared to HLA-B*27:04/B*40:01 ($p = 0.007$, OR=4.833, 95% CI 1.472–15.867). The same to patients with enthesis ($p = 0.037$, OR=3.452, 95% CI 1.044–11.420) (**Table 5**). We did not find other significant peripheral manifestations. One patient with HLA-B*27:04/B*35:05 had all peripheral manifestations (uveitis, peripheral joint involvement, dactylitis, and enthesitis).

All HLA-B*27:05 samples were heterozygote patients. Seventeen patients had clinical information. Among five cases (29.4%) with arthritis, two patients were HLA-B*27:05/B*46:01, and one patient was HLA-B*27:05/B*40:01. There were no different clinical manifestations between B*27:04 and B*27:05 (**Table 4**). Other HLA-B27 subtypes were also heterozygous, and their clinical peripheral phenotypes are shown in **Table 3**. We found one HLA-B*27:02/B*15:01 patient with all the peripheral manifestations.

The Clinical Manifestations in HLA-B27(−) Homozygote and Heterozygote AS Patients

According to high-resolution HLA-B genotypes, in the 36 B27(−) cases with clinical information, the highest HLA-B type was HLA-B*46:01 ($N = 11$, 30.56%). HLA-B*15:02, HLA-B*13:01, HLA-B*38:02, and HLA-B*40:01 were tied for second ($N = 6$). As mentioned earlier, patients with peripheral joint involvement carried HLA-B15 more frequently, especially HLA-B*15:02, which also has an association with the phenotype in B27(−) patients [$p = 0.002_{\text{Fisher}}$, OR=32.5, 95% CI (2.974–355.116)] (**Table 6**). However, the patient with HLA-B*15:02 homozygote was a man who only had axial manifestations in the same way as the HLA-B*27:04 homozygote patient.

DISCUSSION

HLA-B27 as the major gene was closely related to the development of AS. The most prevalent subtypes are HLA-B*27:04 and HLA-B*27:05 in different populations. Other subtypes associated with the disease are B27:02, B*27:15, and so on. In our data, there were B*27:04, B*27:05, B*27:02, and B*27:15 in the patient group, and 88.9% of the patients were HLA-B*27:04. HLA-B27 positive patients had an earlier disease onset and higher family aggregation (12). HLA-B27 negative

TABLE 6 | HLA-B15 and HLA-B*15:02 had association with peripheral joint involvement in HLA-B27(−) AS patients (step 2).

	Peripheral joint involvement	Without peripheral joint involvement	*p*	OR (95% CI)
$B15^{(+)}$ ($N = 14$)	7	7		
$B15^{(-)}$ ($N = 22$)	2	20	0.014 (Fisher)	10 (1.67–60.00)
$B*15:02^{(+)}$ ($N = 9$)	5	4		
$B*15:02^{(-)}$ ($N = 27$)	1	26	0.002 (Fisher)	32.5 (2.974–355.116)

TABLE 5 | Association of HLA-B40 and HLA-B46 with peripheral joint involvement in B27(+) AS patients (step 2).

	Peripheral joint involvement	Without peripheral joint involvement	*p*	OR (95% CI)	Enthesitis	Without enthesitis	*p*	OR (95% CI)
B27(+) AS patients, *n*	48	155	0.002		39	164	0.035	
B27/B40	7	39			9	37		
B27/B46	15	16		3.95 (1.77–8.79)	11	20		2.83 (1.22–6.55)
Other HLA-B types	26	100			19	107		
B27:04 AS patients, *n* (%)			0.007[e]				0.037[e]	
B*27:04/B*40:01 ($N = 35$)	6	29			6	29		
B*27:04/B*46:01 ($N = 24$)	12	12		4.83 (1.47–15.87)	10	14		3.45 (1.04–11.42)

[e]*Chi-squared test between B*27:04/B*40:01 and B*27:04/B*46:01.*

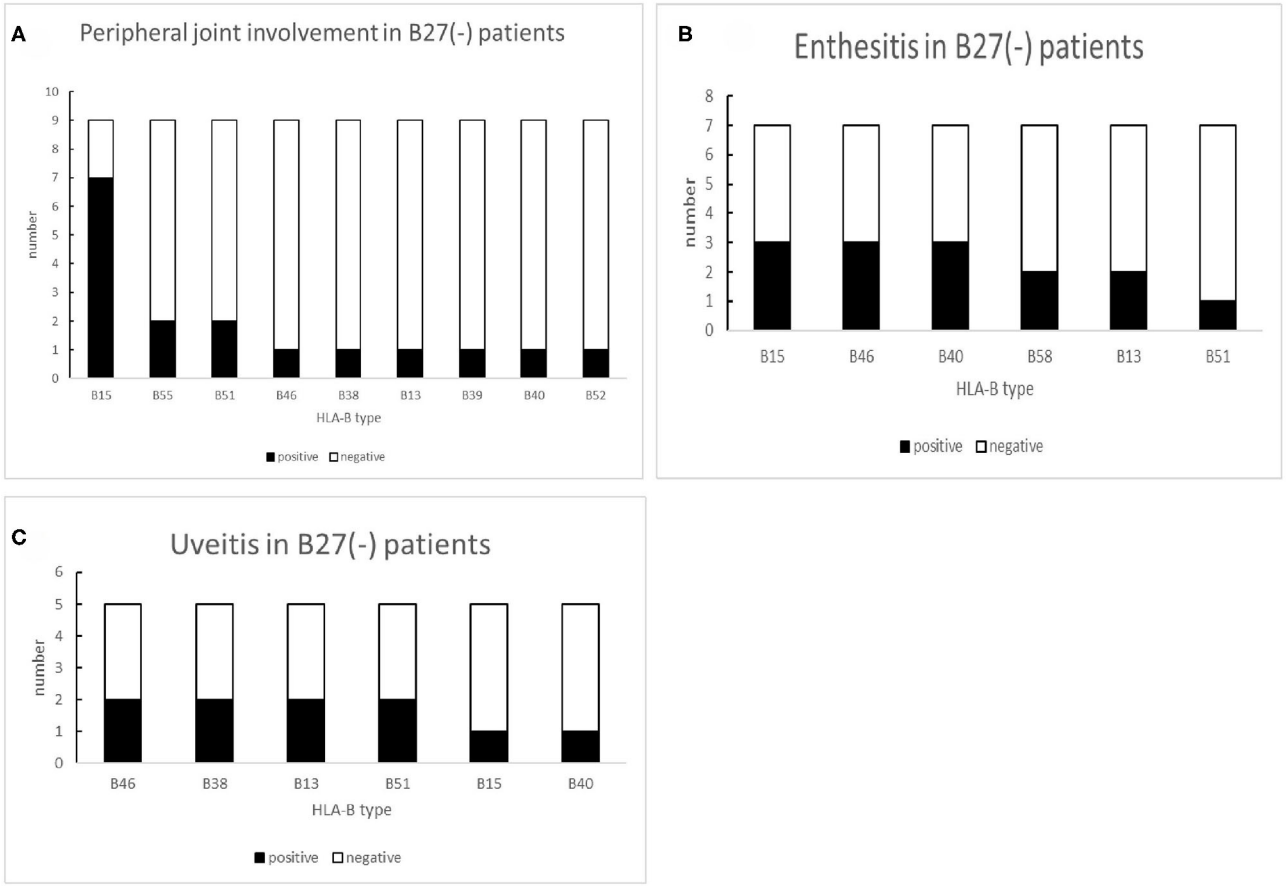

FIGURE 6 | The number of other HLA-B types shown in B27(−) patients with different peripheral manifestations: **(A)** number of other HLA-B types shown in B27(−) patients with peripheral joint involvement, **(B)** number of other HLA-B types shown in B27(−) patients with uveitis, and **(C)** number of other HLA-B types shown in B27(−) patients with enthesis.

patients had a higher frequency of extra-spine manifestations (12). Research about Korean AS patients found that HLA-B27 homozygosity has no significant difference with heterozygosity on the clinical manifestations and radiographic progression (7, 8). Some research found only four homozygous of B*27:04 in 245 HLA-B27-positive AS patients (13). In our study, we found two HLA-B*27:04 homozygous, one HLA-B*27:04/B*27:14, and no homozygote of HLA-B*27:05, 27:02, or 27:15. Only two patients had axial symptoms. Perhaps other factors were associated with peripheral manifestations in AS patients.

In HLA-B27(+) patients, 20.2% of alleles showed as HLA-B40, and the primary subtype was HLA-B*40:01. And 16.9% of HLA-B*27:04 cases were HLA-B*27:04/B*40:01, which were not found in B27(+) controls. Samples with HLA-B*40:01 in HLA-B27(−) controls were more than B27(−) cases—perhaps as a result of the sample size. Other research found that 18.2% of AS patients carried B27/B40 and only 0.4% in healthy controls (14). HLA-B40 can increase HLA-B27 susceptibility to AS (15, 16). The different subtypes had different peripheral manifestations.

HLA-B46 can increase the risk of severe sacroiliitis development related to Japanese psoriatic arthritis (PsA) patients (17). The HLA-A2-B46-DR8 haplotype has a relationship with the levels of complement components (18). HLA-B*46:01 was the only subtype of HLA-B46 found in our data. In HLA-B27 AS, the frequency was second to that of HLA-B40. Relative to other HLA-B alleles, patients with HLA-B*27:04/B*46:01 had a higher prevalence of peripheral joint involvement. HLA-B*46:01 was associated with peripheral joint involvement in HLA-B*27:04 AS patients.

In our data, 11.5% of HLA-B27 patients combined with HLA-B15 and 33.33% in HLA-B27(−) patients. The major subtype was HLA-B*15:02. HLA-B*15:17 was found in seven controls, not AS patients. In undifferentiated SpA, HLA-B15 was increased (19). HLA-B15 can be an independent factor of peripheral SpA (20). In HLA-B27 negative patients, HLA-B15, especially HLA-B*15:02, had a relationship with peripheral joint involvement in patients. HLA-B15 may increase the risk of peripheral joint involvement in HLA-B27 negative patients.

HLA-B35 was associated with AS (21, 22). Previous research found that seven HLA-B27(−) AS families with idiopathic inflammatory bowel disease have a higher frequency of HLA-B15 (21). All five HLA-B*27:04/B*35:01 were patients. Three

HLA-B27(−) patients carried HLA-B*35:03. One heterozygous patient with B*35:05/B*27:04 had multiple peripheral symptoms of uveitis, enthesitis, peripheral joint involvement, and dactylitis

Allele HLA-B51 is associated with Behcet's disease (23), especially in ocular involvement (24). But some results showed that HLA-B27(+)B51(+) is a good factor of Behcet uveitis (25). HLA-B51 was present in autoimmune diseases other than Behcet's disease with high prevalence (26). Eighteen cases showed HLA-B27/B51 (including B*51:01 and B*51:02). Only one patient with HLA-B27:04/B*51:01 had uveitis and dactylitis.

HLA-B38 was associated with clozapine-induced agranulocytosis (27). In the Argentine and Israeli population, the HLA-B38 was associated with PsA (28, 29). But psoriatic arthritis patients with HLA-B38 had less back pain (30). In our data, no patients with HLA-B27/B38 showed psoriatic arthritis.

Seventeen B27(+) patients showed B58. There was no difference between patients and controls. As we all know, Allopurinol-induced severe cutaneous adverse drug reactions (SCAR) is strongly associated with the presence of HLA-B*58:01 (31). But no article has yet reported the relationship between B*58:01 and AS. Further study is necessary to explore the association.

In the present study, we evaluated the HLA-B genotype in AS patients compared to the control group. As a result, we found that more than 98% of the samples were heterozygous in the HLA-B region. HLA-B27 homozygous patients were rare and only had axial manifestations. Based on our study and other reports, for B27(+) people, HLA-B40 can increase the risk of AS. HLA-B40 was the second most common HLA-B subtype in all of the AS patients besides HLA-B27. Then the genotype HLA-B27:04/B*40:01 can improve diagnostic accuracy, and patients with HLA-B*27:04/B*46:01 had a high risk of arthritis and enthesitis. In the HLA-B27(−) group, HLA-B*15:02 was a risk maker of peripheral joint involvement. Perhaps HLA-B*40:01, HLA-B*46:01, and HLA-B*15:02 should be markers included in AS diagnosis value. Due to the limited information in this field and a small number of patients, our results did not show statistical significance in other HLA-B subtypes with peripheral clinical manifestations. There is a need for more samples and further workup on the relationship of the HLA-B heterozygous in AS patients.

In conclusion, our research shows that, besides HLA-B27, other HLA-B types also may impact the AS patient phenotype. It is critical to systematically screen and evaluate the HLA-B genotype in the patients with AS, which may result in an improved accurate diagnosis of the patients.

DATA AVAILABILITY STATEMENT

The authors acknowledge that the data presented in this study must be deposited and made publicly available in an acceptable repository, prior to publication. Frontiers cannot accept a manuscript that does not adhere to our open data policies.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The ethics committee of Third Affiliated Hospital of Sun Yat-Sen University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JG conceived the study and critically revised the manuscript and provided final approval of the manuscript. JW, PZ, and XL were in charge of the experiment. XW performed the analysis. XZ, ZC, QL, LT, QW, and SC were in charge of collecting sample and data. XW wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.568790/full#supplementary-material

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BRIEF RESEARCH REPORT
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Incidence of a First Thrombo-Embolic Event in Patients With Systemic Lupus Erythematosus and Anti-phosphatidylserine/prothrombin Antibodies: A Prospective Study

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Objective: This study aimed to prospectively investigate the incidence of first thromboembolic events (TEs) in a cohort of systemic lupus erythematosus (SLE) patients. The patients were positive for anti-phosphatidylserine/prothrombin (aPS/PT) antibodies and tested negative for anticardiolipin (aCL) and anti-β2–glycoprotein I (aβ2GPI) antibodies [regardless of their Lupus Anticoagulant (LA) status].

Methods: Inclusion criteria included: (a) SLE with no previous TEs; (b) no concomitant anti-thrombotic therapy; (c) isolated confirmed positive test for aPS/PT.

Results: From the total of 52 SLE patients (42, 80.8% women), 18 patients (34.6%) were found to be positive for aPS/PT (IgG/IgM). During a mean follow-up (3.9 ± 1.1 years), 3 TEs occurred (1.3%/year). The overall cumulative incidence of TEs was 5.8% after 2 years, and up to 16.7% when focusing on aPS/PT positive patients. All the TEs events (two cerebrovascular events and one thrombotic kidney microangiopathy) occurred in the aPS/PT positive group. When focusing on IgG aPS/PT, we found that patients who tested positive were at a significantly higher risk for TEs (crude HR 19.6, 95%; CI 1.1 to 357.6; $p < 0.05$) compared to patients with negative aPS/PT.

Conclusion: This study observed a rate of TEs of 1.3%/year, in aPS/PT positive only patients. Our prospective data suggest that aPS/PT might confer an increased risk for the development of TEs in SLE patients.

Keywords: antiphospholipid syndrome, antiphospholipid antibodies, anti-phosphatidylserine/prothrombin, aPS/PT, non-criteria aPL, thrombosis, systemic lupus erythematosus

INTRODUCTION

Multiple positivity in tests investigating the presence of antiphospholipid antibodies (aPL) [criteria aPL comprehend: lupus anti-coagulant (LA), anticardiolipin (aCL), and anti-β2–glycoprotein I (aβ2GPI) antibodies] are now widely recognized as being associated with a higher risk of developing thromboembolic events (TEs). The concomitant presence of all criteria aPL (triple positive patients) is associated with thrombosis and identifies high-risk patients in antiphospholipid syndrome (APS) setting (1). However, some individuals may show a clinical picture that strongly indicates APS even though they are persistently negative for criteria aPL tests. Current research examines testing for other aPL specificities to fill this diagnostic and therapeutic gap. When investigating these so-called "extra-criteria" aPL in a patient with clinical manifestations suggestive of APS, testing for anti-phosphatidylserine/prothrombin (aPS/PT) antibodies has been recommended as a further tool in guiding the management of these patients. It can be particularly relevant when there is an absence of criteria aPL or as a part of risk assessment approaches (2). This approach to testing has been analyzed by two systematic reviews (3, 4), which outline that aPS/PT antibodies might be considered a strong risk factor for TEs independently from sites and type of thrombosis. There is little data, available to provide prospective validation of the role the absence of other aPL tested by β2GPI-dependent assays.

This study prospectively investigates the incidence of first TE in a cohort of systemic lupus erythematosus (SLE) patients positive for aPS/PT antibodies who also tested negative for criteria solid assay (aCL and aβ2GPI antibodies), regardless of their LA status.

METHODS

Inclusion Criteria

Since 2015, aPS/PT has formed part of routine testing in SLE patients as part of the autoantibody screening of consecutive patients attending the S. Giovanni Bosco Hospital (Turin, Italy). The patients included in this study were diagnosed with SLE according to the 1982 revised criteria (5), received prospective follow-up, and fulfilled the following criteria:

1) no previous TEs events;
2) no concomitant anti-coagulant nor anti-platelets therapy;
3) tested negative for criteria aPL solid assay aCL and aβ2GPI (confirmed at least twice, at least 12 weeks apart), regardless of their LA status.

All included patients were tested for aPS/PT, and both IgG and IgM, at study inclusion.

Positive aPS/PT testing was defined as having at least two positive test results (IgG and/or IgM), at least 12 weeks apart. The disposition of patients is illustrated in **Figure 1**.

All subjects provided written consent according to the Declaration of Helsinki. This study was performed according to the local legislation of Rare Diseases in Piedmont (Northwest Italy) (protocol. n. 1577/UC/SAN 11.10.2005).

Data Collection

Data on demographic, and laboratory and clinical features were prospectively collected every 6 months or at the time of any new clinical event for each patient. Patients with a previous history of TEs were excluded based on patient interviews and available hospital records.

Assessed arterial thrombotic risk factors were diabetes mellitus, arterial hypertension, hypercholesterolemia, obesity, smoking habit, and positive family medical history. Assessed venous risk factors were the following: ongoing hormonal replacement therapy, active pregnancy, malignancy, positive family medical history, and thrombophilia (including antithrombin, protein C, or protein S; factor V Leiden; prothrombin G20210A mutation; hyperhomocysteinemia, high factor VIII levels).

Outcome Events

TEs had to be objectively diagnosed during the follow-up. TEs reports include type, site, SLE activity assessed by the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) and ongoing medications at the time of event.

Venous thromboembolism (VTE) was assessed by compression ultrasonography or venography in case of deep vein thrombosis, and spiral tomography, ventilation-perfusion lung scan, or pulmonary angiography in case of pulmonary embolism. Intracerebral thrombosis was assessed by computed tomographic scanning, magnetic resonance imaging, or angiography; retinal thrombosis was evaluated by ophthalmologic examination. Peripheral- or mesenteric- artery thrombosis was documented by arteriography or at the surgery table. Small-vessel thrombosis was evaluated by appropriate imaging study or histopathology in the absence of inflammation in the vessel wall. Acute myocardial infarction was defined in the presence of a typical clinical presentation associated with typical electrocardiographic features and elevated cardiac enzymes (CK-MB or troponins I or T). Stoke/transient ischemic attack was defined according to standard definitions (transient ischemic attack was considered for analysis only if cerebral imaging confirmed cerebral ischemia).

aPL Testing

Complete aPL profile at inclusion in the present study included: LA, aCL IgG/IgM, aβ2GPI IgG/IgM, aPS/PT IgG/IgM, and aβ2GPI Domain 1 (aβ2GPI-D1) IgG.

LA testing was performed according to international guidelines (6). Solid-phase aPL testing was executed with chemiluminescent immunoassay (INOVA Diagnostic) for aCL, aβ2GPI, and aβ2GPI-D1, while aPS/PT testing was performed using ELISA assay (INOVA Diagnostic). The cut-off values were determined by manufacturer recommendations. Cut-off values provided by the manufacturer were independently validated in a cohort of 100 healthy blood donors, and the used values were above the 99th percentile of the distribution. The Global APS Score (GAPSS) was calculated according to Sciascia et al. (7).

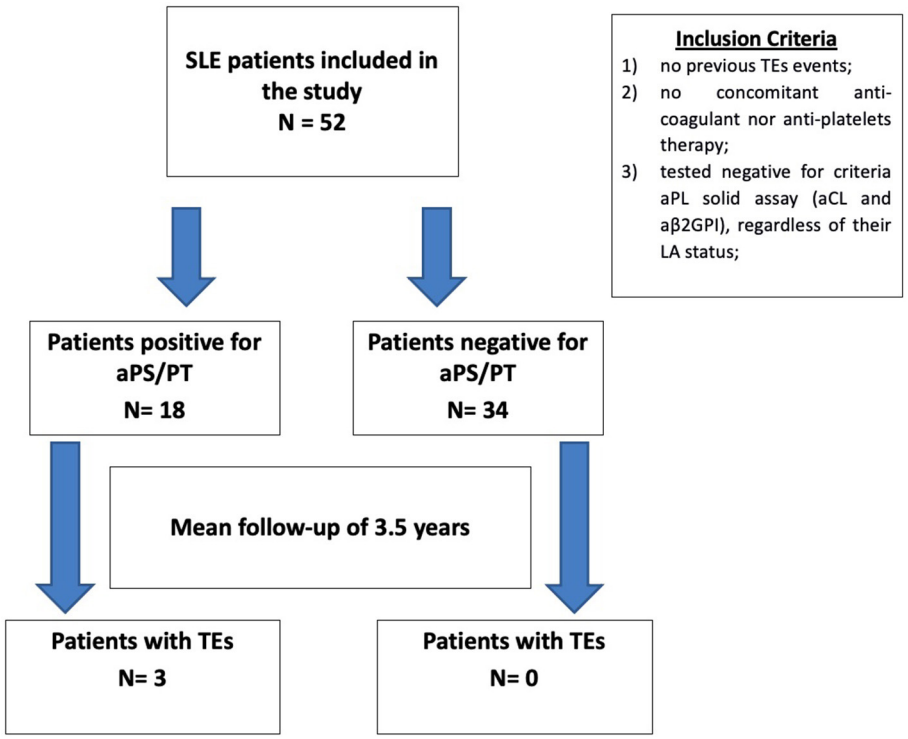

FIGURE 1 | Patients disposition. Description of the patients selected for the study, according to the inclusion criteria. Patients included in the study were then separated according to their anti-phosphatidylserine/prothrombin status (positive/negative), and the number of thrombotic events was recorded retrospectively. aPS/PT, anti-phosphatidylserine/prothrombin; SLE, systemic lupus erythematosus; TE, thrombotic event; aPL, antiphospholipid antibodies; aCL, anticardiolipin; aβ2GPI, anti-β2–glycoprotein I; LA, lupus anti-coagulant.

Statistics

Descriptive statistics are reported as appropriate: categorical data are expressed as frequencies (percentage); continuous data are reported as mean ± *SD*. The Kaplan-Meier survival analysis was used to determine the cumulative incidence of TEs at follow-up. Student's *t*-test was used for normally distributed parameters and the non-parametric Mann–Whitney test for non-normally distributed parameters.

The Cox proportional hazards model was initially included in the statistical plan to detect possible predictors of TEs among the demographic factors. The initial model computed the following variables: age> 50 yrs, sex, active SLE assessed by SLEDAI-2K >6, and any additional thrombotic risk factor (smoking, arterial hypertension, hyperlipidemia, diabetes, immobilization). However, taking into account the rate of observed thrombosis, as the number of primary events per variable can affect the estimation of the subdistribution hazard competing risks model, we decided to keep this analysis as exploratory. A Log-rank test was performed, comparing thrombotic events during the follow-up according to the aPS/PT positivity.

Statistical significance was considered for $p < 0.05$. All analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, USA).

RESULTS

The demographic and clinical characteristics of the included patients are described in **Table 1**.

This study included a total of 52 patients with SLE [42 (80.8%) females]. Of those, 18 patients (34.6%) were found to be positive for aPS/PT (IgG and/or IgM).

During a mean follow-up of more than 3.5 years (3.9 ± 1.1 years), three patients developed TEs (1.3% per year). The overall cumulative incidence of TEs was 5.8% after 2 years, rising to 16.7% when focusing only on aPS/PT positive SLE patients. Details on the three patients who developed a TE are shown in **Table 2**. All the TEs events (two cerebrovascular events and one thrombotic kidney microangiopathy) occurred in the aPS/PT positive group. Two patients, one aPS/PT positive and one aPS/PT negative, experienced superficial thrombophlebitis, not included among endpoints. No patient died and no pregnancy was recorded during the follow-up. To confirm the absence of solid aβ2GPI dependent aPL positive test, all patients were tested for aβ2GPI-D1, and they all had negative results.

No statistically significant difference was observed between patients with TEs compared to those without when dividing for demographic variables (age, sex), SLE features (active SLE assessed by SLEDAI-2K > 6), and arterial and venous risk factors

TABLE 1 | Clinical and demographic characteristics.

	SLE aPS/PT positive		SLE aPS/PT negative	
	(*N* = 18)	**%**	**(*N* = 34)**	**%**
Age				
Years (mean ± *SD*)	47.5 ± 11.5		44.8 ± 13.5	
Female				
(*N*; %)	14	77.8	28	82.4
aPS/PT IgG				
Positive (*N*; %)	11	61.1		
Titer (mean ± *SD*; median [range])	99.8 ± 77.8; 121 [12–229]			
aPS/PT IgM				
Positive (*N*; %)	16	88.9		
Titer (mean ± *SD*; median [range])	126.2 ± 150.8; 131 [8–518]			
Lupus anti-coagulant				
Positive (*N*; %)	5	27.8	12	35.3
SLE manifestation				
Skin (*N*; %)	6	33.3	8	23.5
Joints (*N*; %)	17	94.4	28	82.4
Hematological (*N*; %)	3	16.7	6	17.6
Lupus Nephritis* (*N*; %)	8	44.4	11	32.4
Sierositis (*N*; %)	4	22.2	7	20.6
Follow-up**				
Years (mean ± *SD*; median [range])	3.7 ± 1.2		3.9 ± 1.1	
SLE disease duration				
Years (mean ± *SD*; median [range])	15.7 ± 8.1		19.7 ± 7.2	
Therapy**				
Hydroxychloroquine (*N*; %)	17	94.4	33	97.1
Prednisone < 7.5 mg/die*** (*N*; %)	14	77.8	22	64.7
Cyclophosphamide (*N*; %)	2	11.1	2	5.9
Mycophenolate (*N*; %)	3	16.7	3	8.8
Azathioprine (*N*; %)	6	33.3	10	29.4
Methotrexate (*N*; %)	3	16.7	5	14.7
Rituximab (*N*; %)	5	27.8	10	29.4
Belimumab (*N*; %)	3	16.7	5	14.7
Thrombotic risk factors				
Arterial hypertension (*N*; %)	7	38.9	10	29.4
Hyperlipidemia (*N*; %)	2	11.1	5	14.7
Smoking habit (*N*; %)	3	16.7	5	14.7
Diabetes (*N*; %)	0	0.0	0	0.0
Hormone replacement therapy (*N*; %)	0	0.0	0	0.0
Inherited thrombophilia (*N*; %)	0	0.0	0	0.0

*Biopsy-proven.

**After aPS/PT testing.

*** For at least 80% of the observation time.

No statistical difference was observed between the two groups.

S.D., standard deviation; N., number; aPS/PT, anti-phosphatidylserine/prothrombin; Ig, immunoglobulins; SLE, Systemic Lupus Erythematosus.

(presence of any additional risk factor, to include smoking, arterial hypertension, hyperlipidemia, diabetes, immobilization).

Although we observed a trend for aPS/PT in conferring an increased risk for TEs (crude HR 12.9, 95% CI 0.7–236.7; $p = 0.08$), the results failed to reach statistical significance. When focusing on IgG aPS/PT, we found that patients who tested positive were at a significantly higher risk for TEs (crude HR 19.6, 95% CI 1.1–357.6; $p = 0.04$) compared to aPS/PT negative patients. When taking the whole follow-up period into account by log-rang analysis, patients with aPS/PT presented with a shorter time free from events. Patients with TEs had a higher GAPSS when compared to those without [6 ± 2.6 vs. 2 ± 5.4; $p = 0.09$]; however, this failed to reach statistical significance.

TABLE 2 | Main clinical characteristics of the three patients who developed thromboembolic events.

Patient	Previous clinical SLE manifestation	TEs	aPL profile	Treatment at the time of TE	SLEDAI-2K time of last appointment before TE
#1, F, 49 yrs	Joint, malar rash, oral afthosis	Extended ischemic stroke involving cortico-subcortical occipital area at the level of the left hippocampal gyrus and the internal capsular area	Inconstant LA, aPS/PT IgG, IgM	HCQ, PDN 5 mg	0 (last appointment 2 months before)
#2, F, 36 yrs	Oral aphthosis, photosentivity, malar rash, pleurlal-pericarditis, LN (class IV+V), join, skin	Ischemic stroke (middle cerebral artery territory)	LA, aPS/PT IgG, IgM	HCQ, PDN 5 mg	2 (low C3, last appointment 3 months before)
#3, F, 38 yrs	Photosentivity, malar rash, sub-acute skin rash, pericarditis, joints	Renal TMA	LA, aPS/PT IgG, IgM	HCQ, PDN 5 mg, MTX	4 (low C3, positive anti-dsDNA, last appointment 2 months before)

TMA, thrombotic microangiopathy; aPL, antiphospholipid antibodies; LA, lupus anti-coagulant; aPS/PT, anti-phosphatidylserine/prothrombin; SLE, systemic lupus erythematosus; TE, thrombotic event; F, Female; SLEDAI, systemic lupus erythematosus disease index; Ig, immunoglobulins; HCQ, hydroxychloroquine; PDN, prednisone; MTX, Methotrexate; anti-dsDNA, anti-double strand DNA.

DISCUSSION

In clinical practice, assessing thrombotic risk is challenging for patients who tested negative for aβ2GPI-dependent aPL. The clinical course in persons with high-risk aPL profiles (triple positive patients) has been well-described (8); the role of extra-criteria aPL, with or without concomitant positive LA, and their clinical impact on positive subjects has been the subject of debate over the last decades, with heterogeneous conclusions (2).

To address this issue, we prospectively evaluated a cohort of SLE patients followed at our Center. They were homogeneous in terms of strict inclusion criteria and negative for solid assay criteria aPL (aCL and aβ2GPI antibodies), regardless of their LA status. Besides, aPS/PT positivity tests were confirmed 12 weeks apart. Our results show a relevant incidence of TEs during the follow-up period, with the incidence of TEs at 5.8% after 2 years. The annualized incidence of TEs in SLE patients negative for aβ2GPI-dependent aPL testing was 1.3%. When focusing on patients who tested positive for aPS/PT, the incidence of TEs rises to 16%, with an annualized incidence of 2.8%, with aPS/PT IgG isotype strongly associated with an increased thrombotic risk. To our knowledge, this is the first prospective clinical study that addresses the incidence of TEs in patients positive for aPS/PT and negative for aβ2GPI-dependent aPL testing. Interestingly, in our cohort, LA positivity did not seem to confer an additional risk for TEs.

Additionally, in a prospective study (9), Ruffatti et al. reported that arterial hypertension and LA positivity were independent risk factors for thrombosis when investigating the risk factors for a first thrombotic event in aPL antibody carriers, most of whom had an associated autoimmune disease. While it is clear that LA positivity is associated with TEs (10), managing patients with isolated LA still requires some considerations (11). Investigating the comprehensive aPL profile of patients/carriers should be mandatory, as the isolated positivity for LA has not been unanimously associated with thrombosis (12) or with clinical manifestations of APS (13). Similar findings were observed in the Leiden thrombophilia case-control study (14), which showed that LA positivity in the absence of aβ2GPI or anti-prothrombin antibodies was not associated with an increased risk for deep vein thrombosis. The association of aPS/PT with thrombosis, especially venous thrombosis, was stronger in the LA positive patients than in LA negative subjects. We observed that aPS/PT was independently associated with thrombosis and pregnancy loss after multivariate analysis (15).

This study has some limitations, including the number of observed events in the relatively short follow-up and sample size (albeit in line with the low prevalence of APS, especially when focusing on subgroups of patients with specific aPL profiles). For instance, while no statistical significance was found when looking at the higher levels of GAPSS in patients with TEs, this probably was due to sample size. Furthermore, patients included in the study had SLE in association with APS, which could have influenced the outcome. These observations require further validation in cohorts of patients without concomitant SLE. Investigation of any change in aPS/PT titres after the second confirmation was outside the scope of this study. These aspects were, however, counterbalanced by the use of strict inclusion criteria (including aPS/PT positivity confirmation at least 12 weeks) and the prospective nature of the study. Finally, since consecutive patients with SLE who met the inclusion criteria were prospectively enrolled in the study, some degree of variability in follow-up length was present. Future studies with a larger sample population, homogenous follow-up duration, and accordingly designed statistical analysis plans are warranted to obtain definite conclusions.

This study observed a rate of TEs of 1.3% each year only in aPS/PT positive patients. This prospective data is validated by previous retrospective studies (3, 4) and suggests that aPS/PT might confer an increased risk for the development of TEs in SLE patients. Future research should investigate whether

SLE patients with aPS/PT could benefit from tailored primary thrombo-prophylaxis strategies to include anti-platelet agents. Large clinical trials are needed in the future to test this hypothesis.

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 human participants were reviewed and approved by Local legislation of Rare Diseases in Piedmont (Northwest Italy) (protocol. n. 1577/UC/SAN 11.10.2005). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS and MR designed the study and made the figures. IC, ER, AB, SF, AV, and DRos carried out clinical follow-up, collected data, reviewed the manuscript, and interpreted data. SS, DRoc, and MR analyzed the data. DRoc and SS drafted and revised the paper. All authors approved the final version of the manuscript.

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Early Initiation of Anticoagulation Improves the Long-Term Prognosis in Patients With Antiphospholipid Syndrome Associated Portal Vein Thrombosis

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Objectives: Portal vein thrombosis (PVT) is a rare and severe clinical phenotype of antiphospholipid syndrome (APS) with a poor prognosis. Anticoagulation therapy is efficient but is associated with potentially severe bleeding episodes, especially for those patients with thrombocytopenia. We conducted this case-control study to explore the clinical features and associated factors of PVT in APS patients, the re-canalization rate of the PVT after anticoagulation and investigate the beneficial effects of early initiation of anticoagulation in patients with APS associated PVT.

Methods: We enrolled patients with APS associated PVT as the case group, and age-, and entry-time-matched APS patients without PVT (1:2) as the control group. We explored the associated factors of PVT in APS patients using multivariate logistic regression analysis. The re-canalization rate of the PVT after anticoagulation was analyzed using the survival analysis.

Results: A total of 34 patients (8 males and 26 females) with APS-PVT were enrolled, with a median follow-up time of 3 years (1.5, 7 years). Multivariate logistic regression analysis showed that thrombocytopenia (OR 6.4, 95%CI 1.561–26.218, $P = 0.01$), hypersensitive c-reactive protein >3 mg/L (OR 4.57, 95%CI 1.426–14.666, $P = 0.011$), anti β2GPI positive (OR 5, 95%CI 1.816–13.772, $P = 0.002$) and aPL double-positive (OR 4.08, 95%CI 1.312–12.429, $P = 0.013$) were independent associated factors for PVT in APS. Survival analysis revealed that effective anticoagulation could increase re-canalization rate significantly (log-rank $p = 0.001$), with better prognosis (lower mortality rate, log-rank $p = 0.045$).

Conclusions: PVT could be the first presentation of APS with insidious onset and atypical clinical symptoms and easily be misdiagnosed. For patients with APS, double aPLs positive, thrombocytopenia, and inflammation could be the associated factors of PVT. Early diagnosis and anticoagulation treatment can bring thrombus re-canalization thereby significantly improving the prognosis.

Keywords: antiphospholipid syndrome, portal vein thrombosis, anticoagulation, portal hypertension, thrombosis

KEY-POINTS

- PVT could be the first thrombotic event of APS, usually had insidious onset with atypical clinical symptoms and easily be misdiagnosed.
- For patients with APS, the double aPLs positive, thrombocytopenia, and inflammation could be the risk factor of PVT.
- Early diagnosis and anticoagulation treatment can bring thrombus re-canalization thereby significantly improving the prognosis, with a lower mortality rate.

INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent arterial venous thrombosis, habitual abortion, and/or thrombocytopenia and persistent antiphospholipid antibodies (aPLs) positive in the blood. PVT is a rare, serious, and highly heterogeneous phenotype of APS (1). Portal venous system thrombosis (PVT) includes the thrombus in the portal vein, the superior mesenteric vein/splenic vein, and the inferior mesenteric vein. According to the course of thrombosis, portal vein thrombosis is divided into acute PVT, chronic PVT, and portal vein degeneration. Some patients had insidious onset with atypical clinical symptoms and easily be misdiagnosed. PVT has been related to liver dysfunction, neoplasm, genetic factors (2, 3), hemodynamic factors, and hypercoagulability states (4). Antiphospholipid antibodies also have been implicated as one of the causes of PVT in a few previous studies (5, 6).

It is recommended that anticoagulation should be given for 3–6 months if acute PVT was detected early. If prothrombotic factors are identified, anticoagulation should be given lifelong (7). Anticoagulation therapy is efficient, however, may be associated with potentially severe harmful effects, especially bleeding episodes for those patients with thrombocytopenia. Also, the literature lacks information about the safety and long-term results of anticoagulation in patients of APS associated PVT. Most clinical evidence and treatment experience about PVT came from PVT caused by abdominal infections, tumors, or cirrhosis. Only a few studies have been reported about PVT due to APS. And most are only case reports (8–10), there is only one cross-sectional study of 32 patients (11). This study aimed to explore the clinical features and associated factors of PVT in APS patients, the re-canalization rate of the PVT after anticoagulation, and the beneficial effects of early initiation of anticoagulation in patients with APS associated PVT.

METHODS

Patient Recruitment

We utilized the Hospital Inpatient Information Retrieval System to identify the patients with APS associated PVT admitted to the Peking Union Medical College Hospital (PUMCH) from January 2012 to December 2019. A case-control study was conducted to explore the clinical features and associated factors of PVT in APS patients. Patients with APS associated PVT were defined as the case group, and age-, sex-, and entry-time-matched APS patients without PVT at the ratio of 1:2 as the control group. The diagnosis of APS was confirmed according to the 2006 Sydney classification criteria for definite antiphospholipid syndrome (12) and PVT was confirmed according to the 2009 American College of Liver Diseases (AASLD) criteria. An acute PVT was defined if symptoms developed <60 days before hospital assessment and the absence of or insignificant portal collaterals on imaging and no evidence of portal hypertension including splenomegaly and oesophageal varices (7). Patients with alcoholic cirrhosis, viral hepatitis induced postnecrotic cirrhosis, tumorous obstruction, and patients with a history of abdominal surgery before PVT were excluded. Medical records were reviewed for medical history, results of antiphospholipid antibodies (aPL) testing, including lupus anticoagulant (LAC), anticardiolipin (ACL), and anti-β2-glycoprotein I (antiβ2GPI), IgG or IgM autoantibodies. Subjects were considered aPL positive if at least one of these autoantibodies, at least 12 weeks apart were documented. IgG/IgM antibody of ACL and anti-β2-glycoprotein I were tested by enzyme linked immunosorbent assay (ELISA). We considered positive those titers reported as medium (20–40) or high (>40), being low titers considered as negative. Dilute Russell viper venom time (dRVVT) testing and activated partial thromboplastin time were measured, where LAC was considered positive if the ratio of dRVVT time >1.20. Regular imaging was performed to monitor the outcome of PVT. The data of the re-canalization rate of the PVT after anticoagulation were also collected. The date of entry was the date of the first diagnosis of PVT. Patients were followed every 3–6 months until death, or the end of the study (July 2019). Effective anticoagulation was defined as immediate and sufficient anticoagulant therapy for at least 6 months with a target international normalized ratio (INR) of 2.0–3.0 from the time of PVT diagnosis. This study was approved by the Medical Ethics Committee of the Peking Union Medical College Hospital, which was the lead site, and all patients provided written informed consent.

Statistical Analysis

Continuous variables were presented as means and standard deviation (SD) for normally distributed data, and medians and interquartile range (IQR) (P25, P75) for all other data, whereas categorical variables were presented as number (percentage). In bivariate analysis, continuous variables were compared with the use of the Student's *t*-test or Mann–Whitney test, while categorical variables were compared using the chi-square test or Fisher's exact test. Variables were entered into the univariable (UV) logistic regression model. A multivariate (MV) logistic regression model was then constructed using a stepwise forward selection procedure among those candidate variables with the significance level $p < 0.10$ in the UV logistic regression analysis. Continuous variables are converted to binary or ordered multiple variables when entered into UV or MV logistic regression models. Odds ratios and 95% confidence intervals were calculated. Survival analysis using the log-rank test was applied to compare the accumulated recanalization rate between effective and invalid anticoagulation groups. The *p*-value was two-tailed and defined as significant if the value was <0.05. SPSS

software, version 23 (Chicago, IL, USA) used for all the statistical descriptions, analyses, and inferences.

RESULTS

Clinical Manifestations

A total of 34 patients confirmed with PVT from 187 APS patients were identified, of which 18 patients were primary APS, 14 patients were secondary to systemic lupus erythematosus (SLE), and 2 patients were secondary to Sjogren's syndrome. Sixty-eight age-, sex-, and entry-time-matched APS patients without PVT were selected as the control group, of which 32 patients were primary APS, and 36 patients were secondary to SLE. Comparison of demographic characteristics, clinical, and laboratory manifestations between the case (APS patients with PVT) and control (APS patients without PVT) groups were shown in **Table 1**. There were no significant differences in gender, age at study entry between the case and control groups. In the case group, there were 8 males and 26 females, with a mean age of 40.35±13.029 years, disease course 0.08 years (0, 1.13 years), and median follow-up was 3 years (1.5, 7years). Triple aPLs were positive in 7 cases and double aPLs positive in 15 cases. 11 cases were acute thrombosis, 23 cases chronic thrombosis, and 7 cases portal vein cavernoma. Among the case group, thrombosis of portal veins was initially demonstrated using Doppler ultrasound in 14 cases and computed tomography angiography in 20 cases.

PVT could be the first presentation of APS with insidious onset and atypical clinical symptoms. PVT was the first thrombotic event of APS in 21 patients; 2 patients had other types of thrombosis (myocardial infarction, deep venous thrombosis, respectively) as the first event; and 11 patients presented as thrombocytopenia first. As for the first symptoms of PVT, the presentation with abdominal distention (14 cases) and pain (10 cases) were more common, the presentation with variceal bleeding (5 cases) was less common, and 7 cases were asymptomatic. Among the case group, 17 cases were complicated with portal hypertension. Eighteen cases had dilated esophageal or gastric veins, 22 cases with splenomegaly, 5 cases of splenectomy, and 9 cases of liver cirrhosis (**Table 2**). Four of these 34 patients presented with intestinal infarction, 3 of whom required an intestinal resection.

Multivariate logistic regression analysis showed that thrombocytopenia (OR 6.4, 95%CI 1.561–26.218, $P = 0.01$), hypersensitive c-reactive protein >3 mg/L (OR 4.57, 95%CI 1.426–14.666, $P = 0.011$), anti β2GPI positive (OR 5, 95%CI 1.816–13.772, $P = 0.002$), and aPLs double positive (OR 4.08, 95%CI 1.312–12.429, $P = 0.013$) were independently associated factors for PVT in APS, as shown in **Table 3**.

Treatment and Prognosis

Twenty-nine of 34 patients received anticoagulation therapy, five patients did not receive anticoagulation treatment because of a high risk of gastrointestinal bleeding (**Table 3**). Sixteen cases began effective anticoagulation therapy immediately at the diagnosis of thrombus and for at least 6 months. Anticoagulation therapy consisted of the initial administration of intravenous unfractionated heparin in 29 patients, with a median duration of 19 days (range, 7–60 days), followed by oral anticoagulation in all patients, 25 patients with warfarin and 4 patients with rivaroxaban. Rhinorrhea occurred in one of the patients receiving anticoagulation. No surgical thrombectomy was performed. No thrombolytic therapy was administered. Among the 34 patients, 7 patients got thrombus complete recanalization and 10 patients partial recanalization, 5 patients died.

We compared the rate of aPLs, complications and treatment between groups of recanalization and no recanalization in APS patients with PVT (**Table 4**). There is a significant difference in the rate of effective anticoagulation between groups ($p < 0.005$), which means effective anticoagulation could increase the rate of recanalization. Survival analysis revealed that effective anticoagulation could increase recanalization rate (**Figure 1**) significantly (log-rank $p = 0.001$), with a lower mortality rate (log-rank $p = 0.045$; **Figure 2**). There was no significant

TABLE 1 | Comparison of demographic characteristics, clinical and laboratory manifestations between APS patients with and without PVT.

Variable	APS patients with PVT ($n = 34$)	APS patients without PVT ($n = 68$)	*p*-value
Female, *n* (%)	26 (76.5)	54 (79.4)	0.734
Age, mean (SD), years	40.35 ± 13.029	40.25 ± 13.453	0.97
Disease course, median (P25, P75), years	0.08 (0, 1.13)	4 (1, 9.75)	**<0.001**
Follow-up time, median (P25, P75), years	3 (1.5, 7)	1 (0, 4)	0.861
BMI, median (P25, P75), kg/m^2	21.36 (18.55, 25.28)	22.43 (20.73, 25.95)	0.788
Arterial thrombosis, n (%)	6 (17.6)	26 (38.2)	0.035
Venous thrombosis, n (%)	34 (100)	19 (27.9)	**<0.001**
Pregnancy loss, n (%)	1 (2.9)	15 (22.1)	0.018
PAPS, n (%)	18 (52.9)	32 (47.1)	0.575
SLEDAI (for SLE-related APS), median (P25, P75)	5 (1, 10) ($n = 14$)	2 (0, 2) ($n = 36$)	**0.004**
SLICC (for SLE-related APS), median (P25, P75)	1 (0, 2) ($n = 14$)	0 (0, 1) ($n = 36$)	0.196
Thrombocytopenia, *n* (%)	11 (32.4)	6 (8.8)	**0.003**
Hypoalbuminemia, *n* (%)	16 (47.1)	1 (1.5)	**<0.001**
ESR >20 mm/h, *n* (%)	12 (35.3)	14 (20.6)	0.108
hsCRP>3 mg/L, *n* (%)	15 (44.1)	9 (13.2)	**0.001**
Total cholesterol >5.7 mmol/L, *n* (%)	5 (14.7)	2 (2.9)	**0.027**
Total triglycerides>1.7 mmol/L, *n* (%)	6 (17.6)	10 (14.7)	0.7
ACL positive, *n* (%)	20 (58.8)	41 (60.3)	0.886
anti β2GPI positive, *n* (%)	24 (70.6)	20 (29.4)	**<0.001**
LAC positive, *n* (%)	19 (55.9)	36 (52.9)	0.779
aPL double positive, *n* (%)	15 (44.1)	17 (25)	**0.05**
aPL triple positive, n (%)	7 (20.6)	17 (25)	0.62

Values in bold are statistically significant at $p < 0.05$.

APS, antiphospholipid syndrome; PAPS, primary antiphospholipid syndrome; PVT, portal vein thrombosis; SD, standard deviation; BMI, body mass index; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index; ESR, erythrocyte sedimentation rate; hsCRP, hypersensitive C-reactive protein; LAC, lupus anticoagulant; ACL, anticardiolipin; antiβ2GPI, anti-β2-glycoprotein I.

TABLE 2 | The clinical features, treatment and prognosis of APS patients with PVT.

	n (*N* = 34)	%
Stage at recognition		
Acute	11	32.35
Chronic	23	67.65
The first symptom of APS		
PVT	21	61.76
Other types of thrombosis	2	5.88
Thrombocytopenia	11	32.35
The first symptom of PVT		
Abdominal pain	10	29.41
Abdominal distention	14	41.18
Gastrointestinal bleeding	5	14.71
Asymptomatic	7	20.59
Complications		
Portal hypertension	17	50.00
Dilated esophageal or gastric veins	18	52.94
Splenomegaly	22	64.71
Splenectomy	5	14.71
Liver cirrhosis	9	26.47
Treatment		
Effective anticoagulation	16	47.06
Invalid anticoagulation	13	38.24
No anticoagulation	5	14.71
Outcomes		
Complete re-canalization	7	20.59
Partial re-canalization	10	29.41
No re-canalization	12	35.29
Death	5	14.71

APS, antiphospholipid syndrome; PVT, portal vein thrombosis.

TABLE 3 | Univariate and multivariate logistic regression analyses for variables predictive of PVT in APS patients.

Variables	UV		MV	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
BMI>25 kg/m^2	0.64 (0.25, 1.65)	0.358		
PAPS	1.27 (0.56, 2.89)	0.576		
Thrombocytopenia	4.94 (1.64, 14.9)	**0.005**	6.40 (1.56, 26.22)	**0.01**
ESR >20 mm/h	2.1 (0.84, 5.26)	0.112		
hsCRP>3 mg/L	5.18 (1.95, 13.72)	**0.001**	4.57 (1.43, 14.67)	**0.011**
Total cholesterol >5.7 mmol/L	5.69 (1.04, 31.05)	**0.045**		
Total triglycerides>1.7 mmol/L	1.24 (0.41, 3.76)	0.701		
ACL positive	0.94 (0.41, 2.18)	0.886		
Anti β2GPI positive	5.76 (2.33, 14.22)	**<0.001**	5.00 (1.82, 13.77)	**0.002**
LAC positive	1.13 (0.49, 2.58)	0.779		
aPL double positive	2.37 (0.99, 5.66)	0.052	4.08 (1.34, 12.43)	**0.013**
aPL triple positive	0.78 (0.29, 2.11)	0.621		

Values in bold are statistically significant at $p < 0.05$.
UV, Univariate; MV, multivariate; OR, odds ratio; CI, confidence interval; BMI, body mass index; PAPS, primary antiphospholipid syndrome; ESR, erythrocyte sedimentation rate; hsCRP, hypersensitive C-reactive protein; LAC, lupus anticoagulant; ACL, anticardiolipin; antiβ2GPI, anti-β2-glycoprotein I.

TABLE 4 | The rate of aPLs, complications and treatment between groups of re-canalization and no re-canalization in APS patients with PVT.

	Complete/Partial re-canalization (*n* = 17)		Recurrence/No re-canalization/Death (*n* = 17)		*P*
	n	%	*n*	%	
Acute	4	23.53	7	41.18	0.465
Gastrointestinal bleeding	2	11.76	3	17.65	1
Portal hypertension	9	52.94	8	47.06	0.732
Liver cirrhosis	4	23.53	5	29.41	1
Effective anticoagulation	14	82.35	2	11.76	**<0.001**
ACL	10	58.82	10	58.82	1
aβ2GP1	11	64.71	13	76.47	0.708
LAC	9	52.94	10	58.82	0.73
Splenomegaly	12	70.59	10	58.82	0.721
Blood system involvement	10	58.82	12	70.59	0.721

Values in bold are statistically significant at $p < 0.05$.
LAC, lupus anticoagulant; ACL, anticardiolipin; antiβ2GPI, anti-β2-glycoprotein I.

difference in accumulated no portal hypertension/ cirrhosis rate (log-rank $p = 0.32$; **Figure 3**) and comprehensive adverse events (portal hypertension, cirrhosis, and death) rate (log-rank $p = 0.12$; **Figure 4**) between the effective anticoagulation group and invalid anticoagulation group.

DISCUSSION

This is the first case-control study of the clinical characteristics and prognosis of PVT in APS patients. We found that PVT could be the first thrombotic event of APS, usually had insidious onset with atypical clinical symptoms and easily be misdiagnosed. Clinicians should pay more attention to APS patients combined with PVT. For patients with APS, the double aPLs positive, thrombocytopenia, and inflammation could be the associated factors of PVT. Early diagnosis and anticoagulation treatment can bring thrombus re-canalization thereby significantly improving the prognosis, with a lower mortality rate.

PVT could be the first thrombotic event of APS and PVT is not always symptomatic or result in complications like variceal bleeding and ascites (13). In our study, 21 patients had PVT as the first thrombotic event of APS. And seven patients were asymptomatic. Most of them were underdiagnosed or misdiagnosed at first. In other words, in cases recognized at a stage of cavernoma, the initial episode of portal venous thrombosis probably escaped attention, because signs and symptoms were mild or non-specific or because of inadequate imaging studies. Harmanci et al. (13) reported that the most

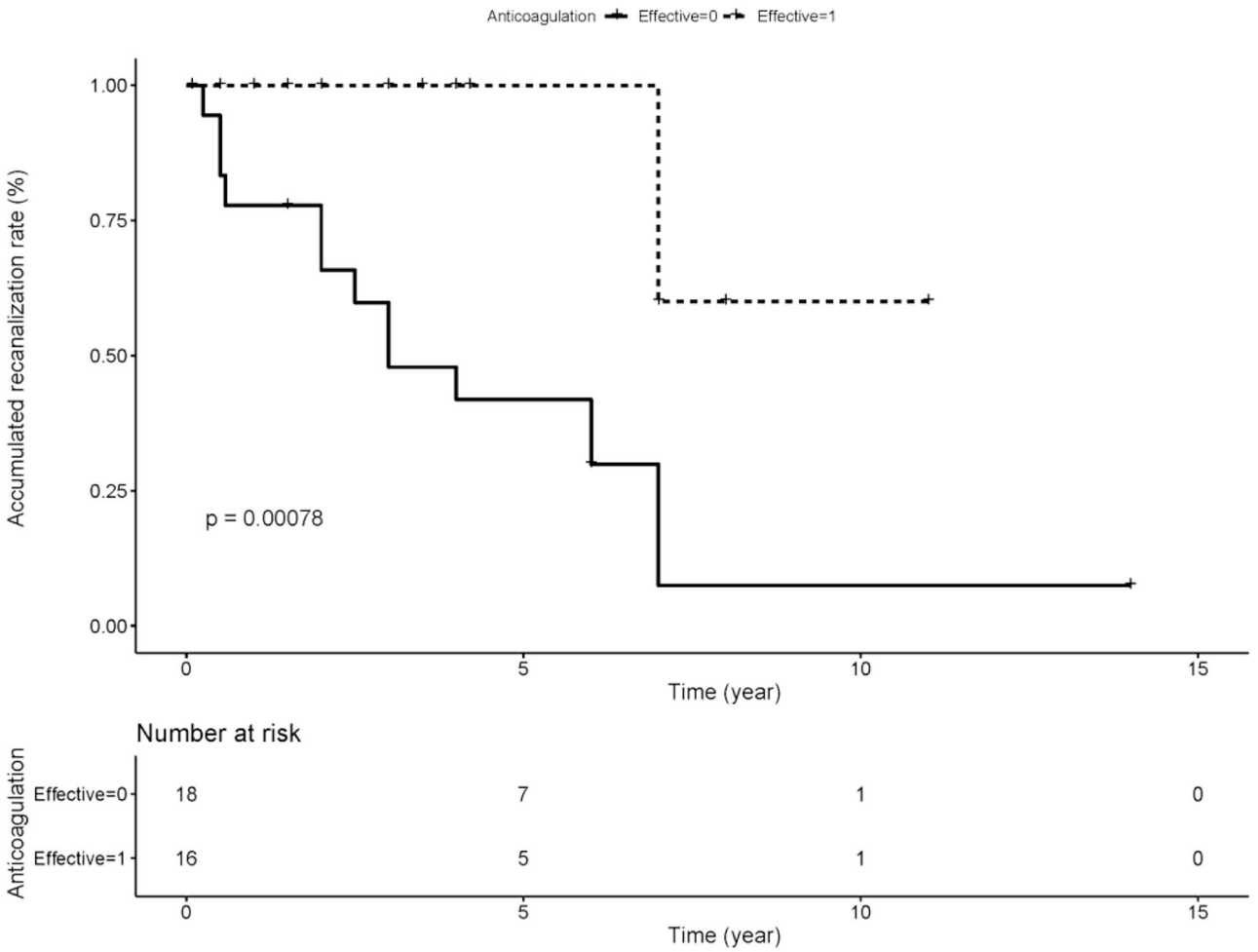

FIGURE 1 | Difference of accumulated re-canalization rate between groups receiving effective anticoagulation ($n = 16$) and not ($n = 18$). Effective anticoagulation was defined as immediate and sufficient anticoagulant therapy for at least 6 months with a target international normalized ratio (INR) of 2.0–3.0 from the time of portal vein thrombosis (PVT) diagnosis.

common presentation of PVT is variceal bleeding followed by pancytopenia due to hypersplenism. It may be a good practice to select the potential patients according to their co-morbid conditions, degree of thrombocytopenia, and the condition of varices. Subtle laboratory abnormalities could lead to missed diagnoses and symptomatic portal hypertension is often indicative of the late stage of the PVT. Fortunately, with the advent and wide distribution of USG and Doppler-USG, the condition is becoming diagnosed earlier and a patient presenting with ascites (which is a late finding in course of PVT) is almost not seen. Esophageal and gastric varices related bleeding contribute to the most important cause of morbidity and hospitalization in this group of patients. Different from cirrhotic patients, the risk of variceal bleeding is much lower (14).

We found that double aPLs positive is an associated factor of PVT in APS patients. The presence of aPLs is associated with an increased risk of arterial and venous thrombosis, thrombocytopenia, and recurrent abortions (15). Two large series found aPL in 4 and 11% of patients with PVT (2, 3). There were two reports of PVT with APS based on the presence of LAC (8, 10). However, Janseen et al. (16) found LAC in 4.7% of 42 patients with Budd Chiari syndrome but none of 92 patients with PVT. Austin et al. found that IgA aCL may trigger thrombosis in small portal vein radicles, which drain the inflamed small intestine, leading to liver injury with consequent hyperplasia of the surrounding tissue (17). A meta-analysis (18) found that the risk of Budd–Chiari syndrome and non-cirrhotic PVT might be increased by positive IgG aCL but not IgM aCL, LAC, aβ2GPI, or β2GPI ox-LDL. But the association between aPLs and PVT in liver cirrhosis was unclear (19). The pathogenesis of aPL induced thrombosis is complex and involves the activation of platelet and neutrophil and injury of the endothelium, finally resulting in an abnormal coagulation cascade (15). 44% of "triple-positive"

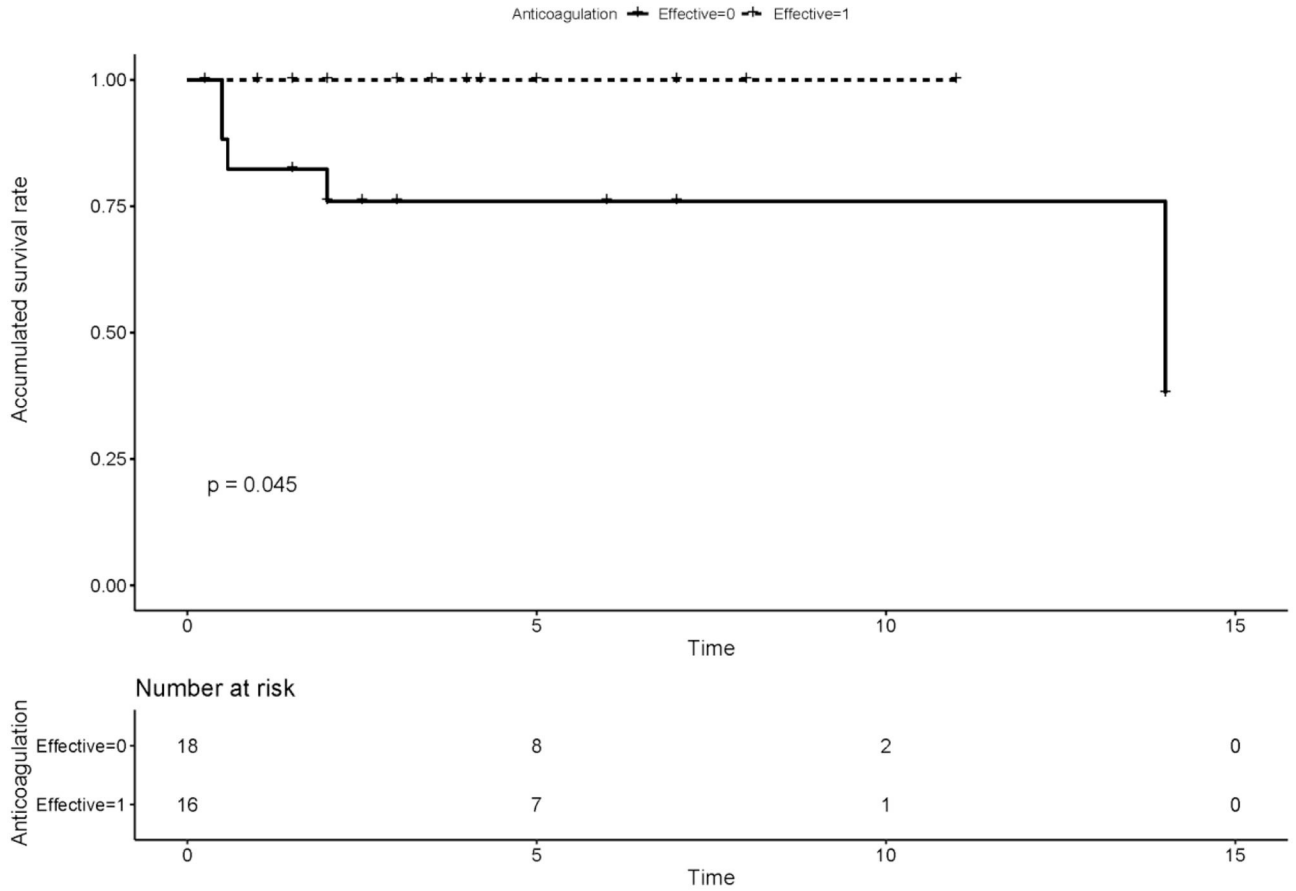

FIGURE 2 | Difference of accumulated survival rate between groups receiving effective anticoagulation ($n = 16$) and not ($n = 18$). Effective anticoagulation was defined as immediate and sufficient anticoagulant therapy for at least 6 months with a target international normalized ratio (INR) of 2.0–3.0 from the time of portal vein thrombosis (PVT) diagnosis.

APS patients will develop recurrent thrombosis over a 10-year follow-up period, even with the majority being prescribed anticoagulants (20).

Our study also shows that thrombocytopenia and inflammation (hsCRP) were associated factors of PVT in APS patients. The direct binding of aPLs could lead to platelet activation and aggregation, which eventually leads to thrombocytopenia (21). The thrombosis process also consumes a large number of platelets. Cirrhosis, hypersplenism, bone marrow hematopoietic inhibition, and drugs (such as heparin) may also be involved in the process of thrombocytopenia. Therefore, thrombocytopenia has no protective effect on thrombosis. It means that hemorrhage and hypercoagulability exist at the same time, which is a high-risk factor for recurrence of thrombus and requires more attention in clinical practice. In our study, elevated CRP is an independently associated factor of PVT. A large number of studies have shown a close relationship between inflammatory status and venous thrombosis. We have already found that lupus patients with higher hsCRP have a high risk of pulmonary embolism (22). The inflammatory status could lead to pro-coagulant disorders, affect vascular homeostasis, or elevate blood coagulability through decreased blood flow speed (23), all of which contributed to the thrombotic events.

The outcome of PVT might vary from one patient to another and depend on early anticoagulation and sustained re-canalization of the portal and mesenteric veins. In the current study, 14 in 16 patients receiving effective anticoagulation got complete or partial re-canalization. Re-canalization of the portal vein on anticoagulant therapy could also prevent the development of portal hypertension (13). There have been reports of re-canalization of PVT after anticoagulation (7). However, the clinical setting of hypersplenism with low platelet counts combined with esophageal varices raises concerns about the safety of anticoagulation. A retrospective analysis (14) got the conclusion that anticoagulation was not found to be a risk factor for bleeding in non-cirrhotic PVT, while no anticoagulation resulted in more thrombotic recurrences as expected. Pharmacological prophylaxis of early anticoagulation can also decrease the incidence of PVT in cirrhotic patients (24). Anticoagulation is also recommended for APS patients with thrombotic manifestations to prevent recurrence or extension of

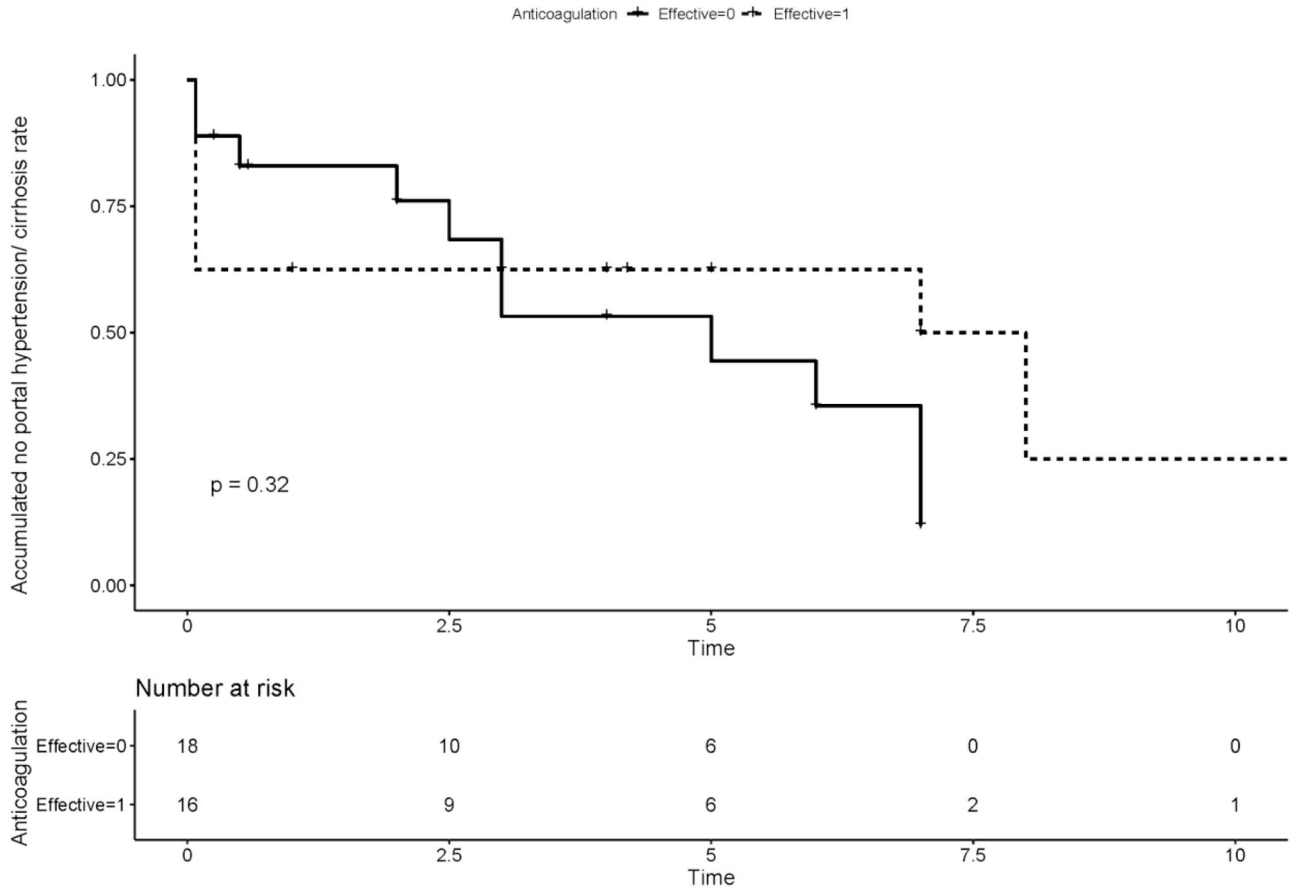

FIGURE 3 | Difference of accumulated no portal hypertension/cirrhosis rate between groups receiving effective anticoagulation ($n = 16$) and not ($n = 18$). Effective anticoagulation was defined as immediate and sufficient anticoagulant therapy for at least 6 months with a target international normalized ratio (INR) of 2.0–3.0 from the time of portal vein thrombosis (PVT) diagnosis.

thrombosis (25). According to current recommendations, APS patients with venous thromboembolism are best treated with standard-intensity oral anticoagulation at a target INR of 2.0–3.0 (26). However, since PVT patients, especially those with liver cirrhosis, also have a high risk of bleeding, the most suitable INR still needs more clinical research to finalize. The duration of anticoagulation has been a topic of debate; some authors recommend anticoagulation for 3–6 months in patients with a first venous thromboembolic event and a transient/reversible precipitating factor and in whom aPL becomes negative over time (27). However, some studies have shown that the risk of relapse events is significantly increased after stopping anticoagulation therapy in those with aPL (28). The choice of anticoagulant type is also a problem. The possibility of direct oral anticoagulants (DOACs) as secondary thromboprophylaxis in APS patients has been controversial. The results of open-label RCT (TRAPS trial) failed to demonstrate non-inferiority of the DOAC rivaroxaban to dose-adjusted vitamin K antagonists (VKAs) for thrombotic APS, as well as showing an increased risk of stroke with rivaroxaban (29). The results of the TRAPS study led EULAR to recommend against the use of rivaroxaban in APS patients with triple aPL positivity and a history of arterial thrombosis (30).

Our research had several limitations. The major limitation of the study is the retrospective design. Some irregular anticoagulation therapy cannot be fully reported. We only tested the total titer of the antiphospholipid antibodies, but not the specific IgG, IgA, and IgM type. Secondly, we study a small number of patients due to the low incidence of APS-PVT. Despite these limitations, our study is the first study reporting the outcome of anticoagulation therapy in APS-PVT patients. We still need larger prospective studies in the future to ensure the association between re-canalization and anticoagulation to support the recommendation of early anticoagulation therapy.

In conclusion, portal vein thrombosis is a rare and severe subtype of APS with a poor prognosis. The disease usually had insidious onset with atypical clinical symptoms

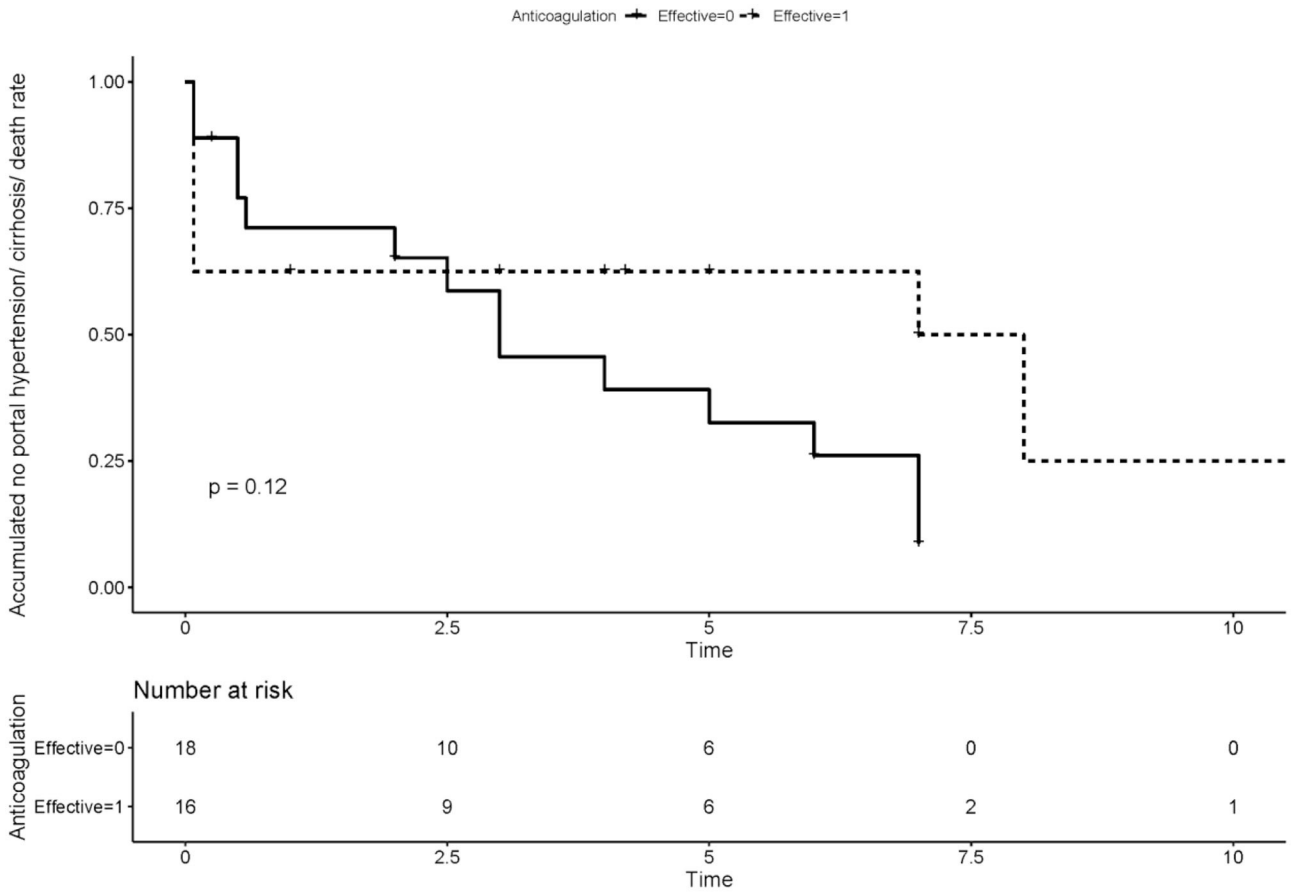

FIGURE 4 | Difference of accumulated no comprehensive adverse events (portal hypertension, cirrhosis, and death) rate between groups receiving effective anticoagulation ($n = 16$) and not ($n = 18$). Effective anticoagulation was defined as immediate and sufficient anticoagulant therapy for at least 6 months with a target international normalized ratio (INR) of 2.0–3.0 from the time of portal vein thrombosis (PVT) diagnosis.

and easily be misdiagnosed. Those APS patients with abdominal symptoms should be paid more attention. We should pay more attention to patients with PVT and we suggest patients with PVT screen aPLs routinely. Early diagnosis and anticoagulation treatment can bring thrombus re-canalization thereby significantly improving the prognosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HY, JZ, ML, and XZ designed the study. HY, JZ, CH, XT, ML, and XZ collected the data and performed research. HY and JZ carried out data analyses. HY drafted the manuscript. JZ and ML helped to explain the critical points in the manuscript. All authors approved the final version of the manuscript.

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frontiers in Medicine

REVIEW
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The Use of Glucocorticoids in Lupus Nephritis: New Pathways for an Old Drug

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Glucocorticoids therapy has greatly improved the outcome of lupus nephritis patients. Since their discovery, their adverse effects have counterbalanced their beneficial anti-inflammatory effects. Glucocorticoids exert their effects through both genomic and non-genomic pathways. Differential activation of these pathways is clinically relevant in terms of benefit and adverse effects. Ongoing aims in lupus nephritis treatment development focus on a better use of glucocorticoids combined with immunosuppressant drugs and biologics. Newer regimens aim to decrease the peak glucocorticoid dose, allow a rapid glucocorticoid tapering, and intend to control disease activity with a lower cumulative glucocorticoid exposure. In this review we discuss the mechanisms, adverse effects and recent strategies to limit glucocorticoid exposure without compromising treatment efficacy.

Keywords: glucocorticoids, lupus nephritis, systemic lupus erythematosus, prednisone, methylpredisolone, steroids, adverse effect

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INTRODUCTION

Cortisone ("compound E" or *17-hydroxy-11-dehydrocorticosterone*) was identified in the 1930's by Edward Kendall and Tadeusz Reichstein, and later purified and synthesized in the 1940's. Compound E had strong anti-inflammatory effects but also potent mineralocorticoid effects which manifested as fluid retention, hypertension, and hypokalemia. Compound E was first applied for treatment in 1948 by Philip Hench. At that time, a young woman with severe rheumatoid arthritis would become the first patient treated with cortisone. The anti-inflammatory effects of the cortisone were remarkable but so were the adverse effects (1, 2).

Subsequently, other glucocorticoid (GC) preparations were developed for treating autoimmune diseases, including systemic lupus erythematosus (SLE) (3). The use of these anti-inflammatory steroids in lupus nephritis (LN) dramatically improved survival. For example, survival was 17% at 5 years in the pre-glucocorticoid era, but 55% at 5 years after introduction of glucocorticoids (4, 5). The addition of immunosuppressive drugs to GC, and later on, the development of biologic drugs, have transitioned LN management to one focused on improving kidney outcomes while minimizing adverse events. In this review, we discuss the use of GCs from mechanisms, adverse events to management of lupus nephritis, and current strategies to limit toxicity of these drugs.

MECHANISM OF ACTION: THE CLINICAL RELEVANCE OF THE GENOMIC AND NON-GENOMIC MECHANISMS

Glucocorticoids are involved in regulatory processes throughout the body, such as energy and lipid metabolism, and adaptation to stress. Two of their most important effects are their strong anti-inflammatory and immunosuppressive effects, evident at concentrations above the physiological glucocorticoid levels (6).

Glucocorticoids and synthetic glucocorticoids have two mechanisms of action: the genomic and non-genomic mechanisms (**Figure 1**) (7). Genomic mechanisms are activated after GC, as lipophilic molecules, cross the cell membranes and bind to the multiprotein complex of chaperones (e.g., Hsp40, Hsp56, Hsp70, and Hsp90), immunophilins that act as co-chaperones (e.g., p23, FKBP51, FKBP52), and the intracellular cytoplasmic glucocorticoid receptor (cGR). After binding and subsequent dissociation from these proteins, the complex GC-cGR translocates to the nucleus and binds to DNA binding sites known as *glucocorticoid response elements*. The final result is a decreased transcription of genes encoding inflammatory cytokines (e.g., interleukin-6, interleukin-8, tumor necrosis factor-a), a process known as *transrepression*; and an increased transcription of anti-inflammatory genes (e.g., interleukin-10, IκB, annexin A1), known as *transactivation* (8).

Genomic mechanisms are generally evident 30 min after GC administration. By contrast, a second type of non-genomic mechanisms produce effects within minutes after the administration. These non-genomic effects are mediated through changes in cellular membranes, inactivation of the phospholipase A2 enzyme, and interaction with membrane glucocorticoid receptors (mGR). Second messengers include kinases, such as the p38 MAP kinase. The final effect is decreased lymphocyte activity and proliferation (9).

Identification of genomic and non-genomic mechanisms is clinically important due to the differential adverse effect profile and differential activation exerted by currently used glucocorticoid dosages and preparations (**Figure 1**). Genomic effects are activated with low (<7.5 mg prednisone equivalent per day) to moderate (7.5–30 mg prednisone equivalent per day) GC doses, and cGRs are progressively saturated with high-doses above 30 to 50 mg per day (10). From this pharmacologic concept, prednisone doses above 50 mg per day approach the ceiling of cGR saturation, with limited additional anti-inflammatory benefit, yet increasing the risk for adverse effects. As will be further discussed, some adverse effects, such as avascular bone necrosis, are dependent on the peak GC dose and duration of high-dose exposure (tapering speed) (**Figure 2**).

Non-genomic mechanisms are activated with very-high GC dosages, such as those reached with methylprednisolone pulses. This activation starts at prednisone dosages of 100 mg, and reaching its maximum around 250 to 500 mg. In contrast to effects mediated by genomic mechanisms, non-genomic mechanisms are thought to be associated with less adverse effects, at least in part due to the short duration of administration (11).

The relative activation of these genomic and non-genomic pathways differs among different GC preparations. For example, dexamethasone and methylprednisolone activate the non-genomic pathway at a 3-fold greater rate than prednisone (12). Different GC preparations also differ in potency (expressed relative to hydrocortisone), mineralocorticoid effects, and duration of suppression of the hypothalamic-pituitary-adrenal axis (13). Other factors, such as time of administration (less suppression when administered in the morning) and their chronopharmacology, contribute to the degree of GC-axis suppression and in consequence to the severity of adverse effects, but are beyond the scope of this review (14).

Understanding these mechanisms is important to develop strategies to limit GC toxicity. As shown in **Figure 2**, GC administration strategies used in recent clinical trials have included intravenous methylprednisolone pulses, which activate non-genomic pathways, followed by lower peak oral GC dosages and a faster tapering of oral GCs. This strategy aims to maintain treatment efficacy while limiting GC-related adverse effects.

GLUCOCORTICOID-RELATED ADVERSE EVENTS

Both disease activity and glucocorticoid exposure have been associated with organ damage in SLE (15, 16). As patients with higher degree of disease activity are usually treated with higher GC doses, many of the reported studies suffer of confounding by indication (i.e., patients with more severe activity are administered higher GC doses). Also, as damage is frequently measured through indices that group several manifestations [e.g., the SLICC/ACR damage index (SDI)], it is difficult to distinguish organ damage caused by prednisone from that caused by disease activity or concomitant immunosuppressive medications (17). Finally, many studies also suffer from time bias, as the contribution of disease activity to damage is usually higher at earlier stages, while GC-related damage is greater at later stages (17).

Organ damage occurs in 50% of patients with SLE within 5-years of SLE diagnosis (18), with reported increased risk of 2.8% for each 1 mg prednisone per day (19). Organ damage has been reported to be minimized by achieving disease remission (15, 20), and by using maintenance doses of prednisone lower than 6.0 to 7.5 mg per day (21–23).

GC-related adverse effects have also been classified into those related to high dosing over a short period of time, and those related to cumulative GC doses. **Table 1** summarizes the reported GC adverse effects according to the use of intravenous methylprednisolone pulses, the peak oral-GC dose, the duration of exposure to high-GC doses, and the GC cumulative dose.

INFECTIONS

Infections have been frequently associated with the peak dose of GC and the duration of exposure to high GC doses. Infections continue to be a major cause of hospitalization and mortality in

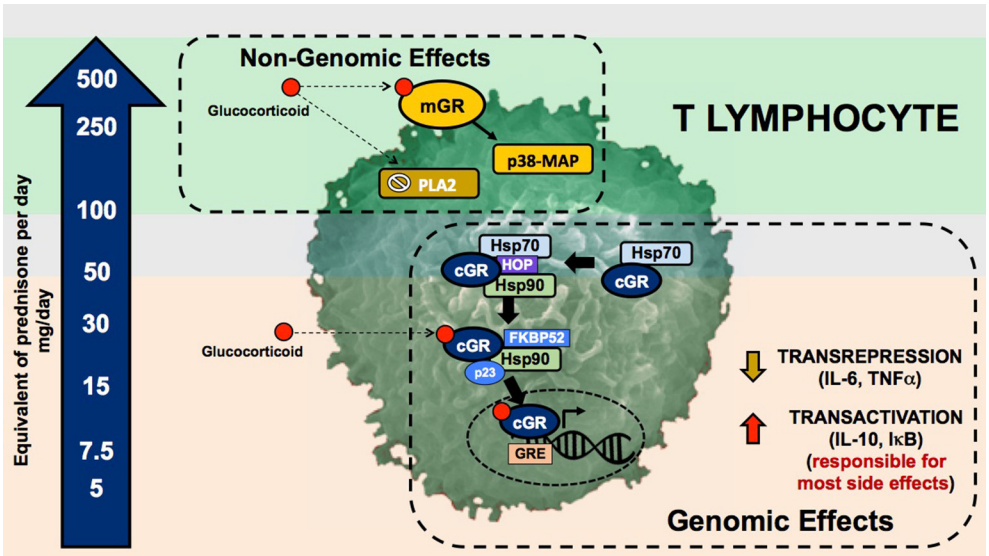

FIGURE 1 | Genomic and non-genomic mechanisms of glucocorticoids. Glucocorticoid genomic pathway is mediated through the cytoplasmic glucocorticoid receptor (cGR) leading to the mechanisms of gene transactivation and transrepression. The non-genomic pathway is mediated through the membrane glucocorticoid receptor (mGR), inhibition of the phospholipase A2, and changes in cell membranes. The arrow in the left depicts the dose of prednisone required to activate these pathways. The upper and lower gray zones represent the doses were genomic (lower gray zone) and non-genomic (upper gray zone) are fully saturated without added benefit and with higher incidence of adverse effects. mGR, membrane glucocorticoid receptor; PLA2, phospholipase A2; cGR, cytoplasmic glucocorticoid receptor; GRE, glucocorticoid response element; Hsp70·HOP·Hsp90, multiprotein complex including chaperones such as heat shock proteins and the glucocorticoid receptor; Hsp90·FKBP52·p23, multiprotein complex including chaperones, co-chaperones, and the glucocorticoid receptor.

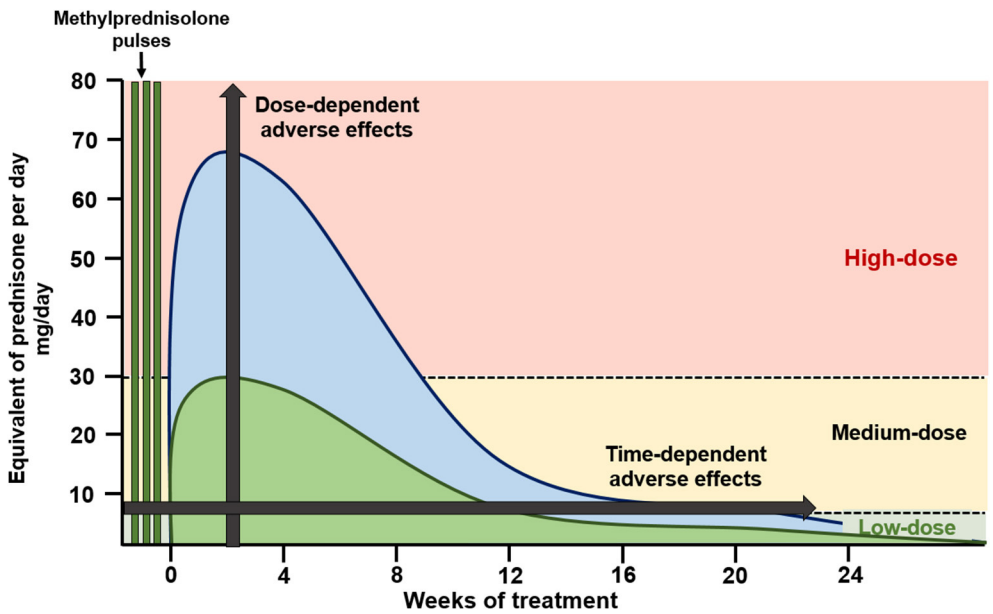

FIGURE 2 | Glucocorticoid dosing in induction of remission schemes. High-dose oral glucocorticoid schemes (blue) apply starting doses of oral glucocorticoids at 0.8–1.0 mg/kg/day, with slow tapering, reaching low glucocorticoid doses by 24 weeks of therapy. Recent schemes (green) apply methylprednisolone pulses followed by medium starting doses of oral glucocorticoids (<0.5 mg/kg/day) with a faster tapering, reaching low glucocorticoid doses by 12 weeks of therapy.

SLE (24–26). An increased incidence of these infections occurs in patients with kidney disease (27). Although bacterial infections in lungs, skin, and urinary tract are far more frequent (25, 28, 29), the risk for both bacterial and opportunistic infections increases progressively with the use of medium- to high-dose of GC (30–33). The risk of infections associated with high-dose GC

TABLE 1 | Reported associations between glucocorticoid (GC) administration and adverse effects.

Methylprednisolone pulses	High GC doses	Longer time under high GC doses	Cumulative GC dose
Acute cardiovascular events	Cardiovascular events	Cardiovascular events	Cardiovascular events
Acute cerebrovascular event	Cerebrovascular events	Bacterial and opportunistic infection	Hypertension
Uncontrolled glucose	Insulin resistance	Insulin resistance	Insulin resistance
Uncontrolled hypertension	Cushingoid features	Cushingoid features	Skin thinning, bruising
	Peptic ulcer disease	Weight gain	Hypertension
	Myopathy	Dyslipidemia	Osteoporosis and vertebral fractures
	Mood disorders	Glaucoma	Sleep disorders
	Psychiatric	Osteoporosis	Avascular necrosis
	Sleep disorders		
	Avascular necrosis		

administration seems to be independent of the use of other immunosuppressive medications (21).

Studies of infections with administration of methylprednisolone pulses have also been confounded by indication, due to the traditional administration of this treatment in combination with other aggressive immunosuppressive regimens to sicker patients (32, 34). Some studies suggest that the risk of infection is lower with the use of methylprednisolone pulses of less than 1.5 g in total (35, 36). It has also been hypothesized that the shorter duration of pulse therapy (3–5 days) may limit the prolonged suppression of T-cell responses, which usually peaks after 21 days of GC administration (37). Therefore, methylprednisolone pulses of less than 1.5 g in total followed by reduced oral GC may potentially decrease the incidence of steroid induced infections. Additional preventive measures include vaccination and the use of prophylactic antibiotics and antivirals when indicated (38, 39).

BONE DISEASE

Avascular bone necrosis occurs in 5–15% of patients with SLE. It is most commonly found in the femoral head, but may occur in other weight-bearing joints, and may occur bilaterally (40–42). The pathophysiology of avascular bone necrosis is not fully understood and suggested mechanisms are reviewed elsewhere (43). As for infections, avascular bone necrosis has been reported to occur more frequently in association with lupus nephritis (44, 45). Also, it has been associated with GC pulse therapy (46), the peak initial GC dose (47, 48), and the high cumulative GC doses in the first months of treatment (40, 49).

The prevalence of osteoporosis in SLE is 10 to 20%, with up to 20% of patients experiencing vertebral fractures (50). Glucocorticoids increase bone resorption and reduce bone formation. The former is more pronounced in the first months of steroid use while the latter becomes predominant with chronic GC use (51). Osteoporosis and vertebral fractures have been associated with higher GC doses, cumulative doses, and prolonged administration (52). The risk of osteoporotic fractures has been estimated to increase 4.2% for each 1 mg per day of prednisone (19). As bone loss develops over a long-time, many studies with short follow-up fail to assess the impact of GC therapy on bone density. Assessment of risk for fractures and of the need for concomitant preventive therapies including calcium, vitamin D, and bisphosphonates are recommended for all patients on GC therapy and are reviewed elsewhere (53, 54).

Metabolic Disease

Long-term and high-dose GC therapy are associated with pro-atherogenic disturbances that characterize the metabolic syndrome (55). This syndrome occurs in 30 to 40% of patients with SLE and has been associated with higher disease activity, past or present history of LN, and higher oral doses of GCs. Its prevalence varies according to age and ethnicity as expected (56, 57).

Insulin resistance increases in patients with SLE on oral GC above 7.5 mg per day (58). Furthermore, the risk of diabetes increases 2- to 4-fold in non-diabetic patients with SLE, especially with increasing years of chronic GC use (59–61). In patients with pre-existing diabetes, exacerbation of the disease is particularly severe in patients with poor glycemic control at baseline (62, 63).

Hypertension is common in SLE and LN patients, with a prevalence up to 70% when assessed by 24-h blood pressure monitoring (64). Acute exacerbation of hypertension is frequent during pulse GC therapy. Although hypertension during an active LN is mediated by salt-sensitive mechanisms (65), the risk of hypertension has been also reported to be higher in patients exposed to GC, and has been associated with the duration of exposure and the daily dosage of GCs (61, 66, 67).

Glucocorticoids contribute to weight gain by increasing the appetite for high caloric, high fat food intake (68, 69). The weight gain is characterized by central hypertrophy of adipose tissue with concomitant thinning of peripheral subcutaneous adiposity, providing a lipodystrophic appearance (Cushingoid phenotype) (70). Up to 60–70% of patients prescribed long-term GCs report weight gain (52), and this effect has been associated with doses of GC above 5 mg per day (52, 61).

CARDIOVASCULAR DISEASE

It is known that the incidence of cardiovascular events is increased in SLE, particularly, in patients with lupus nephritis and chronic kidney disease (23). Although it is difficult to

differentiate the effect of disease activity, traditional risk factors, and treatment-related factors; the use of medium- to high-dose GCs has been associated with increased cardiovascular events, subclinical atherosclerosis such as carotid intima-media thickening, severity of coronary calcifications, and severity of arterial stiffness (71, 72). The risk of cardiovascular events is estimated to increase 5-fold in SLE patients taking >20 mg per day of prednisone (23), and 3-fold in those who develop cushingoid features (73). Cardiovascular events may be reduced by administering lower peak GC doses, faster GC tapering, and by limiting cumulative dose. In fact, reductions in cumulative oral GC were associated with lower incidence of cardiovascular events in a reported cohort study (74).

STRATEGIES TO MINIMIZE GLUCOCORTICOID EXPOSURE DURING THE INDUCTION PHASE OF TREATMENT

The treatment of lupus nephritis has been traditionally divided into an induction phase of intense immunosuppression, aimed to quickly suppress inflammation, followed by a prolonged maintenance phase, directed to consolidate response and to prevent disease flares (75). For the induction phase, current guidelines recommend the use of medium to high-dose GCs, combined with an immunosuppressant such as mycophenolate mofetil, cyclophosphamide, and more recently, calcineurin inhibitors (38, 39). Next, we describe strategies aimed to reduce exposure while keeping treatment efficacy. These strategies have 3 main objectives: (1) reducing the peak GC dose, (2) reducing the duration of exposure to high-dose GC via a faster GC tapering, and (3) limiting the cumulative dose from prolonged administration.

THE USE OF INTRAVENOUS METHYLPREDNISOLONE PULSES TO LIMIT GLUCOCORTICOID EXPOSURE

Administration of methylprednisolone pulses may allow the use of lower initial oral GC doses (lower peak GC dose) with a faster tapering schedule (lower exposure to high GC doses). Several clinical studies in lupus nephritis have included methylprednisolone pulses, followed by moderate (≤0.5 mg/kg/day) doses of oral GCs (**Figure 3**) (76–78). The Euro Lupus Nephritis Trial (ELNT) scheme (77) included three 750 mg methylprednisolone pulses, followed by 0.5 mg/kg/day prednisone slowly tapered to 10 mg/day by 6 months. This trial reported renal response rates (complete and partial) around 20 and 50% at 6- and 12-months, respectively, and long-term preservation of kidney function (77, 79).

The MYLUPUS trial (80) is the only randomized clinical trial that compared the efficacy of medium-dose oral GC therapy to high-dose GC. In this trial, all patients received three 0.5 g methylprednisolone pulses plus extended-release mycophenolate acid. Subjects were randomized to either high-dose oral GC scheme (starting dose 1 mg/kg/day) or to a reduced-dose oral GC scheme (starting dose ≈0.5 mg/kg/day). Complete and total response rates were similar at 6 months, 19 vs. 21% and 67 vs. 56%, respectively, in both groups.

In a trial evaluating a combination of calcineurin inhibitor, mycophenolate mofetil and GCs vs. intravenous cyclophosphamide, all patients received three 0.5 g/day methylprednisolone pulses followed by 0.6 mg/kg oral

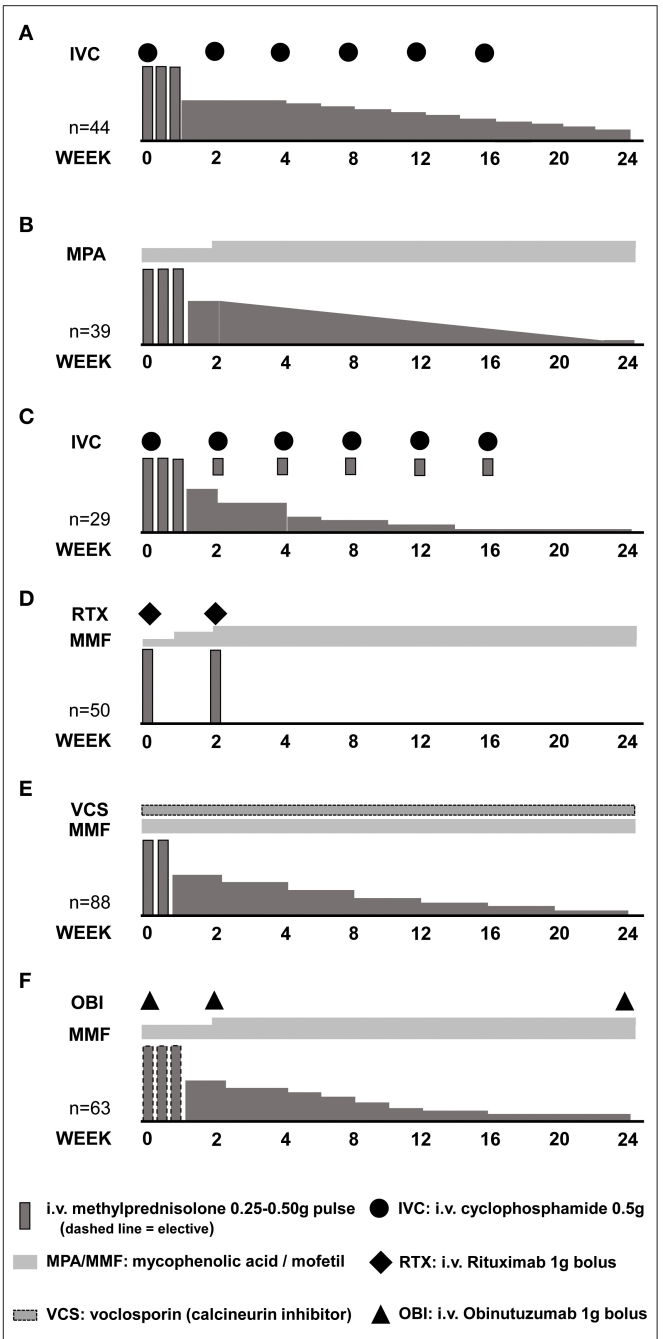

FIGURE 3 | Induction of remission schemes in several studies. **(A)** Euro Lupus Nephritis Trial low-dose cyclophosphamide arm; **(B)** MYLUPUS reduced-dose glucocorticoid arm; **(C)** Lupus-Cruces protocol; **(D)** RITUXILUP protocol; **(E)** AURA-LV study voclosporin-treated arm; **(F)** NOBILITY study obinutuzumab-treated arm.

prednisone slowly tapered to 10 mg/day by week 16. Response rates of 84 and 63% at 6-months, were documented in the multi-targeted therapy and cyclophosphamide groups, respectively, and of 78% in both groups by 2 years of therapy (81–83).

More recent clinical trials have used lower doses of methylprednisolone pulses combined with a lower and faster oral GC tapering. In the AURA-LV (84) and AURORA (NCT03021499) trials evaluating combination therapy of voclosporin (a novel calcineurin inhibitor) with mycophenolate mofetil and oral GC, patients were treated with two 0.25–0.5 g methylprednisolone pulses, followed by a fixed 20–25 mg/d starting oral prednisone rapidly tapered to 5 mg by 12 weeks. Among clinical trials in LN, these two trials used the lowest peak oral GC doses and the faster tapering (**Table 2**). At 12 months, complete and total renal response rates of 49 vs. 24%, and 67 vs. 48%, respectively, were observed in the multi-targeted treatment and control groups in the AURA-LV trial (84).

Uncontrolled single center experiences also suggest that treatment with methylprednisolone pulses allows a safe administration of lower starting oral GCs, and a faster tapering without compromising response and possibly reducing adverse effects. For example, the "Lupus Cruces" protocol for class III or IV LN includes the administration of three methylprednisolone pulses between 0.25–0.50 g, and an extra pulse of 0.1 g along with each cyclophosphamide bolus, following the ELNT scheme. The starting oral GC in this protocol was below 30 mg per day. In two reports, including 15 and 29 patients, response rates of 60 and 80%, and 86 and 87%, have been achieved at 6- and 12-months, respectively, with relapse rates below 15%. More importantly, the incidence of GC-related adverse effects was reduced to 7%, a significantly lower percentage when compared to that of historical or concurrent cohorts treated with higher doses of oral GC (48, 90).

Therefore, as evidenced in clinical trials and single-center experiences, the use of methylprednisolone pulses may allow reducing the starting oral GC doses and the duration of the exposure to high GC doses by allowing a faster tapering.

THE USE OF "REDUCED-DOSE" INTRAVENOUS METHYLPREDNISOLONE PULSES

There are no specific reports evaluating the dose of methylprednisolone in lupus nephritis. Although the use of methylprednisolone pulses has been associated with higher risk of infection in some cohort studies (32), these studies do not control for methylprednisolone dose. A small clinical trial including 21 patients with SLE (6 of them with nephritis) suggested that clinical outcomes are similar when using three daily 100 mg vs. 1 g methylprednisolone pulses. However, this trial did not control for other important variables such as concomitant treatment (91). While quality evidence is still low to support lower doses of methylprednisolone pulses, pharmacologic studies suggest that pulse doses above 0.5 g provide little additional anti-inflammatory benefit, and as mentioned earlier, may be associated with a higher incidence of adverse effects.

MEDIUM-DOSE GLUCOCORTICOID IN COMBINATION WITH NEW IMMUNOSUPPRESSANT DRUGS AND BIOLOGICS

Combination therapy of mycophenolate mofetil, calcineurin inhibitors, and glucocorticoids may facilitate the use of lower GC doses. As previously mentioned, the AURA-LV trial used a forced reduced steroid taper along with mycophenolate ± voclosporin. The multi-targeted group showed 67% response rate by 12 months of treatment (84).

A recent trial evaluated the combination therapy of obinutuzumab, a novel B-cell depleting therapy, with mycophenolic acid analogs. All patients received a starting oral prednisone dose of 0.5 mg/kg with a fast taper to 7.5 mg by week 12, and optional methylprednisolone pulses. This trial has reported 52-week CR rates of 35%, maintained at 40 and 41% by 76 and 104 weeks, respectively (89).

Other immunosuppressants such as Janus kinase inhibitors, spleen tyrosine kinase inhibitors and biologics such as anifrolumab and ustekinumab, are being tested in patients with LN. Their addition might also facilitate the use of lower dose glucocorticoids in the future.

SCHEMES FREE OF ORAL GLUCOCORTICOIDS

After initial reports describing the potential use of rituximab without increasing GC dose in renal (92) and non-renal lupus (93), the UK group from the Imperial College in London reported their first 50 patient experience with the RITUXILUP scheme (86). This regimen consists of 2 doses of rituximab 1 g administered with 0.5 g methylprednisolone followed by mycophenolate mofetil and no oral glucocorticoids. The initial report, which included class III, IV, and V LN patients, showed 6-month complete and total response rates of 32 and 62%, respectively. During follow-up, kidney function was preserved in most patients, with 22% of patients experiencing nephrotic relapses. Importantly, unlike the LUNAR trial (94) that failed to demonstrate a benefit of added rituximab to the standard of care therapy, depletion of B-cells to <5 B lymphocytes/mL was achieved in 93% of patients. The importance of B-cell depletion is supported by a sub-analysis from the LUNAR trial showing that complete response was more frequent in those subjects with B cell depletion (95). Therefore, although not yet demonstrated in a clinical trial, the RITUXILUP scheme supports the concept that the use of biologic drugs may facilitate the administration of GC free regimen in some patients with LN.

Targeting the activated complement system with complement inhibitors may also promote GC-reduced or GC-free regimens. Although complement inhibition in lupus nephritis has been used in a few case reports (96, 97), particularly in the context of concomitant thrombotic microangiopathy, the CLEAR (98)

TABLE 2 | Estimated cumulative glucocorticoid doses in a 24-week period for a 60 kg patient in different induction to remission schemes.

Regimen	Methylprednisolone total cumulative dose (g)	Oral prednisone total cumulative dose (g)	Oral prednisone average dose (mg/day)	Total GC dose (g)
Modified NIH, 2001 (76)	9.00	2.84	16.9	11.8
ELNT, 2002 (77)	2.25	3.12	18.5	5.37
ALMS, 2009 (85)	–	4.27	25.4	4.27
MYLUPUS, 2011 (80)	1.50	2.14	12.7	3.64
RITUXILUP, 2013 (86)	1.00	–	–	1.00
LupusCRUCES, 2014 (48)	1.50-3.00	1.30-1.50	8.0-9.0	2.80–4.50
Chinese multitarget, 2015 (81)	1.5	3.25	16.2	4.75
4+2 Rituximab, 2015 (87)	2.70	2.52	15.0	5.22
AURA-LV, 2019 (84)	1.00	1.33	7.9	2.33
BLISS-LN, 2020 (88)	0.50–3.00*	3.12–4.27	18.5–25.4	3.12–4.27
NOBILITY, 2020 (89)	0.75–3.00*	1.79–1.93	10.6–11.5	1.79–1.93

*Methylprednisolone pulses elective at discretion of the investigator.

and ADVOCATE (99) studies in ANCA-associated vasculitis suggest this may be an approach worth investigating in lupus nephritis. In these studies, administration of avacopan (an oral complement C5aR inhibitor) along with cyclophosphamide or rituximab, allowed the administration of a GC-free regimen with higher remission rates at 52 weeks of follow-up in patients with ANCA-associated vasculitis (99).

CONCOMITANT USE OF ANTIMALARIALS

The use of antimalarial in all patients with SLE and lupus nephritis is recommended in recent guidelines (38, 39). Although unexplored in controlled trials, combination schemes with antimalarial may add to the use of lower doses of GC by an enhanced effect for remission (100–102). Other demonstrated benefits from antimalarial, as the protective effect for damage accrual (103, 104), infections (33), and mortality (105), may add to the potential benefit of GC-reduced regimens.

GLUCOCORTICOIDS DURING THE MAINTENANCE PHASE OF TREATMENT

Maintenance therapy in lupus nephritis aims to consolidate the response obtained after the induction phase of therapy, and to prevent systemic and renal relapses. Current guidelines suggest tapering glucocorticoids to "the lowest possible dose" and to consider discontinuation after 12 months of complete remission (39).

Although there is no solid evidence in lupus nephritis, the CORTICOLUP trial (106) evaluated discontinuation of steroid in stable SLE patients (34–41% had history of LN). In this trial, patients receiving 5 mg of prednisone who have been stable for 1 year (the median quiescence duration was ≈5 years) were randomized to suspend or continue prednisone at the same dose. Disease flares were observed in 27% of patients who suspended prednisone vs. 7% in those who continued prednisone at 5 mg per day (RR 0.2, 0.01–0.7, $p = 0.003$). Only 3 patients had renal flares and the study was underpowered to evaluate the subgroup of patients with LN. Noteworthy, there were no differences in adverse events or damage accrual in both groups measured using the glucocorticoid toxicity index (107) and the SDI, respectively.

This study suggests that a low-dose of glucocorticoids at 5 mg per day may be safe and keeps patients free from disease flares. In other studies, the longer duration of the GC therapy before suspension has also been associated with less disease flares (108). A recent EULAR expert consensus suggested that at ≤5 mg/day, there is a low level of harm related to GC's main adverse effects (109), however, acknowledges that the actual risk of harm is patient-specific. Therefore, long-term glucocorticoid therapy must be balanced individually considering individual risk factors for flares (e.g., partial instead of complete response, persistently low C3), against individual risk factors for GC related adverse effects (e.g., age, cumulative GC dose, cardiovascular risk factors, presence of metabolic disease, etc.).

STRATEGIES TO MINIMIZE CORTICOSTEROIDS DURING THE MAINTENANCE PHASE

Antimalarial Treatment

Antimalarials have been associated with lower incidence of disease flares in several observational cohort studies (110, 111). Moreover, reports of successful withdrawal of therapy in SLE patients have repeatedly found antimalarial treatment and duration of remission as the main factors associated with decreased odds of flares (112, 113). Also, as previously mentioned, antimalarials may reduce long-term damage from the disease activity (114).

BIOLOGICS FOR MAINTENANCE THERAPY

Although evidence is still scarce, there is growing data suggesting that the use of certain biologics during the maintenance phase may aid in achieving sustained remission. For patients already on glucocorticoids, the RITUXIRESCUE regimen includes the administration of rituximab and methylprednisolone without increasing oral GC dose. This regimen showed a response rate of 78% in LN relapses, furthermore it allowed reduction or discontinuation of oral GC in more than 50% of patients during follow up (92).

An Italian strategy consisting of four 375 g/m^2 rituximab doses reinforced by two additional doses at 1 and 2 months after (the 4+2 rituximab scheme), showed no flares during follow-up without the need for additional maintenance therapy beyond 5 mg of prednisone per day (87). Other small reports have highlighted the potential role of rituximab as a maintenance drug allowing glucocorticoid suspension (115). Therefore, although rituximab has not been tested for maintenance in a clinical trial, its use may aid in preventing flares during GC withdrawal.

In the BLISS-LN trial, the addition of belimumab to standard of care therapy (MMF or cyclophosphamide plus GC) showed a better response and a stable glomerular filtration rate beyond the induction phase, for up to 2 years of follow up (88). Furthermore, there have been small reports (116–118) suggesting that belimumab therapy may allow reduction or suspension of maintenance GCs, but this remains to be further studied.

Likewise, the NOBILITY trial has reported that the addition of obinutuzumab to standard of care therapy favored a sustained response, better glomerular filtration rate, and better serological profile at 76 weeks and onwards (89). This suggests that B cell targeted therapy may potentially facilitate GC withdrawal or at least a safe reduction to <5 mg/d of prednisone.

FUTURE STEPS AND A WORD OF CAUTION

Although recent advances in drug development in lupus nephritis promote the use of lower glucocorticoid doses, we must acknowledge that "one size does not fit all" patients. For example, patients with severe lupus nephritis presenting with a glomerular filtration rate below 30 mL/min/1.73 m^2 have been excluded from most clinical trials, and there are no data to support the effectiveness of reduced glucocorticoid doses in this group of patients. Moreover, many of the published studies are single-center and observational reports subject to bias. Therefore, caution and case-by-case evaluation is recommended in selecting an appropriate glucocorticoid therapy.

Future studies in lupus nephritis will likely aim at using the lowest effective dose of glucocorticoids or glucocorticoid-free regimens. Studying the safety and efficacy of calcineurin inhibitors, biologic drugs or perhaps complement inhibitors in combination with standard of care therapy might lead successfully to this aim.

CONCLUSIONS

The anti-inflammatory properties of GC have always been counterbalanced by their side effects. Adverse effects may be associated with peak doses, time under high doses, or cumulative doses. An objective for current and future management of lupus nephritis is to develop strategies that increase response to therapy with the least glucocorticoid exposure.

AUTHOR CONTRIBUTIONS

JM-V and IA designed the concept, planned, and performed this work.

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Longitudinal Analysis of Anti-cardiolipin and Anti-β2-glycoprotein-I Antibodies in Recent-Onset Systemic Lupus Erythematosus: A Prospective Study in Swedish Patients

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Background: Anti-phospholipid syndrome (APS) and systemic lupus erythematous (SLE) are autoimmune disorders that often co-occur. Anti-phospholipid antibodies (aPL) are typical of both conditions and may be associated with vascular events and pregnancy-related morbidities. Whereas, aPL-screening is mandatory for individuals with suspected SLE, the clinical value of longitudinal aPL analyses in established SLE is unclear.

Methods: We investigated the occurrence and variation of IgG/IgA/IgM anti-cardiolipin (aCL) and anti-β2-glycoprotein-I (anti-β2GPI) antibodies, using both the manufacturer's cut-off and a cut-off based on the 99th percentile of 400 apparently healthy donors, in recent-onset SLE. Furthermore, we evaluated the relationships between aPL levels and SLE/APS manifestations, as well as the pharmacotherapy. Patients with SLE who met validated classification criteria were included in this prospective study ($N = 54$). Samples were obtained at 0, 6, 12, 24, 36, 48, 60, 72, 84, and 96 months after SLE diagnosis.

Results: Depending on the cut-off applied, 61.1 or 44.4% showed a positive result for at least one aPL isotype or the lupus anticoagulant test over time. Median values for all six aPL isotypes numerically decreased from inclusion to last follow-up, but none of the isotypes met statistical significance. Seroconversion (from positive to negative, or the opposite direction) was occasionally seen for both aCL and anti-β2GPI. IgA and IgM anti-β2GPI were the most common isotypes, followed by IgM aCL. Presence of IgG aCL associated significantly with myocardial infarction and miscarriage, and IgG/IgA anti-β2GPI with miscarriage.

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Conclusion: aPL were common during the first years of SLE. Even though the levels fluctuated over time, the patients tended to remain aPL positive or negative. Repeated aPL testing in the absence of new symptoms seems to be of uncertain value in patients with recent-onset SLE.

Keywords: anti-phospholipid antibodies, anti-cardiolipin antibodies, anti-β2-glycoprotein-I antibodies, cardiovascular events, longitudinal analysis, pregnancy morbidities, systemic lupus erthematosus, antiphospholipid antibody syndrome

INTRODUCTION

Although antiphospholipid syndrome (APS) and systemic lupus erythematous (SLE) are distinct autoimmune disorders, they share several features and are often regarded as two sides of the same coin (1, 2). Indeed, the clinical presentations of Libman-Sacks endocarditis, livedo reticularis, migraine or autoimmune cytopenia, together with laboratory findings in relation to complement consumption, antinuclear and antiphospholipid antibodies (aPL), are commonly seen in both conditions (3). Regardless of whether APS appears on its own or concomitantly with (secondary to) other autoimmune conditions, it is classified based on the presence of one or more repeated aPL tests and at least one of two major clinical manifestations, thrombosis or pregnancy complications.

The validated APS classification criteria from 2006 include immunoglobulin (Ig)G/IgM anti-cardiolipin antibodies (aCL), IgG/IgM anti-β2-glycoprotein-I antibodies (anti-β2GPI), and the lupus anticoagulant (LA) test (4). The percentage of subjects with SLE who occasionally or continuously test positive for aPL has been estimated as in the range of 30–40%, and about half of these patients eventually fulfill the APS criteria (5–7).

There has been intense discussion regarding "seronegative" APS, since some patients with a clinical APS phenotype show negative results for the classical aPL tests (8). Indeed, antibodies directed against other proteins of the coagulation cascade, such as prothrombin and phosphatidylserine-prothrombin complexes, are examples of other aPL that are not (yet) included in APS classification criteria (1). This is because they are not used routinely, or there is some uncertainty as to their clinical significance, or there is a lack of standardized testing. Some studies have shown that one of the five domains of β2GPI, domain I, is of particular importance for the pathogenesis of APS (9, 10). Antibodies directed against this specific domain appear to be strongly associated with thrombosis, as well as with obstetric complications, as compared to antibodies that target the entire β2GPI molecule (11).

In SLE, the presence of aPL has been associated with thrombosis and/or pregnancy morbidities, as well as with worse prognosis involving increased damage accrual (12–15). A positive LA test has been identified as the single laboratory finding with the highest predictive value regarding future organ damage in patients with SLE (16). In contrast to the 2006 APS classification criteria and older SLE criteria sets, the 2012 Systemic Lupus International Collaborating Clinics (SLICC) and the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria include the IgA isotype for aCL and anti-β2GPI (2, 17). Some reports have suggested that the IgA isotype has additional value for the risk assessment of thrombosis and pregnancy morbidities (18–20). Other data indicate that the significance of IgA aPL varies across different ethnicities (21, 22). Our own recent study based on a Swedish (mainly Caucasian) SLE population concluded that IgA aPL alone are of additional value only for patients with a high probability of APS, despite negative test outcomes for IgG/IgM aCL, anti-β2GPI and LA (23). Similar data were recently reported from a large Chinese population (24).

Whereas, screening for aPL is mandatory during the investigation of individuals with suspected recent-onset APS or SLE, the clinical value of longitudinal aPL analyses in those patients with an established SLE diagnosis remains uncertain. Already in 1989, fluctuations of aCL levels over time in subjects with SLE were reported, and the variability of anti-β2GPI levels was also highlighted (25, 26). However, data regarding longitudinal prospective studies of aPL in patients with recent-onset SLE are limited (27). In primary APS, <10% of patients switched from being aPL positive to negative over 5 years of follow-up (28).

The primary aim of the present study was to investigate the presence and fluctuation of aPL isotype (IgG, IgA, and IgM) levels in patients with recent-onset SLE. The secondary aims were to evaluate the potential relationships between aPL levels and smoking habits, pharmacotherapy, SLE and APS manifestations, clinical and laboratory markers of disease activity, and organ damage accrual.

MATERIALS AND METHODS

Subjects

The 54 patients with newly diagnosed SLE (≤6 months of symptoms) included in this study have been described previously (29). All the subjects met the 1982 ACR criteria and/or the 2012 SLICC criteria and had taken part in the prospective follow-up program KLURING (Swedish acronym for Clinical LUpus Register In Northeastern Gothia) at the Rheumatology Clinic, Linköping University Hospital (30). APS was classified according to the Sydney criteria (4). Clinical follow-up data and serum samples (stored at −70°C) were collected from the time of SLE diagnosis (Month 0) and thereafter, in most cases yearly.

Pharmacotherapy [azathioprine, belimumab, cyclophosphamide, hydroxychloroquine (HCQ), methotrexate, mycophenolate mofetil, rituximab, prednisolone and sirolimus] was recorded at each visit. The daily dose of prednisolone

was calculated as a continuous variable. SLE disease activity was assessed using the clinical SLE disease activity index 2000 (cSLEDAI-2K) (which excludes items for low complement levels and positive anti-dsDNA) (31). Organ damage, required to have been persistent for ≥6 months, was recorded annually by the SLICC/ACR damage index (SDI), which encompasses damage in 12 defined organ systems (32). A detailed description of the study population is given in **Table 1**.

aPL Assays

Sera for the detection of IgG/IgA/IgM aCL and anti-β2GPI were available from the time-points of 0, 6, 12, 24, 36, 48, 60, 72, 84, and 96 months after the diagnosis of SLE. The analyses were performed by the personnel at the accredited Clinical Immunology laboratory at Linköping University Hospital using a fluoroenzyme-immunoassay (Phadia-250 instrument; ThermoFisher Scientific Phadia AB, Uppsala, Sweden). The cut-offs for each aPL isotype were set according to the manufacturer's instructions; for IgG and IgM aCL, positive results were defined as ≥10 U/mL and for IgA aCL ≥14 U/mL. For IgG, IgA and IgM anti-β2GPI, positive results were defined as ≥7 U/mL. Outcomes below the cut-off for each antibody were ascribed half the cut-off value in the statistical analyses. To comply with the Sydney criteria of APS, cut-offs based on the 99th percentile of apparently healthy donors ($N = 400$; 50% males, 50% females) were set and positive results were defined as follows: IgG aCL ≥24 U/mL, IgA aCL ≥17 U/mL, IgM aCL ≥30 U/mL, IgG anti-β2GPI ≥18 U/mL, IgA anti-β2GPI ≥9 U/mL, and IgM anti-β2GPI ≥6 U/mL (4). All samples were analyzed at the same occasion to minimize inter-assay variation.

The results of the LA tests, which were performed using the dilute Russell's viper venom time (dRVVT) method at the Clinical Chemistry laboratory at Linköping University Hospital, were retrieved from the medical records.

Statistics

For comparisons of aPL levels between groups, the Mann-Whitney *U*-test was used. Associations between aPL positivity (categorical variable) and APS-related events, SLE manifestations and organ damage were examined with the χ^2-test, or Fisher's exact-test when appropriate ($N \leq 5$). *P*-values ≤ 0.05 were considered statistically significant. Statistical analyses were performed using the SPSS software ver. 26.0.0.0 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism ver. 8.4.3 (GraphPad Software Inc., San Diego, CA). Graphs were created using GraphPad Prism ver.8.4.3 (GraphPad Software).

Ethics Statement

Oral and written informed consent was obtained from all the participants. The study protocol was approved by the Regional Ethics Review Board in Linköping (Decision Nr. M75-08/2008).

RESULTS

Prevalence of aPL

Table 2 lists the prevalence rates of aPL among the participating subjects at inclusion and longitudinally using the manufacturer's cut-offs or the more stringent 99th percentile cut-offs shown in parenthesis. Overall, 38.9% (24.1%) were ever positive for any aCL isotype. Regarding anti-β2GPI, 42.6% (37.0%) of the 54 patients showed ever positivity for any anti-β2GPI isotype. A positive LA test was recorded for 16 cases (29.6%). LA was initially controlled in all patients adjacent to the time-point of SLE diagnosis as part of clinical routine. In all patients with

TABLE 1 | Characteristics of the included patients with recent-onset SLE ($N = 54$).

	At inclusion	At last follow-up
Background variables		
Age, mean (range), years	44 (18–82)	
Gender, female, *N* (%)	45 (83.3)	
Ever smoker (former or current), *N* (%)	23 (42.6)	
Body mass index, mean (range)	25.1 (17.8–40.4)	
Caucasian ethnicity, *N* (%)	51 (94.4)	
Clinical APS phenotypes, *N* (%)		
APS (clinical diagnosis)	8 (14.8)	
APS (defined by classification*)	7 (13)	
cSLEDAI at inclusion, mean (range)	2.6 (0–20)	0.4 (0–8)
SDI at last visit, mean (range)	N/A	0.8 (0–5)
Low complement (%)	24 (44.4)	15 (27.8)
Meeting SLICC-12 criteria, *N* (%)	52 (96.3)	
Meeting ACR-82 criteria, *N* (%)	44 (82.0)	47 (87.0)
Number of fulfilled ACR-82 criteria, mean (range)	4.4 (3–9)	4.6 (3–9)
Clinical SLE phenotypes (ACR-82 definitions), *N* (%)		
Malar rash	17 (31.5)	17 (31.5)
Discoid lupus	6 (11.1)	6 (11.1)
Photosensitivity	30 (55.6)	30 (55.6)
Oral ulcers	8 (14.8)	9 (16.7)
Arthritis	41 (75.9)	41 (75.9)
Serositis	18 (33.3)	19 (35.2)
Renal disorder	6 (11.1)	8 (14.8)
Neurological disorder	1 (1.9)	2 (3.7)
Hematological disorder	25 (46.3)	30 (55.6)
Immunological disorder	27 (50.0)	30 (55.6)
Anti-nuclear antibody#	54 (100)	54 (100)
Immunomodulatory therapies, *N* (%)		
Azathioprine	4 (7.4)	3 (5.6)
Belimumab	0	1 (1.9)
Cyclophosphamide	1 (1.9)	0
Hydroxychloroquine	41 (74.1)	41 (74.1)
Methotrexate	7 (13.0)	4 (7.4)
Mycophenolate mofetil	0	3 (5.6)
Prednisolone, median dose (range) in mg	5 (0–60)	5 (0–30)
Rituximab	1 (1.9)	0
Sirolimus	0	1 (1.9)

**According to Sydney criteria (4).*

#Positive by immunofluorescence microscopy (IF-ANA).

ACR, American College of Rheumatology; APS, anti-phospholipid syndrome; cSLEDAI, clinical SLE disease activity 2000 (SLEDAI-2K) score; MI, myocardial infarction; N/A, not applicable; SDI, SLICC/ACR damage index; SLICC, systemic lupus international collaborating clinics.

TABLE 2 | Frequencies of aPL using either the manufacturer's or the 99th percentile's cut-off by each antiphospholipid antibody isotype in the included patients with recent-onset SLE.

	At inclusion, manufacturer's cut-off, *N* (%)	Ever positive, manufacturer's cut-off, *N* (%)	At inclusion, 99th percentile cut-off, *N* (%)	Ever positive, 99th percentile cut-off, *N* (%)
aCL isotypes				
Any aCL	15 (27.8)	21 (38.9)	10 (18.5)	13 (24.1)
IgG	7 (13.0)	11 (20.4)	5 (9.3)	7 (13.0)
IgA	4 (7.4)	4 (7.4)	3 (5.6)	3 (5.6)
IgM	11 (20.4)	14 (25.9)	5 (9.3)	8 (14.8)
IgG, IgA	2 (3.7)	2 (3.7)	2 (3.7)	2 (3.7)
IgG, IgM	4 (7.4)	5 (9.3)	2 (3.7)	3 (5.6)
IgM, IgA	2 (3.7)	2 (3.7)	1 (1.9)	1 (1.9)
IgG, IgM, IgA	1 (1.9)	1 (1.9)	1 (1.9)	1 (1.9)
anti-β2GPI isotypes				
Any anti-β2GPI	17 (31.5)	23 (42.6)	16 (29.6)	20 (37.0)
IgG	8 (14.8)	9 (16.7)	3 (5.6)	5 (9.3)
IgA	14 (25.9)	16 (29.6)	11 (20.4)	12 (22.2)
IgM	8 (14.8)	12 (22.2)	9 (16.7)	13 (24.1)
IgG, IgA	4 (7.4)	6 (11.1)	1 (1.9)	2 (3.7)
IgG, IgM	3 (5.5)	5 (9.3)	2 (3.7)	3 (5.6)
IgM, IgA	7 (13.0)	8 (14.8)	6 (11.1)	7 (13.0)
IgG, IgM, IgA	3 (5.5)	5 (9.3)	2 (3.7)	2 (3.7)
LA	N/A	16 (29.6)	N/A	16 (29.6)
Triple-positive*	N/A	8 (14.8)	N/A	8 (14.8)

N/A, Not applicable; LA, Lupus anticoagulant test.

**Positive for any isotype of aCL, any isotype of anti-β2GPI combined with positive lupus anticoagulant test.*

a positive LA test, the test was confirmed positive with a new sample and use of the same assay (dRVVT).

Longitudinal aPL Analyses and Seroconversion

Longitudinal data for the positive aPL isotypes for aCL and anti-β2GPI, separately, are illustrated in **Figure 1**. Complete data for 50/54 patients (92.6%) were available up to 36 months from the time of SLE diagnosis. By comparing the inclusion sample with the last follow-up, four cases converted from positive aCL to negative and three patients showed the opposite conversion (from negative to positive) (**Figure 1A**). Regarding anti-β2GPI, four subjects converted from positive to negative and four patients initially showed positive anti-β2GPI at inclusion but were negative at last follow-up (**Figure 1B**). Median values for all six aPL isotypes numerically decreased from inclusion to last follow-up, but none met statistical significance. IgG anti-β2GPI were borderline significant ($p = 0.068$).

Seven patients tested positive for IgG aCL at inclusion (five patients using the 99th percentile cut-off) and five were still positive at the last follow-up visit (three patients using the 99th percentile cut-off). Only one of the four IgA aCL-positive cases at inclusion was persistently positive, regardless of which cut-off that was used. Of the 11 cases that were IgM aCL-positive at inclusion (five patients using the 99th percentile cut-off), six were still positive at the last follow-up visit (one patient using the 99th percentile cut-off). Initially, 72.2% (83.3% using the 99th percentile cut-offs) were aCL-negative and 61.1% (85.2% using the 99th percentile cut-off) were still negative at the last follow-up.

Of the eight IgG anti-β2GPI-positive cases at inclusion (three patients using the 99th percentile cut-off), only two (one patient using the 99th percentile cut-off) were persistently positive. Regarding the IgA anti-β2GPI-positive subjects, ten out of 14 (seven patients of 11 using the 99th percentile cut-off) were persistently positive. Of the eight IgM anti-β2GPI-positive cases at inclusion (nine patients using the 99th percentile cut-off), three were persistently positive at the last follow-up visit regardless of cut-off applied. Initially, 68.5% (72.2% using the 99th percentile cut-off) were anti-β2GPI-negative and 31 subjects were still negative at the last follow-up (34 using the 99th percentile cut-off).

Over time, 33 (61.1%) out of 54 patients showed a positive result for at least one aPL isotype or the LA test [24 patients using the 99th percentile cut-offs (44.4%)]. The distributions of the aPL-positive cases are illustrated in Venn diagrams with the different cut-offs applied (**Figure 2**).

Longitudinal aPL and Clinical Outcome

Table 3 shows detailed information on aPL positivity (manufacturer's cut-off applied) and the vascular events experienced by the included patients during the study period. Ever positivity for IgG aCL as a categorical variable

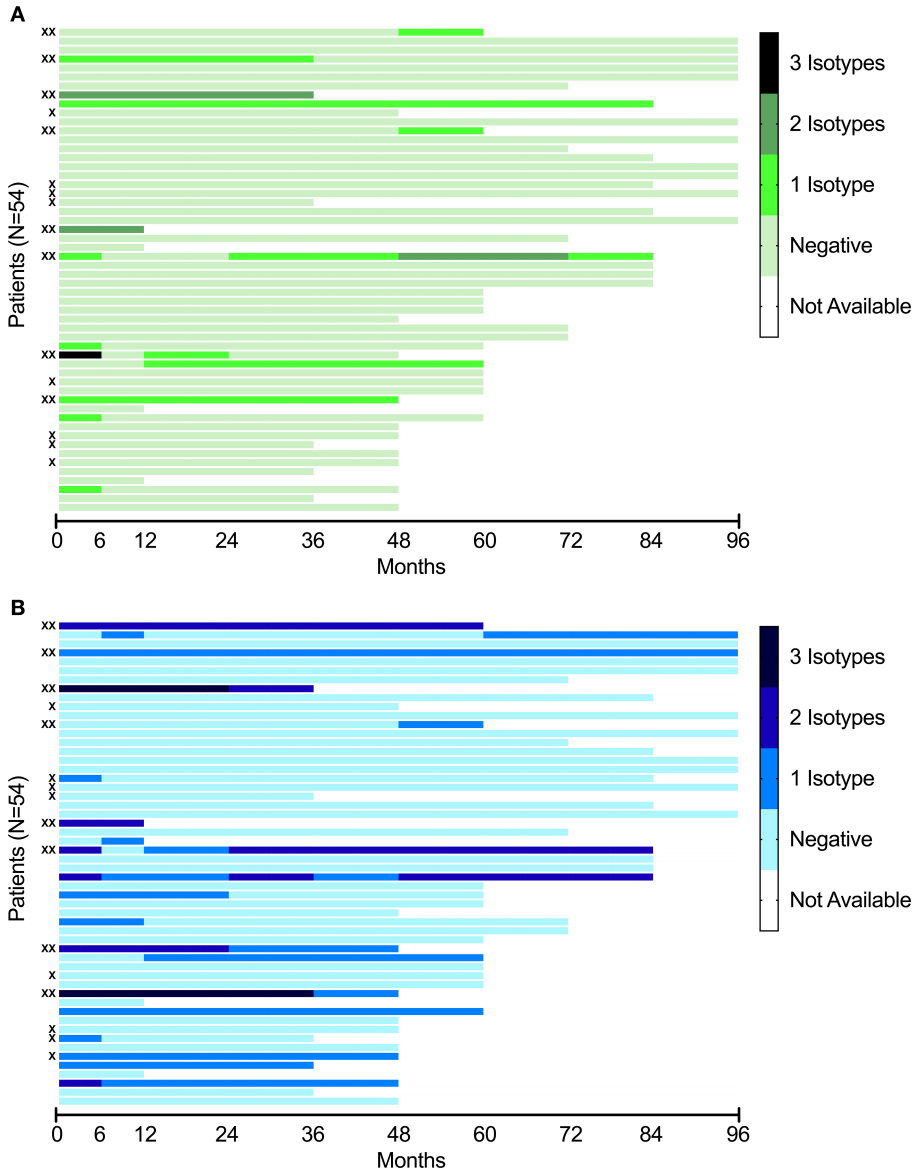

FIGURE 1 | (A) Longitudinal analysis of aCL of three isotypes (IgG, IgA, IgM) in the included patients with recent-onset SLE ($N = 54$). Each line represents a single patient, in the same order as in this figure **(B)**. **(B)** Longitudinal analysis of anti-β2GPI of three isotypes (IgG, IgA, IgM) in the included patients with recent-onset SLE ($N = 54$). Each line represents a single patient, in the same order as in this figure **(A)**. **X** indicate cases with a positive LA test. **XX** indicate triple-positive subjects (individuals positive for any isotype of aCL, any isotype of anti-β2GPI combined with a positive LA test).

was significantly associated with any vascular event [i.e., cerebrovascular lesions, transient ischemic attack, myocardial infarction (MI), pulmonary embolism and deep vein thrombosis], as compared with the IgG aCL-negative cases ($p < 0.002$).

The median longitudinal IgG aCL levels were significantly higher among cases with MI compared with cases without MI (55 vs. 11 U/mL; $p < 0.0001$) (**Supplementary Figure 1A**). Females who reported late miscarriage (≥10th week of pregnancy) had significantly higher median levels of IgG aCL (188 vs. 10 U/mL; $p < 0.007$), IgG anti-β2GPI (49 vs. 4 U/mL, $p < 0.003$) and IgA anti-β2GPI (15 vs. 7 U/mL; $p < 0.005$), compared with women who did not suffer late miscarriage (**Supplementary Figures 1B–D**).

aPL Levels Over Time in Relation to Received Pharmacotherapy

To assess the impact of administered pharmacotherapy on aPL levels, the patients were categorized into different groups. Those who received continuous HCQ therapy showed significantly higher median levels of IgM anti-β2GPI (7 vs. 3 U/mL; $p < 0.0001$) and IgM aCL (11 vs. 5 U/mL; p

< 0.02) compared with the group that received another Disease-Modifying Anti-Rheumatic Drug (DMARD) or no DMARD (**Supplementary Figures 1E,F**).

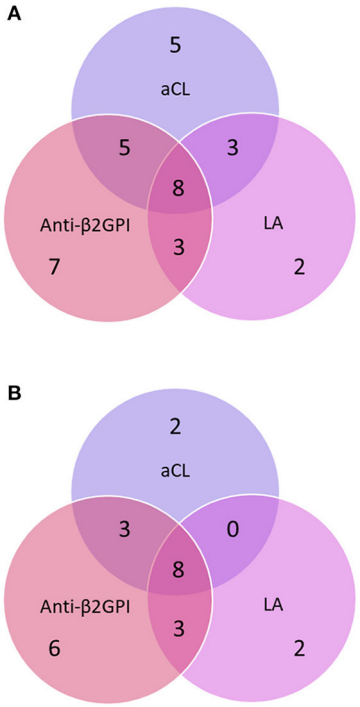

FIGURE 2 | (A) Distribution of anti-phospholipid antibodies (aPL) using the manufacturer's cut-offs among patients who tested positive on at least one occasion during the study period. **(B)** Distribution of anti-phospholipid antibodies (aPL) using the 99th percentile cut-offs among patients who tested positive on at least one occasion during the study period. The circles represent IgG/IgA/IgM anti-cardiolipin antibody (aCL), anti-β2-glycoprotein-I (anti-β2GPI) and the lupus anticoagulant (LA) test.

No significant association was found regarding the levels of any aPL isotype and the prescribed daily dose of prednisolone.

aPL Levels in Relation to Clinical Phenotypes (Classification Criteria)

When using the aPL results as categorical variables, an inverse association between IgG anti-β2GPI and photosensitivity ($p < 0.008$) was observed. In line with this finding, a positive LA test was inversely associated with photosensitivity ($p < 0.05$). IgA anti-β2GPI levels were significantly associated with leukopenia ($p < 0.04$), and IgM anti-β2GPI levels were inversely associated with the presence of Raynaud's phenomenon ($p < 0.05$). Finally, the levels of IgM aCL were significantly associated with hypocomplementemia (low C3 and/or C4) ($p < 0.03$).

aPL Levels in Relation to Gender, Smoking Habits, and Organ Damage

No significant associations regarding aCL, anti-β2GPI and LA were observed in relation to gender. Similarly, smoking habits did not show any significant associations with aPL levels. However, the presence of IgG anti-β2GPI at any time-point during the follow-up period was significantly associated with severe damage accrual (SDI score ≥4; $p < 0.03$).

DISCUSSION

The main goal of this prospective study was to evaluate the occurrence and variations of aPL over time among individuals who were recently diagnosed with SLE. Longitudinal studies of aPL are uncommon and most of them have had a specific focus, such as investigating aPL levels from conception and onwards in relation to adverse pregnancy outcomes (27, 33, 34). Depending on which cut-offs that were applied, our data demonstrate a

TABLE 3 | Vascular events vs. antiphospholipid antibodies (manufacturer's cut-off applied) in the included patients with recent-onset SLE during the study period (*N* = 54).

Type of VE	*N*, %	aPL negative* (*N* = 21)	aCL (*N* = 21)			Anti-β2GPI (*N* = 23)			LA (*N* = 16)	Triple-positive# (*N* = 8)
			IgG	IgA	IgM	IgG	IgA	IgM		
All VEs	13 (24.1)	3 (23)	6 (46)	0 (0)	3 (23)	4 (31)	3 (23)	4 (31)	6 (46)	2 (15)
Any arterial event	4 (7.4)	1 (25)	2 (50)	0 (0)	1 (25)	1 (25)	0 (0)	0 (0)	2 (50)	1 (25)
CVL	4 (7.4)	1 (25)	2 (50)	0 (0)	1 (25)	1 (25)	0 (0)	0 (0)	2 (50)	1 (25)
Ischemic stroke	3 (5.6)	1 (33)	2 (67)	0 (0)	1 (33)	1 (33)	0 (0)	0 (0)	1 (33)	1 (33)
TIA	3 (5.6)	0 (0)	1 (33)	0 (0)	0 (0)	1 (33)	0 (0)	0 (0)	2 (67)	0 (0)
MI	2 (3.7)	0 (0)	2 (100)	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	1 (50)	1 (50)
Any venous event	5 (9.3)	1 (20)	2 (40)	0 (0)	2 (40)	1 (20)	1 (20)	3 (60)	1 (20)	1 (20)
PE	3 (5.6)	0 (0)	1 (33)	0 (0)	1 (33)	0 (0)	1 (33)	2 (67)	1 (33)	0 (0)
DVT	2 (3.7)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)
Late miscarriage (at or after gestational week 10)	2 (4.4)	0 (0)	2 (100)	0 (0)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)

**Continuously negative for aCL, anti-β2GPI and LA.*

#Positive for any isotype of aCL and any isotype of anti-β2GPI, combined with a positive lupus anticoagulant test.

CVL, cerebrovascular lesion; DVT, deep vein thrombosis; MI, myocardial infarction; PE, pulmonary embolism; TIA, transient ischemic attack; VE, vascular event.

prevalence for aPL of 44–61% (any isotype of aCL, anti-β2GPI or LA at least once during follow-up). This broad range of aPL prevalence underlines the importance of choosing a relevant cut-off. Although the Sydney criteria postulate a 99th percentile (or 40 units) cut-off, according to the widely used external control program UK NEQAS many labs seem to use the cut-offs that are recommended by the manufacturer and these are usually lower (normally corresponding to the level slightly above of the 95th percentile) (4). Lower frequencies of aPL in SLE than we achieved here have previously been reported in cross-sectional studies (5). Three to four cases seroconverted in either direction for both aCL and anti-β2GPI, whereas the median aPL levels numerically decreased over time. Although the aPL levels fluctuated over time, the patients tended to remain aPL-positive or aPL-negative and the clinical value of repeated aPL-testing in the absence of thromboembolic events or new symptoms appears to be limited. However, this study was not powered to fully evaluate how the fluctuation of the antibody levels lead to a variation in the risk of clinical events.

We confirm some established relationships, such as the significantly higher aPL levels in patients with certain APS-related events, such as late miscarriage and MI (35). The strongest associations with vascular events were found for the IgG isotype of aCL and anti-β2GPI, which is in line with observations made by others (1, 6, 36). LA positivity was not significantly associated with any clinical outcome, which was unexpected. This may be attributable to the fact that the LA results were retrieved from the medical records and not analyzed continuously at all visits. As only patients initially testing positive for LA were re-tested, we cannot exclude that subjects initially testing negative for LA could have shown a positive LA test later on. In addition, a limited number of vascular events and a rather high prevalence of recorded LA positivity could have contributed to the lack of associations between LA and APS-related events. Furthermore, biased LA results due to ongoing anticoagulation during follow-up could not be excluded. Nevertheless, an observed association, which has been reported previously, was the relationship between hypocomplementemia and IgM aCL, indicating more pronounced activation of the classical pathway in aPL-positive SLE patients compared to those patients without aPL (1, 37, 38).

Regarding aPL isotypes, the most frequently detected aCL isotype "at inclusion" and during follow-up was IgM, and for anti-β2GPI it was IgA. While the overlap between isotypes was substantial (**Table 2**), the overlap between aCL, anti-β2GPI and LA appeared to be less extensive. For instance, exclusive anti-β2GPI positivity was detected in seven of the 33 aPL-positive patients (21%) using manufacturer's cut-offs. Compared to previous studies of SLE as well as of primary APS, and regardless of cut-off applied, we obtained a higher percentage of IgA anti-β2GPI-positive cases. The reason for this is not obvious. We have previously reported that analyzing IgA aPL in addition to IgG and IgM in patients with SLE has limited clinical value (23). In a Spanish setting, Ruiz-Irastorza et al. investigated the prevalence of IgG/IgM aCL and/or LA among patients with incident SLE and found that 36% were positive (12). During a mean follow-up of almost 10 years, 43% of the patients tested positive for aCL and/or LA at any time-point. Although the follow-up was significantly shorter in our study, similar results were achieved, with ∼39% testing positive for aCL and/or LA over time.

Some studies have indicated that patient ethnicity influences both the aPL levels and aPL isotypes. In a North American setting, Caucasian patients with SLE were shown to have higher levels of IgG aPL whereas African-American patients with SLE showed higher levels of IgA aPL (21). In addition, the presence of anti-β2GPI (all three isotypes) was suggested to be more common among Caucasians than African-Americans. In addition, a recent study has concluded that IgA aPL is more common in Sudanese patients with SLE than in Swedish patients with SLE (39). Furthermore, in the Sudanese control group, IgA aPL was more commonly detected than in the Swedish controls (22). This indicates that the results obtained herein, being based mainly on samples from Caucasian individuals, cannot necessarily be extrapolated to other ethnicities.

An additional aim of this study was to evaluate potential associations between aPL levels and the use of pharmacotherapy. In a comparison of patients who received HCQ continuously and patients prescribed other DMARDs or no DMARD, significantly higher IgM aCL and anti-β2GPI levels were found in the HCQ group, whereas no differences were found for the other isotypes (**Supplementary Figures 1E,F**). Partly in contrast to our findings, other groups have reported decreasing levels of IgG aCL and IgG/IgM anti-β2GPI in SLE subjects who received HCQ therapy compared with those not treated with HCQ (40). Sciascia et al. reported reduced levels of aCL and anti-β2GPI following belimumab therapy, and co-treatment with HCQ was suggested to have an even greater potential to reduce aPL levels than belimumab alone (41, 42). It is important to keep in mind that, although aPL herein were analyzed at the same occasion in stored samples, the clinicians also achieved aPL results as part of clinical routine. The indication for HCQ therapy might thus have been dependent on aPL positivity and the estimated future risk of thrombosis. In addition, we acknowledge that a non-adherence assessment for background therapies was not performed in the present study. It is well-known that adherence to HCQ is far from optimal among patients with SLE (43).

This study has certain limitations. Relatively few patients were included and the number of APS-related events was low. The latter may be attributed to the close monitoring and generally well-controlled patients. As mentioned, the study was underpowered to evaluate how fluctuations of aPL levels could lead to changed risks of clinical events. The results for LA were cross-sectional and based on "ever positivity." In contrast, the prospective study design, a study population unbiased from prior organ damage at inclusion and the monitoring of patients at a single rheumatology unit constitute major strengths. Furthermore, the fact that all samples were analyzed by an accredited laboratory and at the same occasion, thereby minimizing the inter-assay variation, was advantageous. The study population had good coverage, with very few missing values during the first 36 months. However, over time further cases were lost to follow-up, mainly due to death or migration (missing data are indicated in **Figure 1**). Finally, all new SLE patients with

recent-onset of disease at our unit during the study period were included, excluding the risk of selection bias.

CONCLUSIONS

This study demonstrates that the presence of aPL in patients with SLE is common at disease onset, and that the percentage of aPL-positive cases increases slightly over time, which is similar to what has been observed in primary APS (28). Previously reported associations between increased levels of IgG aCL and MI, as well as with late miscarriage were confirmed in the present study. In addition, the IgG and IgA anti-β2GPI levels were significantly higher among females who suffered a late miscarriage. Although the aPL levels fluctuated over time, the majority of the patients tended to remain aPL-positive or aPL-negative. Based on our data, we conclude that in the absence of new symptoms or before a planned pregnancy, the value of repeated aPL-testing is limited for patients with SLE but larger longitudinal studies are warranted to shed further light upon this issue.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Ethics Review Board in Linköping (Decision Nr. M75-08/2008). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MF and CS: conceptualization and supervision. MF and CD: methodology and project administration. MF and TW: formal analysis, data curation, and visualization. TW: writing—original draft preparation. MF, CD, and CS: writing—review and editing. All authors: validation, investigation, and have read and agreed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.646846/full#supplementary-material

Supplementary Figure 1 | Anti-phospholipid antibody levels in relation to vascular events, pharmacotherapy received and late miscarriages. Statistically significant associations are noted for: **(A)** IgG anti-cardiolipin (aCL) versus (vs.) myocardial infarction; **(B)** IgG aCL vs. late miscarriage; **(C)** IgG anti-β2-glycoprotein-I (anti-β2GPI) vs. late miscarriage; **(D)** IgA anti-β2GPI vs. late miscarriage; **(E)** IgM anti-β2GPI vs. continuous hydroxychloroquine (HCQ) therapy; and **(F)** IgM aCL vs. continuous HCQ therapy.

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Adverse Health-Related Quality of Life Outcome Despite Adequate Clinical Response to Treatment in Systemic Lupus Erythematosus

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Objective: To determine the prevalence of adverse health-related quality of life (HRQoL) outcomes in patients with SLE who achieved an adequate clinical response after a 52-week long standard therapy plus belimumab or placebo, and identify contributing factors.

Methods: We included patients who met the primary endpoint of the BLISS-52 (NCT00424476) and BLISS-76 (NCT00410384) trials, i.e., SLE Responder Index 4 (total population: $N = 760/1,684$; placebo: $N = 217/562$; belimumab 1 mg/kg: $N = 258/559$; belimumab 10 mg/kg: $N = 285/563$). Adverse HRQoL outcomes were defined as SF-36 scale scores $\leq$ the 5th percentile derived from age- and sex-matched population-based norms, and FACIT-Fatigue scores <30. We investigated factors associated with adverse HRQoL outcomes using logistic regression analysis.

Results: We found clinically important diminutions of HRQoL in SLE patients compared with matched norms and high frequencies of adverse HRQoL outcomes, the highest in SF-36 general health (29.1%), followed by FACIT-Fatigue (25.8%) and SF-36 physical functioning (25.4%). Overall, frequencies were higher with increasing age. Black/African American and White/Caucasian patients reported higher frequencies than Asians and Indigenous Americans, while Hispanics experienced adverse HRQoL outcome less frequently than non-Hispanics. Established organ damage was associated with adverse physical but not mental HRQoL outcomes; particularly, damage in the cardiovascular (OR: 2.12; 95% CI: 1.07–4.21; $P = 0.032$) and musculoskeletal (OR: 1.41; 95% CI: 1.01–1.96; $P = 0.041$) domains was associated with adverse SF-36 physical component summary. Disease activity showed no impact on HRQoL outcomes. In multivariable logistic regression analysis, addition of belimumab to standard therapy was associated with lower frequencies of adverse SF-36 physical functioning (OR: 0.59; 95% CI: 0.39–0.91; $P = 0.016$) and FACIT-F (OR: 0.53; 95% CI: 0.34–0.81; $P = 0.004$).

Conclusions: Despite adequate clinical response to standard therapy plus belimumab or placebo, a substantial proportion of SLE patients still reported adverse HRQoL

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outcomes. While no impact was documented for disease activity, established organ damage contributed to adverse outcome within physical HRQoL aspects and add-on belimumab was shown to be protective against adverse physical functioning and severe fatigue.

Keywords: systemic lupus erythematosus, health-related quality of life, patient-reported outcome, fatigue, biologic drugs, patient perscpective

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a highly heterogeneous clinical presentation. A better understanding of the subtleties of the disease together with improvements in medical care have contributed to prolonged life expectancy for SLE patients over the past decades (1). However, people living with SLE still suffer from substantial diminutions of health-related quality of life (HRQoL) compared with the general population and with other chronic diseases (2).

Divergent disease prevalence, clinical manifestations, disease course and mortality rates between sexes and across ethnic groups contribute to the heterogeneity of SLE (3, 4). Overall, patients of Black/African American or Asian origin have an increased risk for SLE than White/Caucasian individuals, and show more severe disease phenotypes. The implications of these discrepancies across ethnic groups in SLE patients' HRQoL perception have not been thoroughly delineated.

Some studies have demonstrated that conventional synthetic and biological disease-modifying agents contribute to improvements in SLE patients' HRQoL (5–9), and responders to treatment have been shown to report greater improvements than non-responders (10, 11). Although these observations are clinically relevant, improvement following a therapeutic intervention does not necessarily signify that the individual has achieved a satisfactory health perception. In rheumatoid arthritis (RA), significant pain and severe fatigue persist in a substantial proportion of patients who achieve a good clinical response to treatment or remission (12, 13). This paradoxical observation has not been thoroughly explored in SLE.

In the present investigation, we aimed to determine the prevalence of adverse HRQoL outcomes in patients with SLE who achieved an adequate clinical response after a 52-week long period on standard therapy (ST) plus belimumab or placebo within the frame of two phase III clinical trials. We further compared frequencies of adverse HRQoL outcomes across different age categories and ethnic groups, and sought to identify contributing factors.

MATERIALS AND METHODS

Study Design and Population

We designed a *post-hoc* analysis of data from two randomised, double blind, phase III clinical trials, i.e., BLISS-52 (NCT00424476) (14) and BLISS-76 (NCT00410384) (15), which comprised 865 and 819 SLE patients, respectively. Inclusion criteria for both trials were age ≥18 years, SLE diagnosis according to the revised 1997 American College of Rheumatology (ACR) criteria (16), an active disease defined as a Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) (17) score ≥6, and antinuclear antibody (ANA) titre ≥1:80 and/or serum anti-double stranded (ds)DNA antibody level ≥30 IU/mL. Key exclusion criteria included pregnancy, severe active lupus nephritis and active neuropsychiatric SLE. Patients mainly from Asia, Eastern Europe and Latin America were enrolled in BLISS-52, and from North America and Europe in BLISS-76.

The primary endpoint of the trials was achievement of the SLE responder index 4 (SRI-4) at week 52, defined as ≥4 points reduction in the SELENA-SLEDAI score compared with baseline, no new classic British Isles Lupus Assessment Group Index (BILAG) (18) A organ domain score and no more than one new BILAG B organ domain scores compared with baseline, and no worsening in the physician's global assessment (PGA) by ≥0.30 points (range: 0–3) from baseline. Of 1,684 study participants, 760 met the criteria for SRI-4 response at week 52, and constituted the study population of this *post-hoc* analysis. The frequency of SRI-4 responders was 217/562 in the placebo arm, 258/559 in the belimumab 1 mg/kg arm, and 285/563 in the belimumab 10 mg/kg arm. Accordingly, evaluation of adverse HRQoL outcomes was based on patient reports at week 52 from treatment initiation.

Demographics and Clinical Characteristics

We retrieved data on disease activity assessed with the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (19), and data on organ damage assessed with the Systemic Lupus International Collaborating Clinics (SLICC)/ACR Damage Index (SDI) (20).

Patients were stratified into four ancestry groups based on self-reports, i.e., Asian, Black/African American, Indigenous American and White/Caucasian. Additionally, they were stratified into Hispanics and non-Hispanics, as well as subgroups based on their country of residence, as detailed in **Supplementary Table 1**.

Evaluation of HRQoL

HRQoL was assessed utilising the generic instruments Medical Outcomes Study Short Form 36 (SF-36) health survey (21) and Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue (FACIT-F) scale (22). Importantly, the psychometric properties of these two instruments have been reviewed in compliance with the US Food and Drug Administration (FDA) (23) under the auspices of the Outcome Measures in Rheumatology (OMERACT) SLE working group, and are suggested as secondary endpoints in clinical trials (24).

The SF-36 is a questionnaire used for assessment of HRQoL over the preceding 4 weeks. Computation of patients' responses to 36 questions results in eight subscales, each representing a distinct HRQoL aspect, i.e., physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), social functioning (SF), vitality (VT), role emotional (RE) and mental health (MH). SF-36 subscale scores were calculated according to the SF-36v2 manual (25), and transformed to generate subscale scores ranging from 0 to 100. Subsequently, the SF-36 subscale scores are weighted into two summary scores, i.e., the physical component summary (PCS) and mental component summary (MCS). The component summary scores are norm-based, with a mean of 50 and a standard deviation of 10. All subscales contribute to the derivation of PCS and MCS, albeit differently weighted in each one of them. PF, RP, BP, and GH are referred to as the physical aspects, and SF, VT, RE, and MH are referred to as the mental aspects of SF-36. Higher scores in SF-36 items are interpreted as better HRQoL perceptions. As suggested in literature, we determined the minimal clinically important difference (MCID) for PCS and MCS as scores $\geq$2.5 and for SF-36 subscales as scores $\geq$5.0 (26). After management of missing values, all SRI-4 responders had available SF-36 registrations at week 52 ($N = 760$).

The FACIT-F is a survey that evaluates the level of fatigue over the preceding seven days (22). Patient responses to the 13 items of FACIT-F are transformed into a score ranging from 0 (maximal fatigue) to 52 (minimal fatigue). After management of missing values, the number of SRI-4 responders with available FACIT-F registrations at week 52 was 745.

Definition of Adverse HRQoL

To our knowledge, no established definitions of adverse HRQoL outcomes based on specific cut-offs in SF-36 subscale and component summary scores exist for SLE. We created a US population-based reference group, pairwise matched for age and sex with the BLISS study participants, using normative data from the SF-36 health survey user manual (27, 28).

First, we compared the mean SF-36 subscale and component summary scores of SRI-4 responders with the corresponding scores as derived from the age- and sex-matched norms. Next, we determined adverse HRQoL. In a cohort of patients with RA, Druce et al. defined severe fatigue as SF-36 VT scores corresponding to the 5th percentile of VT scores derived from a Scottish general population-based reference group that was matched for age and sex with the patients (29). Following a similar approach, we defined adverse HRQoL outcomes in SF-36 as subscale or component summary scores equal to or less than the normative 5th percentile (NP5), as derived from the reference group described above. Following this process, the NP5 for each SF-36 scale yielded the following values, as previously reported by our group (30): PF $\leq$52.5; RP $\leq$29.8; BP $\leq$38.6; GH $\leq$41.0; VT $\leq$25.4; SF $\leq$46.2; RE $\leq$26.6; MH $\leq$43.4; PCS $\leq$36.0; MCS $\leq$34.5.

FACIT-F values $<$30 represent severe fatigue (22), and herein designated adverse FACIT-F outcome.

Statistics

Data are presented as number (percentage) or mean $\pm$ standard deviation (SD) and, in case of non-normal distributions, the median and interquartile range (IQR) are indicated. Pearson's chi-square or Fisher's exact tests were used to investigate associations between dichotomous variables. Comparisons of continuous data between SLE patients and age- and sex-matched norms, as well as comparisons between baseline and week 52, were performed using the Wilcoxon signed-rank test. Comparisons of continuous data between unrelated groups were conducted using the Mann-Whitney *U* test, and across more than two groups using the Kruskal-Wallis test. Multivariable logistic regression models were created in order to assess independence and priority of potential factors associated with adverse HRQoL outcomes.

P-values $<$0.05 were considered statistically significant. Missing data were imputed using the last observation carried forward (LOCF) or next observation carried backward (NOCB) methods as appropriate. The IBM SPSS version 25 software (IBM Corp., NY, USA) was used for statistical analysis.

Ethics

Data from the BLISS trials were made available by GlaxoSmithKline (Uxbridge, UK) through the Clinical Study Data Request consortium. The BLISS-52 and BLISS-76 study protocols were approved by regional ethics review boards for all participating centres, and the protocol of the present study was approved by the Swedish Ethical Review Authority (2019-05498).

RESULTS

Demographics and Clinical Characteristics

Demographics and clinical characteristics of the SRI-4 responders ($N = 760$) are presented in **Table 1**. They were mainly women (94.3%) of middle-age (37.3 $\pm$ 11.4 years) with a median disease duration of 3.9 (IQR: 1.2–8.5) years. The most represented ancestries were White/Caucasian (46.2%) and Indigenous American (27.6%), whereas 42.0% of the patients reported Hispanic ethnicity. The mean SLEDAI-2K score at week 52 was 3.8 ($\pm$2.9), and 453 patients (59.6%) had zero SDI scores (IQR: 0–1).

HRQoL Outcome at Week 52 of Treatment

As illustrated in **Figure 1**, patients with SLE who achieved SRI-4 response reported worse HRQoL at week 52 from treatment initiation than US population-based norms individually matched for age and sex ($P < 0.001$ for all SF-36 scales), which exceeded the MCID in all SF-36 items but VT (**Figures 1A,B**; **Supplementary Table 2**). The differences were most prominent for GH, RP and PF, yielding 4.2, 3.6, and 3.5 times the MCID lower mean scores than the matched norms, respectively. **Figure 2** delineates proportions of SRI-4 responders who reported adverse HRQoL outcomes at week 52 from treatment initiation. Overall, proportions of patients experiencing adverse HRQoL outcomes were higher within physical vs. mental aspects, with GH (29.1%) and PF (25.4%) being the SF-36 domains

TABLE 1 | Demographics and clinical characteristics of SRI-4 responders in the pooled BLISS study population.

	SRI-4 responders *N* = 760	Hispanics *N* = 319	Non-Hispanics *N* = 441	*P*-value
Patient characteristics				
Age at baseline (years)	37.3 ± 11.4	36.5 ± 10.6	37.9 ± 11.9	0.170
Female sex	717 (94.3%)	302 (94.7%)	415 (94.1%)	0.739
Ancestries				
Asian	144 (18.9%)	0	144 (32.7%)	**<0.001**
Black/African American	55 (7.2%)	99 (31.0%)	252 (57.1%)	**<0.001**
Indigenous American*	210 (27.6%)	15 (4.7%)	40 (9.1%)	**0.022**
White/Caucasian	351 (46.2%)	205 (64.3%)	5 (1.1%)	**<0.001**
Clinical data				
SLE duration at baseline (years)	5.8 (1.2–8.5)	5.1 (1.0–7.8)	6.3 (1.4–9.0)	**0.017**
SLEDAI-2K score				
Baseline	10.7 ± 3.6	10.6 ± 3.5	10.7 ± 3.6	0.888
Week 52	3.8 ± 2.9	3.6 ± 2.7	3.9 ± 3.0	0.332
SDI score				
Baseline	0.7 ± 1.1 0.0 (0.0–1.0)	0.5 ± 1.0 0.0 (0.0–1.0)	0.8 ± 1.2 0.0 (0.0–1.0)	**<0.001**
Week 52	0.7 ± 1.2 0.0 (0.0–1.0)	0.5 ± 1.0 0.0 (0.0–1.0)	0.9 ± 1.3 0.0 (0.0–1.0)	**<0.001**
SDI score > 0				
Baseline	293 (38.6%)	90 (28.2%)	203 (46.0%)	**<0.001**
Week 52	307 (40.4%)	96 (30.1%)	211 (47.8%)	**<0.001**
Serological profile at baseline				
Anti-dsDNA (+)	517 (68.0%)	216 (67.7%)	301 (68.3%)	0.874
Anti-Sm (+)	224 (29.6%); N = 758	109 (34.2%)	115 (26.2%); N = 439	**0.018**
Low C3	311 (40.9%)	122 (38.2%)	189 (42.9%)	0.202
Low C4	395 (52.0%)	165 (51.7%)	230 (52.2%)	0.907
Prednisone eq. dose (mg/day)				
Baseline	11.7 ± 9.0	12.5 ± 9.1	11.1 ± 9.0	**0.028**
Week 52	8.7 ± 6.8; *N* = 754	9.4 ± 7.2; *N* = 318	8.2 ± 6.6; *N* = 436	**0.022**
Antimalarial agents at week 52†	478 (62.9%)	223 (69.9%)	255 (57.8%)	**0.001**
Immunosuppressants at week 52				
Azathioprine	149 (19.6%)	76 (23.8%)	73 (16.6%)	**0.013**
Methotrexate	78 (10.3%)	36 (11.3%)	42 (9.5%)	0.430
Mycophenolic acid	72 (9.5%)	25 (7.8%)	47 (10.7%)	0.190
Other immunosuppressants‡	15 (2.0%)	6 (1.9%)	9 (2.0%)	0.876
Trial intervention				
Placebo	217 (28.6%)	95 (29.8%)	122 (27.7%)	0.524
Belimumab 1 mg/kg	258 (33.9%)	113 (35.4%)	145 (32.9%)	0.465
Belimumab 10 mg/kg	285 (37.5%)	111 (34.8%)	174 (39.5%)	0.190

Data are presented as numbers (percentage) or means ± standard deviation. In case of non-normal distributions, medians (interquartile range) are indicated. In case of missing values, the total number of patients with available data is indicated. Statistically significant P-values are in bold.

**Alaska Native or American Indian from North, South or Central America.*

†Hydroxychloroquine, chloroquine, mepacrine, mepacrine hydrochloride or quinine sulphate.

‡Cyclosporine, oral cyclophosphamide, leflunomide, mizoribine or thalidomide.

C3, complement component protein 3; C4, complement component protein 4; dsDNA, double stranded DNA; SDI, Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; Sm, Smith; SRI-4, SLE Responder Index 4.

yielding the highest frequencies (**Figure 2A**). SF-36 VT scores ≤NP5 were reported by 10.7% of SRI-4 responders, whereas 25.8% reported FACIT-F scores <30. As expected, frequencies of adverse HRQoL outcome at week 52 were lower in SRI-4 responders compared with non-responders with regard to all SF-36 items and FACIT-F score <30 ($P < 0.001$ for all; **Supplementary Table 3**), with the greatest absolute difference observed for SF-36 GH ≤NP5 (29.1% vs. 47.2%). Moreover, SRI-4 responders displayed improvements in all SF-36 subscale scores from baseline through week 52 (**Figure 1B**); while they generally reported similar scores to non-responders at baseline (**Figure 1C**), they scored higher in all subscales at week 52 (**Figure 1D**). Consequently, SRI-4 responders reported lower proportions of adverse HRQoL at week 52 compared

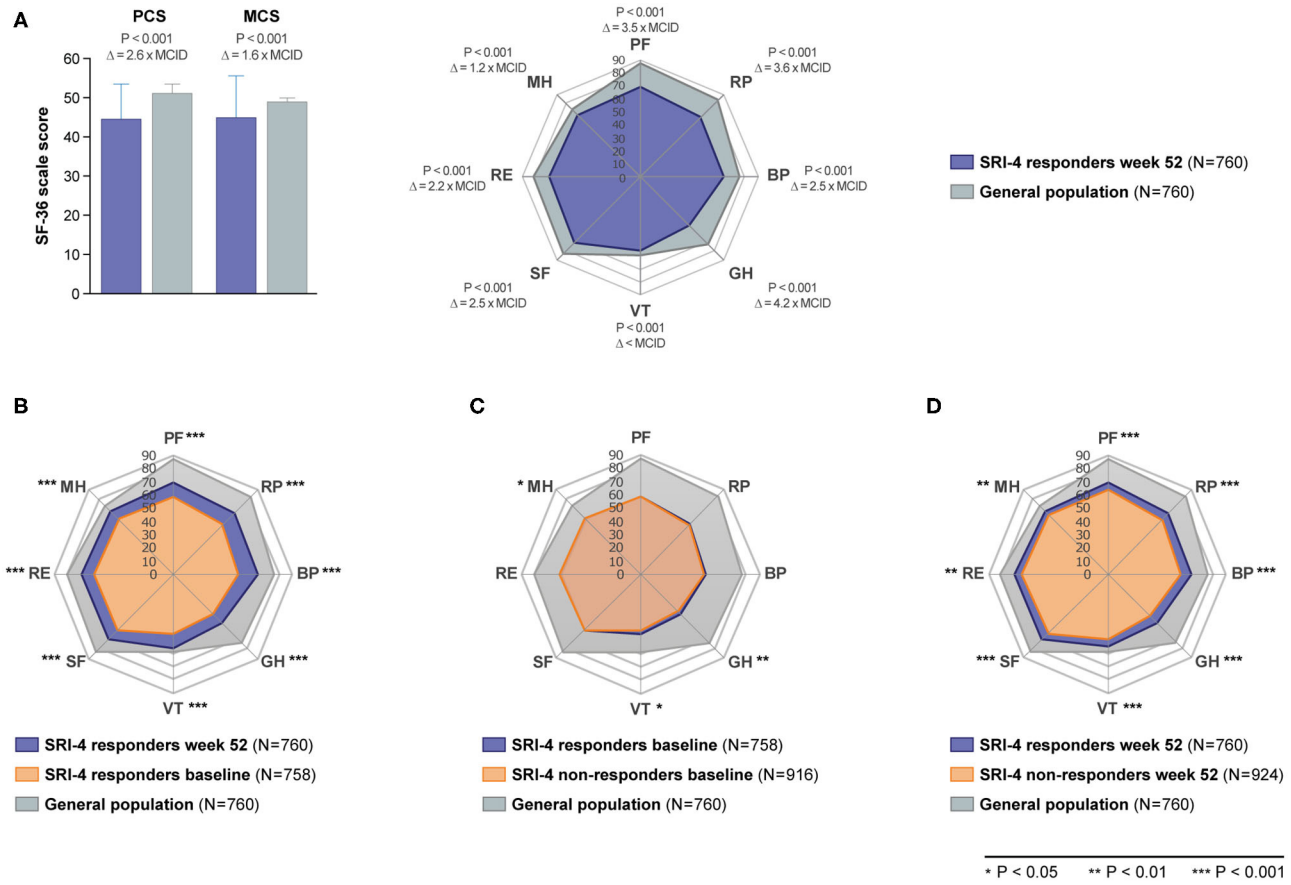

FIGURE 1 | SF-36 scores among SRI-4 responders and non-responders before and after trial intervention. The bar chart **(A)** illustrates comparisons of SF-36 component summary scores between SRI-4 responders at week 52 and age- and sex-matched US population-based norms. The height of the bars represents mean scores, while the height of the whiskers above the bars represents standard deviation. The radial charts illustrate comparisons of mean SF-36 subscale scores between **(A)** SRI-4 responders at week 52 and age- and sex-matched US population-based norms, **(B)** baseline and week 52 in SRI-4 responders, **(C)** SRI-4 responders and non-responders at baseline, and **(D)** SRI-4 responders and non-responders at week 52. Asterisks indicate statistically significant associations. BP, bodily pain; GH, general health; MCID, minimal clinically important difference; MCS, mental component summary; MH, mental health; NP5, normative 5th percentile; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; SLE, systemic lupus erythematosus; VT, vitality; Δ, delta (difference).

with baseline ($P < 0.001$ for all SF-36 items and FACIT-F; **Supplementary Table 4**).

Adverse HRQoL Outcomes Across Age Categories

We observed gradually higher frequencies of adverse HRQoL outcomes within SF-36 physical subscales with increasing age category (**Figure 2B**); of 62 patients aged 55+ years, 26 (41.9%) reported adverse PF and 25 (40.3%) reported adverse GH. The frequency of adverse HRQoL outcomes within SF-36 mental subscales also increased with age, but peaked in the category of 45–54 years. We found greater proportions of patients reporting FACIT-F scores <30 within higher age categories, which however plateaued from 35 years of age (**Figures 2B,C**).

Comparisons Across Ancestries

Table 2 shows demographics and clinical characteristics of the study participants stratified by their ancestry. As delineated in **Figure 3**, proportions of patients with adverse SF-36 PCS differed across ancestries ($P = 0.018$); Black/African Americans showed the highest frequency (21.8%), followed by White/Caucasians (21.4%), while Asian and Indigenous American patients reported the lowest frequencies (12.5 and 12.9%, respectively). Similar patterns were observed for adverse PF ($P = 0.019$) and GH ($P = 0.003$), within which Black/African Americans reported the highest (38.2 and 36.4%, respectively) and Indigenous Americans the lowest (19.0 and 20.5%, respectively) frequencies.

Within the SF-36 mental scales, proportions of patients reporting adverse HRQoL outcomes differed regarding MCS ($P = 0.007$), VT ($P < 0.001$) and MH ($P = 0.002$). With regard to these three SF-36 scales, White/Caucasians showed the highest frequencies (21.4, 16.2, and 14.2%, respectively), whereas Indigenous Americans reported the lowest frequencies in all SF-36 mental subscales, i.e., 8.1% within SF, 5.2% within MH and 3.8% within VT and RE (**Figure 3**).

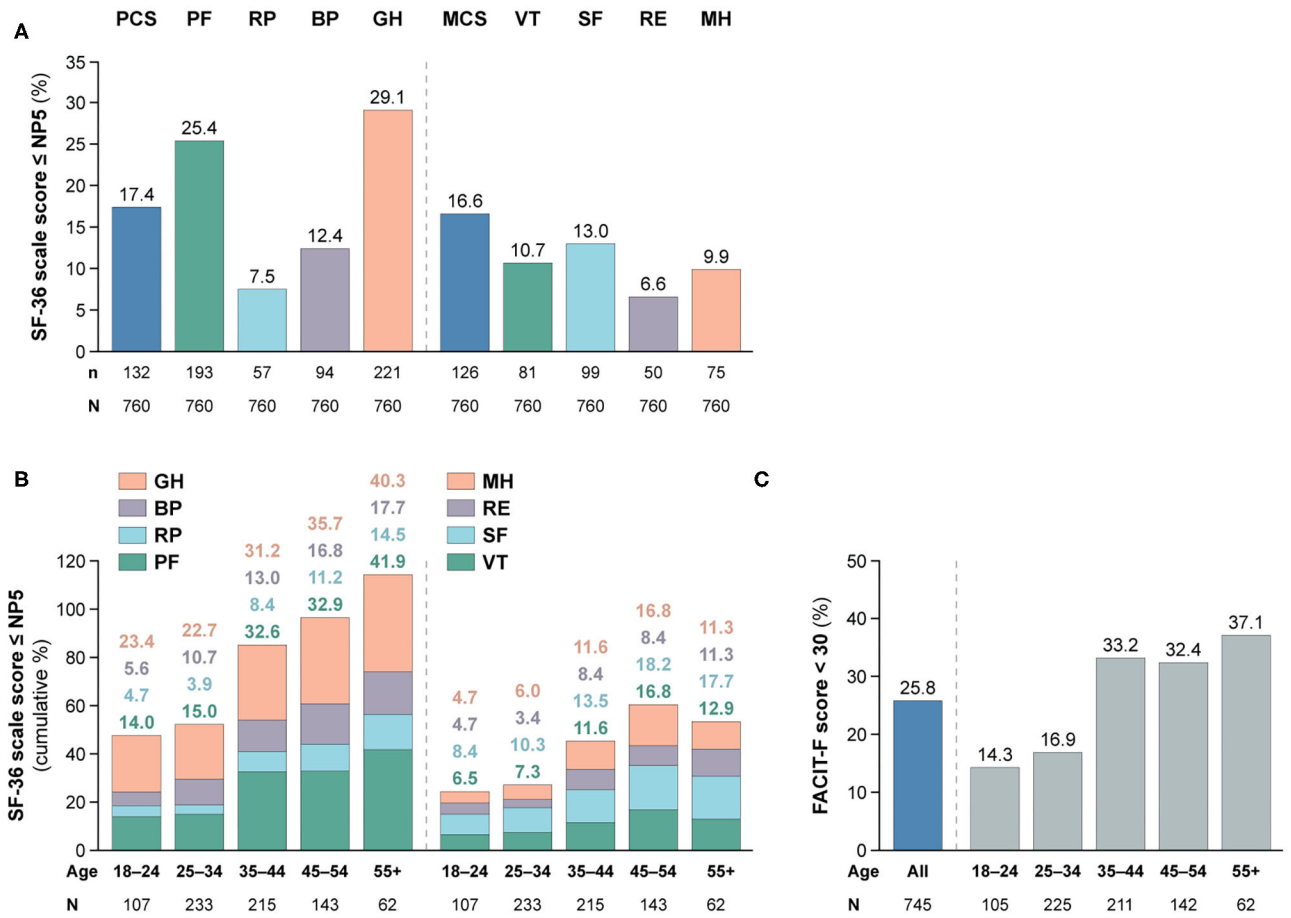

FIGURE 2 | Adverse HRQoL outcome despite clinical improvement. This figure illustrates the prevalence of adverse HRQoL outcomes. Panel **(A)** shows frequencies of adverse HRQoL outcome defined as SF-36 physical and mental scale scores ≤NP5, and panel **(B)** shows their cumulative frequencies within different age categories. Panel **(C)** shows frequencies of FACIT-F scores <30, including frequencies within different age categories. BP, bodily pain; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; GH, general health; MCS, mental component summary; MH, mental health; n, number of patients reporting adverse HRQoL outcomes; N, total number of patients with available data; NP5, normative 5th percentile; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; SLE, systemic lupus erythematosus; VT, vitality.

Comparisons Across Country Groups

A similar analysis, albeit stratifying the patients by country groups, is presented in **Figure 4**. The demographics and clinical characteristics of these groups are shown in **Table 3**.

Patients residing in Canada/USA most frequently reported adverse outcome in all physical SF-36 items (13.2–45.0%), whereas patients from Latin America showed the lowest frequencies (5.5–21.4%). Within mental HRQoL aspects, patients residing in Canada/USA and Europe/Israel most frequently reported adverse VT (19.2 and 17.6%, respectively) and FACIT-F (45.7 and 31.8%, respectively), and the highest frequencies of adverse MH were seen among patients from Europe/Israel (15.5%).

Comparisons Between Hispanics and Non-hispanics

Patients of Hispanic/Latin American ethnicity had shorter disease duration than non-Hispanics (median; IQR: 5.1; 1.0–7.8 vs. 6.3; 1.4–9.0 years; $P = 0.017$), and fewer patients among Hispanics had SDI scores >0 at baseline (28.2 vs. 46.0%; $P < 0.001$) and week 52 (30.1 vs. 47.8%; $P < 0.001$). At week 52, Hispanics were on slightly higher mean prednisone or prednisone equivalent doses (9.4 ± 7.2 vs. 8.2 ± 6.6 mg/day; $P = 0.022$), and a higher percentage among them were on antimalarial agents (69.9 vs. 57.8%; $P = 0.001$; **Table 1**).

As seen in **Figure 5**, frequencies of patients reporting adverse HRQoL outcomes in the physical domains of SF-36 were lower in Hispanics vs. non-Hispanics regarding PCS (12.2 vs. 21.1%; odds ratio, OR: 0.52; 95% confidence interval, CI: 0.35–0.58; $P = 0.001$), PF (19.7 vs. 29.5%; OR: 0.59; 95% CI: 0.42–0.83; $P = 0.002$) and GH (21.0 vs. 34.9%; OR: 0.50; 95% CI: 0.36–0.69; $P < 0.001$). With regard to the mental compartment of SF-36, a lower proportion of Hispanic patients reported adverse VT (4.1%) compared with non-Hispanics (15.4%; OR: 0.23; 95% CI: 0.13–0.43; $P < 0.001$). Likewise,

TABLE 2 | Demographics and clinical characteristics of patients across different ancestries.

	Asian *N* = 144	Black/ African American *N* = 55	Indigenous American *N* = 210	White/ Caucasian *N* = 351	*P*-value
Patient characteristics					
Age at baseline (years)	32.7 ± 9.4	38.5 ± 11.9	36.7 ± 10.4	39.4 ± 12.0	**<0.001**
Female sex	136 (94.4%)	53 (96.4%)	201 (95.7%)	327 (93.2%)	0.556
Clinical data					
SLE duration at baseline (years)	5.0 (0.7–7.5)	6.5 (1.2–9.0)	4.7 (0.9–6.8)	6.7 (1.6–10.4)	**0.002**
SLEDAI-2K score					
Baseline	11.3 ± 3.6	10.3 ± 3.3	10.6 ± 3.5	10.5 ± 3.6	0.060
Week 52	4.5 ± 3.0	3.6 ± 2.6	3.6 ± 2.8	3.7 ± 2.9	0.260
SDI score					
Baseline	0.5 ± 1.0 0.0 (0.0–1.0)	0.9 ± 1.3 0.0 (1.0–1.0)	0.4 ± 0.9 0.0 (0.0–1.0)	0.8 ± 1.3 0.0 (0.0–1.0)	**<0.001**
Week 52	0.6 ± 1.0 0.0 (0.0–1.0)	1.0 ± 1.5 0.0 (1.0–1.0)	0.4 ± 0.9 0.0 (0.0–1.0)	0.9 ± 1.3 0.0 (0.0–1.0)	**<0.001**
SDI score > 0					
Baseline	48 (33.3%)	28 (50.9%)	54 (25.7%)	163 (46.4%)	**<0.001**
Week 52	50 (34.7%)	29 (52.7%)	57 (27.1%)	171 (48.7%)	**<0.001**
Serological profile at baseline					
Anti-dsDNA (+)	122 (84.7%)	37 (67.3%)	141 (67.1%)	217 (61.8%)	**<0.001**
Anti-Sm (+)	55 (38.2%)	22 (40.0%)	74 (35.2%)	73 (20.9%)	**<0.001**
Low C3	88 (61.1%)	18 (32.7%)	86 (41.0%)	119 (33.9%)	**<0.001**
Low C4	85 (59.0%)	17 (30.9%)	113 (53.8%)	180 (51.3%)	**0.005**
Prednisone eq. dose (mg/day)					
Baseline	13.3 ± 9.3	12.6 ± 10.0	12.0 ± 8.2	10.7 ± 9.2	0.236
Week 52	8.4 ± 5.1; N = 143	9.9 ± 8.7; N = 54	9.3 ± 6.7; N = 209	8.3 ± 7.2; N = 348	**0.010**
Antimalarial agents at week 52†	83 (57.6%)	35 (63.6%)	147 (70.0%)	213 (60.7%)	0.072
Immunosuppressants at week 52					
Azathioprine	23 (16.0%)	11 (20.0%)	54 (25.7%)	61 (17.4%)	0.063
Methotrexate	4 (2.8%)	7 (12.7%)	30 (14.3%)	37 (10.5%)	**0.005**
Mycophenolic acid	11 (7.6%)	9 (16.4%)	14 (6.7%)	38 (10.8%)	0.098
Other immunosuppressants‡	2 (1.4%)	2 (3.6%)	3 (1.4%)	8 (2.3%)	0.675
Trial intervention					
Placebo	41 (28.5%)	22 (40.0%)	61 (29.0%)	93 (26.5%)	0.232
Belimumab 1 mg/kg	44 (30.6%)	15 (27.3%)	74 (35.2%)	125 (35.6%)	0.490
Belimumab 10 mg/kg	59 (41.0%)	18 (32.7%)	75 (35.7%)	133 (37.9%)	0.663

Data are presented as numbers (percentage) or means ± standard deviation. In case of non-normal distributions, medians (interquartile range) are indicated. In case of missing values, the total number of patients with available data is indicated. Statistically significant P-values are in bold.

**Alaska Native or American Indian from North, South or Central America.*

†Hydroxychloroquine, chloroquine, mepacrine, mepacrine hydrochloride or quinine sulphate.

‡Cyclosporine, oral cyclophosphamide, leflunomide, mizoribine or thalidomide.

C3, complement component protein 3; C4, complement component protein 4; dsDNA, double stranded DNA; SDI, Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; Sm, Smith.

the proportion of patients with FACIT-F scores <30 was lower among Hispanics (15.7 vs. 33.3%; OR: 0.37; 95% CI: 0.26–0.53; $P < 0.001$).

Factors Associated With Adverse HRQoL

First, we compared demographic and clinical characteristics of patients with adverse vs. non-adverse PCS, MCS and FACIT-F at week 52. Compared with those reporting adverse PCS, individuals with non-adverse PCS were younger and had lower SDI scores (**Supplementary Table 5**). Higher proportions of anti-dsDNA positive (70.7 vs. 55.3%; $P = 0.001$) patients as well as patients with low C3 (43.6 vs. 28.0%; $P = 0.001$) and low C4 (54.8 vs. 38.6%; $P = 0.001$) levels at baseline were seen among patients who reported non-adverse PCS at week 52. Notably, a higher proportion of anti-Sm positive patients was seen within patients who reported non-adverse MCS (31.5 vs. 19.8%; $P = 0.009$; **Supplementary Table 6**). Patients with FACIT-F scores ≥30 at week 52 were younger and had lower SDI scores compared with patients with severe fatigue (**Supplementary Table 7**). Moreover, more patients within the non-severe fatigue group were anti-dsDNA (71.4 vs. 57.8%; $P < 0.001$) and anti-Sm (32.7 vs. 20.8%; $P = 0.002$) positive at

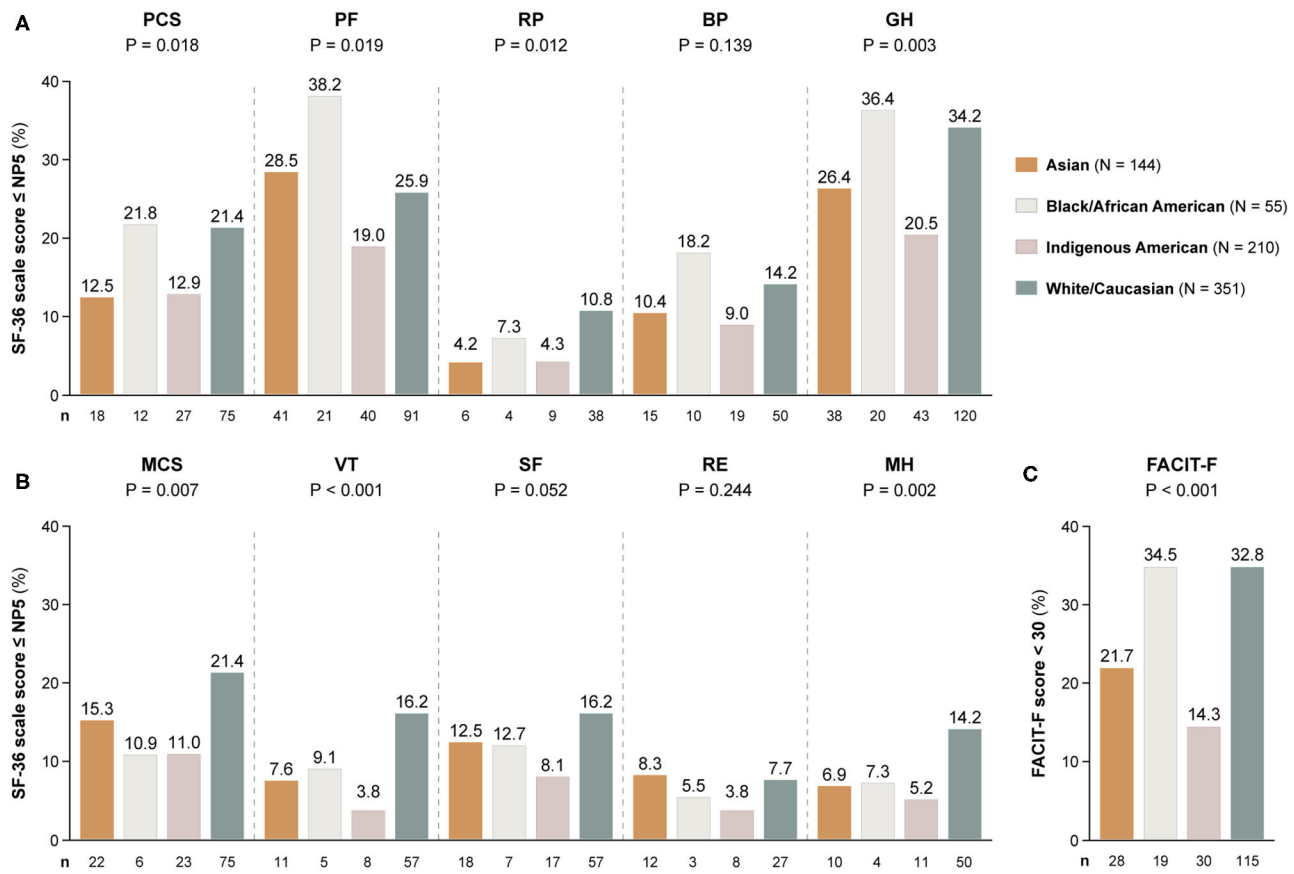

FIGURE 3 | Adverse HRQoL outcome despite clinical improvement across different ancestries. This figure illustrates comparisons of the prevalence of adverse HRQoL outcomes at week 52 from treatment initiation across patients with SLE of Asian, Black/African American, Indigenous American, and White/Caucasian ancestries. **(A,B)** Delineate proportions of patients reporting SF-36 scores below or equal to the 5th percentile of the corresponding scores as derived from a general population of the same age and sex, within physical and mental aspects, respectively. **(C)** Delineates proportions of patients reporting FACIT-F scores <30, signifying severe fatigue. The number of patients reporting adverse HRQoL outcomes (*n*) is indicated below the respective bar. *P*-values derived from chi-square tests. BP, bodily pain; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; GH, general health; MCS, mental component summary; MH, mental health; NP5, normative 5th percentile; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; VT, vitality.

baseline, and more had been treated with belimumab 10 mg/kg (39.8 vs. 29.2%; $P = 0.009$).

Subsequently, we created multivariable logistic regression models to assess independence and account for confounding potentiality. Covariates in the models included age, sex, ancestry, Hispanic ethnicity, SLEDAI-2K and SDI scores at week 52, and the trial intervention, i.e., belimumab 10 mg/kg or 1 mg/kg with placebo as the reference comparator.

Increasing age was associated with adverse HRQoL outcome in all physical and mental SF-36 scales, except for MCS, and with adverse FACIT-F (**Figure 6**). White/Caucasian ancestry was associated with adverse RP (OR: 1.95; 95% CI: 1.06–3.59; $P = 0.033$), VT (OR: 2.17; 95% CI: 1.28–3.68; $P = 0.004$) and MH (OR: 2.37; 95% CI: 1.39–4.05; $P = 0.002$), as well as with FACIT-F scores <30 (OR: 1.47; 95% CI: 1.02–2.11; $P = 0.039$). Conversely, Hispanic ethnicity was associated with lower proportions of adverse PF (OR: 0.59; 95% CI: 0.39–0.88; $P = 0.010$), GH (OR: 0.59; 95% CI: 0.40–0.85; $P = 0.005$) and VT (OR: 0.34; 95% CI: 0.18–0.64; $P = 0.001$), as well as with FACIT-F scores <30 (OR: 0.31; 95% CI: 0.46–0.70; $P < 0.001$).

Increasing SDI scores at week 52, representing organ damage accrued from disease onset until the evaluation, were associated with adverse HRQoL outcomes within physical SF-36 scales, including PCS (OR: 1.25; 95% CI: 1.07–1.45; $P = 0.004$), PF (OR: 1.31; 95% CI: 1.13–1.51; $P < 0.001$) and GH (OR: 1.16; 95% CI: 1.01–1.33; $P = 0.040$), but not within mental SF-36 scales or FACIT-F. We found no significant association between SLEDAI-2K scores and adverse HRQoL outcomes (**Figure 6**). Notably, addition of belimumab 10 mg/kg to ST was associated with lower frequencies of adverse PF (OR: 0.59; 95% CI: 0.39–0.91; $P = 0.016$) and FACIT-F (OR: 0.53; 95% CI: 0.34–0.81; $P = 0.004$). Similar results were seen for age, sex, SLEDAI-2K, SDI and belimumab use when we included country groups instead of ancestries or ethnic origin as covariates in the models (**Supplementary Figure 1**).

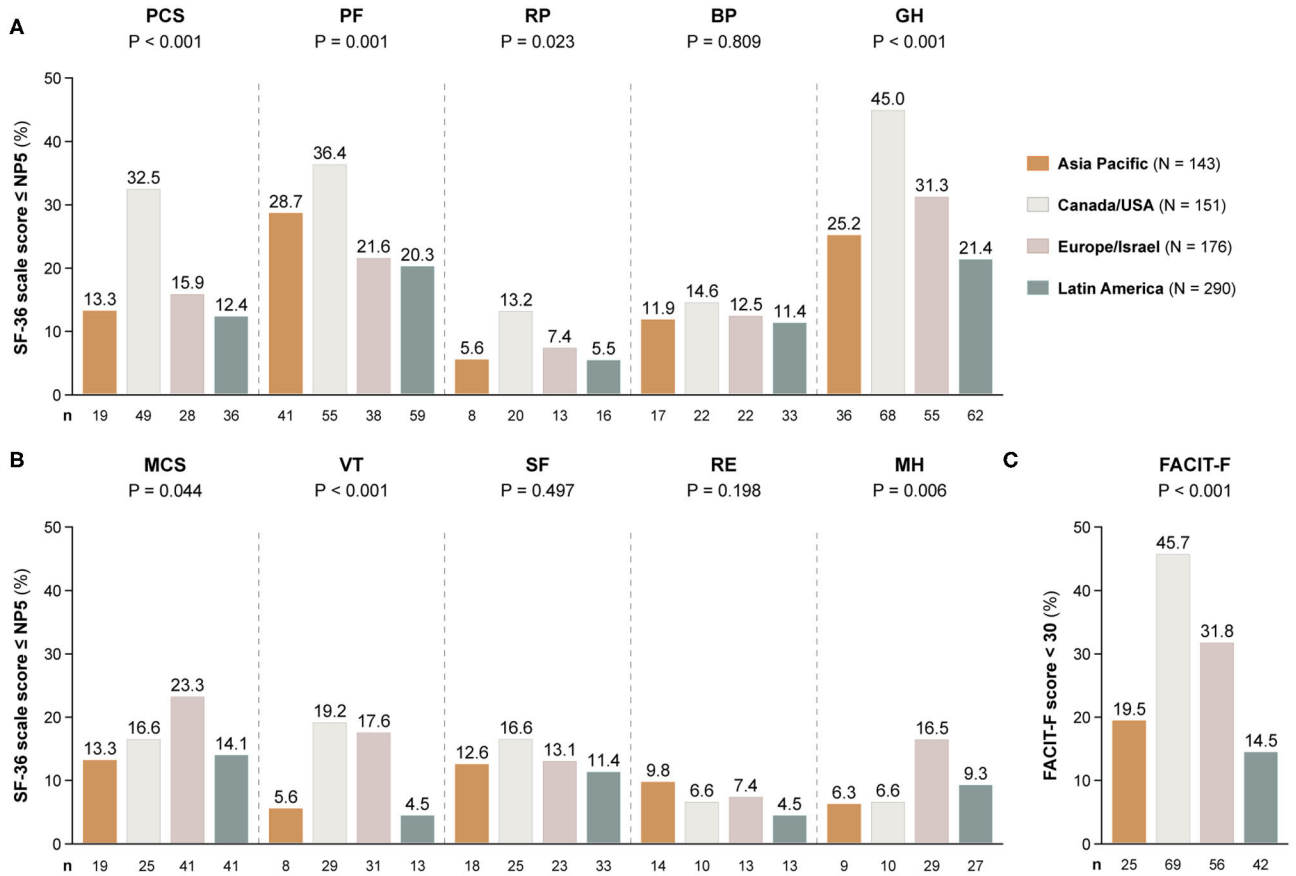

FIGURE 4 | Adverse HRQoL outcome despite clinical improvement across different country groups. This figure illustrates comparisons of the prevalence of adverse HRQoL outcomes at week 52 from treatment initiation across patients with SLE from Asia Pacific, Canada/USA, Europe, and Latin America. **(A,B)** Delineate proportions of patients reporting SF-36 scores below or equal to the 5th percentile of the corresponding scores as derived from a general population of the same age and sex, within physical and mental aspects, respectively. **(C)** Delineates proportions of patients reporting FACIT-F scores <30, signifying severe fatigue. The number of patients reporting adverse HRQoL outcomes (*n*) is indicated below the respective bar. *P*-values derived from chi-square tests. BP, bodily pain; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; GH, general health; MCS, mental component summary; MH, mental health, NP5, normative 5th percentile; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; VT, vitality.

Next, in order to determine the type of established organ damage accrued from disease onset until the time of evaluation (week 52) that accounted for the observed association between SDI scores and adverse HRQoL outcomes, we created separate univariable and multivariable logistic regression models for each one of the SDI organ domains (**Figure 7**, **Supplementary Tables 8–10**). Damage in the neuropsychiatric, cardiovascular, gastrointestinal and musculoskeletal domains was associated with adverse PCS in univariable models; this association remained significant after adjustment for the cardiovascular (OR: 2.12; 95% CI: 1.07–4.21; $P = 0.032$) and musculoskeletal (OR: 1.41; 95% CI: 1.01–1.96; $P = 0.041$) domains. With regard to mental aspects, damage in the neuropsychiatric domain was associated with adverse SF-36 SF (OR: 1.55; 95% CI: 1.01–2.38 $P = 0.044$) and MH (OR: 1.59; 95% CI: 1.00–2.53; $P = 0.050$), whereas renal damage was associated with adverse RE (OR: 3.70; 95% CI: 1.01–13.57; $P = 0.048$) in univariable analyses; however, these associations did not reach statistical significance in the adjusted models. Finally, damage in the neuropsychiatric (OR: 1.56; 95% CI: 1.04–2.16; $P = 0.031$) and gastrointestinal (OR: 2.01; 95% CI: 1.05–3.82; $P = 0.034$) domains was associated with severe fatigue (FACIT-F scores <30) in the univariable but not the multivariable models.

DISCUSSION

Herein, we investigated frequencies of adverse HRQoL outcome and contributing factors in 760 patients with SLE who showed adequate clinical response to a 52-week long intervention with standard therapy along with belimumab or placebo, using previously reported definitions (30). We observed clinically important diminutions of patient-reported HRQoL in multiple physical, mental and social aspects compared with the general population, and high frequencies of adverse HRQoL outcomes, especially within physical domains of SF-36 and FACIT-F. Overall, higher frequencies of adverse HRQoL outcomes were seen with increasing age. Black/African American and

TABLE 3 | Demographics and clinical characteristics of patients across different country groups.

	Asia Pacific *N* = 143	Canada/USA *N* = 151	Europe[#] *N* = 176	Latin America *N* = 290	*P*-value
Patient characteristics					
Age at baseline (years)	32.9 ± 9.9	41.7 ± 11.6	38.1 ± 12.0	36.7 ± 10.7	**<0.001**
Female sex	135 (94.4%)	139 (92.1%)	167 (94.9%)	276 (95.2%)	0.582
Ancestries					
Asian	138 (96.5%)	5 (3.3%)	1 (0.6%)	0	**<0.001**
Black/African American	0	39 (25.8%)	1 (0.6%)	15 (5.2%)	**<0.001**
Indigenous American*	1 (0.7%)	8 (5.3%)	1 (0.6%)	200 (69.0%)	**<0.001**
White/Caucasian	4 (2.8%)	99 (65.6%)	173 (98.3%)	75 (25.9%)	**<0.001**
Hispanic ethnicity	0	34 (22.5%)	0	285 (98.3%)	**<0.001**
Clinical data					
SLE duration at baseline (years)	3.6 (0.8–7.4)	4.9 (1.4–9.6)	5.0 (1.7–10.3)	3.2 (1.0–7.3)	**0.004**
SLEDAI-2K score					
Baseline	11.4 ± 3.6	10.3 ± 3.4	10.5 ± 3.8	10.6 ± 3.5	**0.016**
Week 52	3.8 ± 2.3	3.5 ± 2.5	3.8 ± 3.2	3.3 ± 2.4	0.074
SDI score					
Baseline	0.5 ± 0.9 0.0 (0.0–1.0)	1.1 ± 1.5 0.0 (1.0–1.0)	0.7 ± 1.1 0.0 (0.0–1.0)	0.5 ± 1.0 0.0 (0.0–1.0)	**<0.001**
Week 52	0.6 ± 1.0 0.0 (0.0–1.0)	1.2 ± 1.6 0.0 (0.0–2.0)	0.8 ± 1.1 0.0 (0.0–1.0)	0.5 ± 1.0 0.0 (0.0–1.0)	**<0.001**
SDI score > 0					
Baseline	47 (32.9%)	87 (57.6%)	75 (42.6%)	84 (29.0%)	**<0.001**
Week 52	49 (34.3%)	89 (58.9%)	79 (44.9%)	90 (31.0%)	**<0.001**
Serological profile at baseline					
Anti-dsDNA (+)	119 (83.2%)	80 (53.0%)	122 (69.3%)	196 (67.6%)	**<0.001**
Anti-Sm (+)	53 (37.1%)	36 (23.8%)	36 (20.7%); N=174	99 (34.1%)	**0.001**
Low C3	86 (60.1%)	45 (29.8%)	71 (40.3%)	109 (37.6%)	**<0.001**
Low C4	85 (59.4%)	55 (36.4%)	104 (59.1%)	151 (52.1%)	**<0.001**
Prednisone eq. dose (mg/day)					
Baseline	13.1 ± 9.2	7.1 ± 8.4	12.1 ± 8.0	13.1 ± 9.1	**<0.001**
Week 52	8.4 ± 5.2; N=142	5.3 ± 6.2; N=149	10.0 ± 7.0; N=174	9.9 ± 7.2; N=289	**<0.001**
Antimalarial agents at week 52[†]	85 (59.4%)	107 (70.9%)	84 (47.7%)	202 (69.7%)	**<0.001**
Immunosuppressants at week 52					
Azathioprine	22 (15.4%)	22 (14.6%)	33 (18.8%)	72 (24.8%)	**0.027**
Methotrexate	6 (4.2%)	24 (15.9%)	13 (7.4%)	35 (12.1%)	**0.004**
Mycophenolic acid	8 (5.6%)	23 (15.2%)	24 (13.6%)	17 (5.9%)	**0.001**
Other immunosuppressants[‡]	3 (2.1%)	3 (2.0%)	3 (1.7%)	6 (2.1%)	0.993
Trial intervention					
Placebo	42 (29.4%)	45 (29.8%)	42 (23.9%)	88 (30.3%)	0.472
Belimumab 1 mg/kg	42 (29.4%)	59 (39.1%)	57 (32.4%)	100 (34.5%)	0.343
Belimumab 10 mg/kg	59 (41.3%)	47 (31.1%)	77 (43.8%)	102 (35.2%)	0.069

Data are presented as numbers (percentage) or means ± standard deviation. In case of non-normal distributions, medians (interquartile range) are indicated. In case of missing values, the total number of patients with available data is indicated. Statistically significant P-values are in bold.

**Alaska Native or American Indian from North, South or Central America.*

[†] Hydroxychloroquine, chloroquine, mepacrine, mepacrine hydrochloride or quinine sulphate.

[‡] Cyclosporine, oral cyclophosphamide, leflunomide, mizoribine or thalidomide.

[#] Including Israel.

C3, complement component protein 3; C4, complement component protein 4; dsDNA, double stranded DNA; SDI, Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) Damage Index; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; Sm, Smith.

White/Caucasian patients reported higher frequencies of adverse HRQoL outcomes than Asians and Indigenous Americans, while Hispanic/Latin American patients experienced adverse HRQoL less frequently than non-Hispanics. Importantly, addition of the licenced dose of intravenous belimumab to standard therapy was associated with lower frequencies of adverse physical functioning and severe fatigue.

Improvements in multiple HRQoL aspects following treatment with conventional synthetic or biological disease-modifying agents have been documented in patients with SLE,

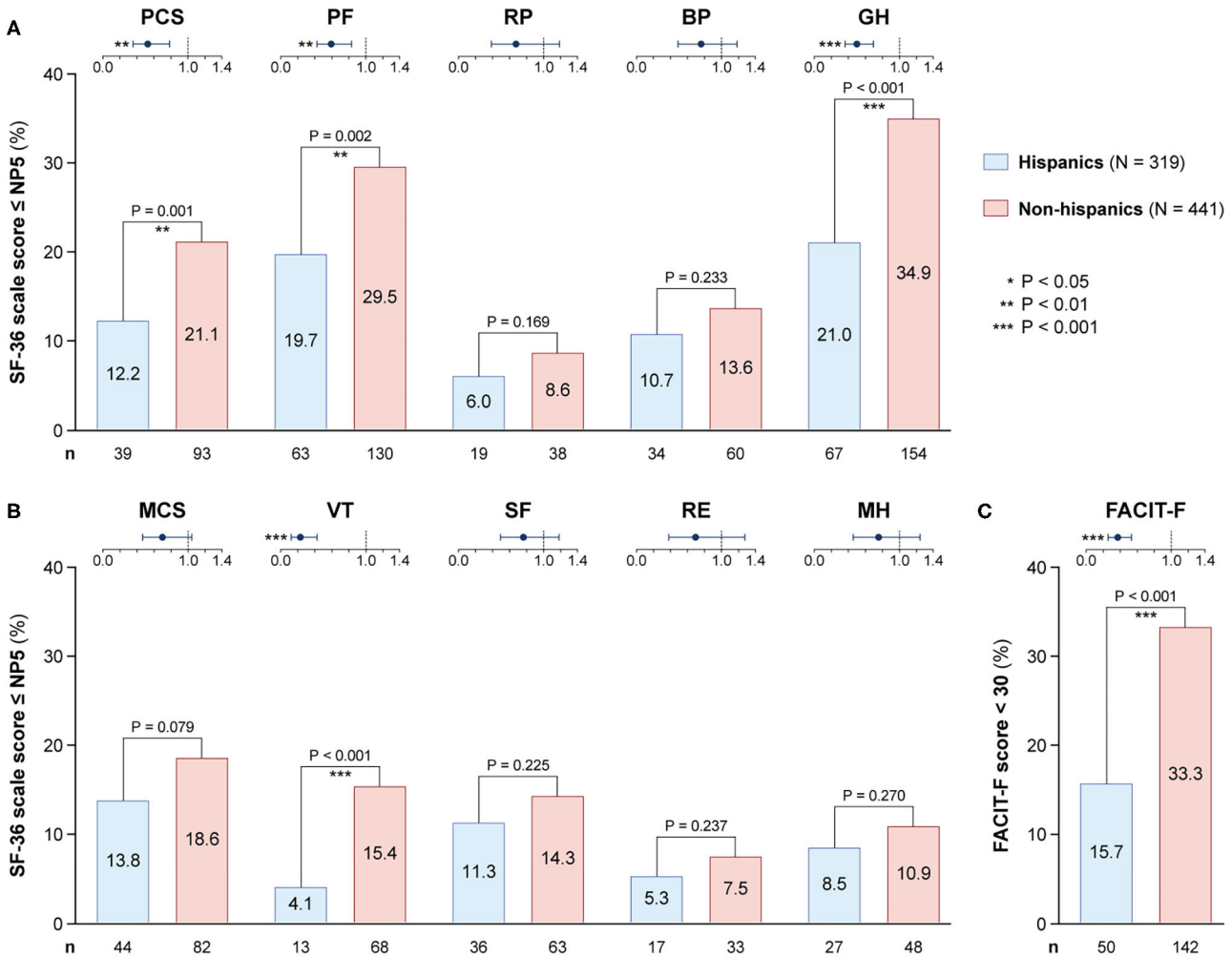

FIGURE 5 | Adverse HRQoL outcome despite clinical improvement in Hispanics versus non-Hispanics. This figure illustrates comparisons of the prevalence of adverse HRQoL outcomes at week 52 from treatment initiation between patients with SLE of Hispanic/Latin American and non-Hispanic ethnicity. **(A,B)** Delineate proportions of patients reporting SF-36 scores below or equal to the 5th percentile of the corresponding scores as derived from a general population of the same age and sex, within physical and mental aspects, respectively. **(C)** Delineates proportions of patients reporting FACIT-F scores <30, signifying severe fatigue. In the forest plots, circles designate the corresponding unadjusted odds ratios and whiskers designate 95% confidence intervals. The number of patients reporting adverse HRQoL outcomes (*n*) is indicated below the respective bar. *P*-values derived from chi-square tests. Significant *P*-values are indicated with asterisks. BP, bodily pain; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; GH, general health; MCS, mental component summary; MH, mental health; NP5, normative 5th percentile; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; VT, vitality.

especially along with clinical improvements or attainment of low disease activity or remission (5–9, 31), which we corroborated in the present study, demonstrating that treatment responders improved in all SF-36 items during the study period and reported higher SF-36 scores than non-responders to treatment. However, it is important to emphasise that improvement does not necessarily reflect a satisfactory health state perception. Studies of patients with RA have demonstrated considerable frequencies of persisting pain and severe fatigue among patients who achieved a good clinical response or remission following treatment (12, 13). In light of the above, we studied the prevalence of HRQoL outcome in patients who met the primary endpoint of two phase III clinical trials of belimumab in patients with SLE. First, we observed clinically important diminutions of patient-reported HRQoL in multiple aspects compared with matched US population-based norms. In general health, role physical and physical functioning, these differences yielded 4.2, 3.6 and 3.5 times the MCID lower scores, respectively. Next, despite conservative definitions of adverse HRQoL outcomes, especially for SF-36, we found high frequencies at week 52 among responders. Ranging from 6.6% (for role emotional) to 29.1% (for general health), these frequencies exceeded the expected frequencies as derived from individually matched US population-based norms in all physical and mental aspects.

Frequencies of adverse HRQoL outcome were more prominent within physical compared with mental domains

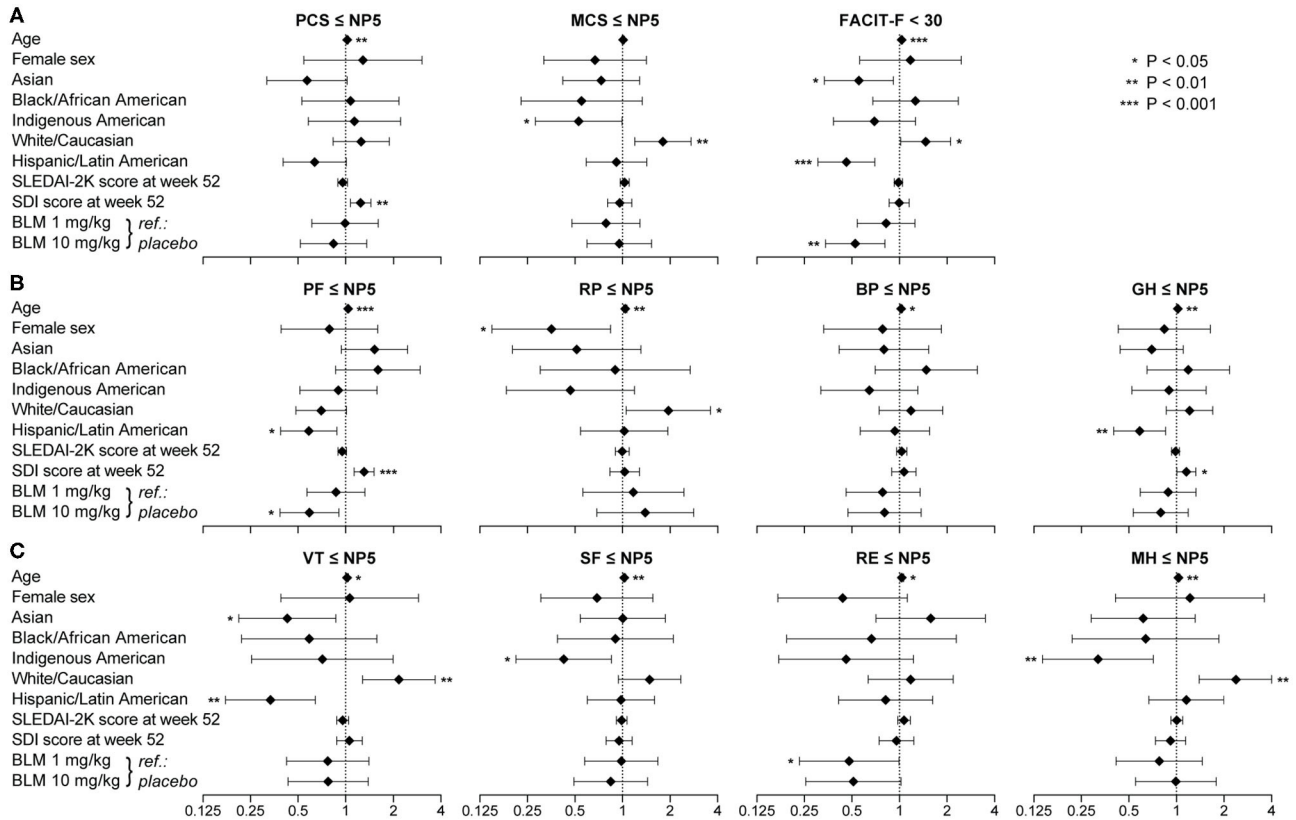

FIGURE 6 | Factors associated with adverse HRQoL outcomes. The forest plots illustrate results from multivariable logistic regression models, with the SF-36 component summary scores ≤NP5 **(A)**, physical subscale scores ≤NP5 **(B)**, mental subscale scores ≤NP5 **(C)** and FACIT-F scores <30 **(A)** at week 52 from treatment initiation as the dependant variables. Diamonds represent odds ratios and whiskers represent 95% confidence intervals. Asterisks indicate statistically significant associations. BP, bodily pain; CI, confidence interval; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; GH, general health; MCS, mental component summary; MH, mental health; NP5, normative 5th percentile; OR, odds ratio; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; VT, vitality.

of SF-36, which is consistent with the general trend in SLE patients as derived from real-world SF-36 data (32, 33). Since SLE is a highly heterogeneous disease, the clinical phenotype is expected to impact on HRQoL. For instance, in a cohort of patients with lupus nephritis, those with active disease reported worse HRQoL in mental aspects than patients with inactive disease, but the groups did not differ in physical health or pain (34). Most study participants in BLISS-52 and BLISS-76 had mucocutaneous (58.8%) and/or musculoskeletal (43.6%) manifestations at baseline, which have been shown to be associated with diminutions in physical HRQoL aspects (35, 36) and may therefore partially explain the observed high frequencies of adverse HRQoL outcomes in physical functioning and general health.

As expected, we observed increasing proportions of patients reporting adverse HRQoL outcomes and severe fatigue with increasing age, especially in physical outcomes, with more than 40% of SRI-4 responders aged 55+ years reporting adverse physical functioning and general health. The association between increasing age and adverse HRQoL outcomes was independent of other factors in multivariable logistic regression models, in line with the known negative impact of age on SLE patient's HRQoL (37). Interestingly, anti-dsDNA positivity at baseline was negatively associated with adverse outcome in physical HRQoL aspects and fatigue, as was anti-Sm positivity in mental HRQoL aspects and fatigue. Given the fact that more patients among responders had received belimumab rather than placebo (14, 15), this finding is in line with what is known about serological activity at baseline portending favourable response to belimumab therapy in clinical (38–40) and HRQoL (41) facets.

Established organ damage (accrued from disease onset until the time of evaluation, i.e., at week 52) in the musculoskeletal, mucocutaneous as well as cardiovascular and peripheral vascular SDI domains was associated with adverse HRQoL outcomes in physical but not mental aspects. By contrast, neuropsychiatric damage was associated with adverse outcome to treatment with regard to social functioning, mental health and fatigue. The latter finding, albeit not reaching statistical significance after adjustment, may provide support for investigation of functional

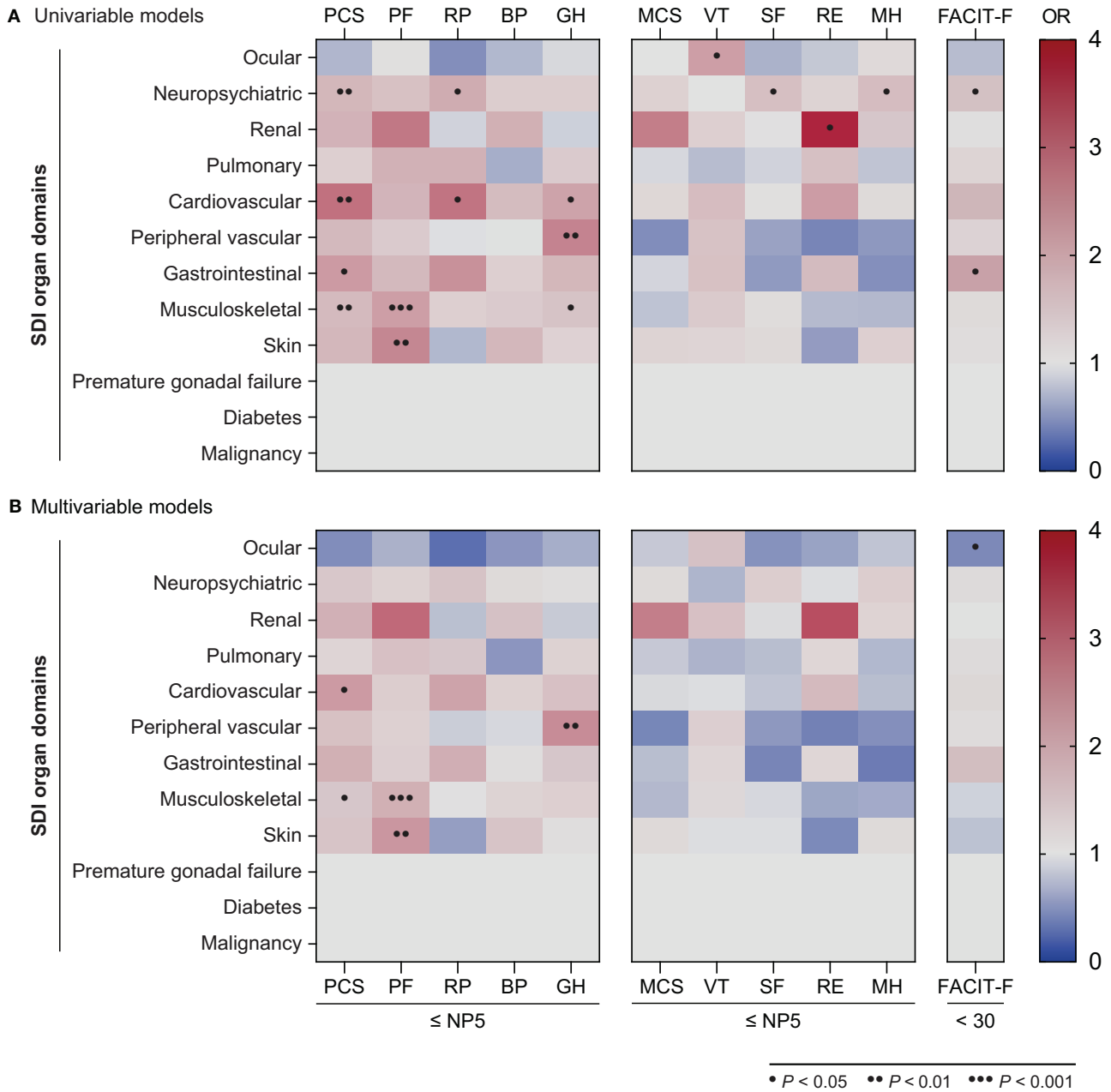

FIGURE 7 | Associations between SDI organ domains and adverse HRQoL outcomes. The heatmaps illustrate ORs deriving from univariable **(A)** and multivariable **(B)** logistic regression models, with the SF-36 scale scores ≤NP5 and FACIT-F scores <30 at week 52 as the dependant variables. Dots indicate statistically significant associations. BP, bodily pain; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; GH, general health; MCS, mental component summary; MH, mental health; OR, odds ratio; PCS, physical component summary; PF, physical functioning; RE, role emotional; RP, role physical; SF, social functioning; SF-36, short form 36 health survey; VT, vitality.

or structural changes in the brain as potential contributors to the prominent fatigue in patients with SLE rather than or along with neuroinflammation, as also implicated in multiple sclerosis (42). Notably, we found no association between the degree of SLE disease activity and adverse HRQoL outcomes. Data in previous literature are conflicting regarding the effect of disease activity and organ damage on HRQoL, with some studies implicating a negative impact (43–45) and others reporting no evident connexion (37, 46, 47). These discrepancies could be partly explained by the different instruments used to measure patients' HRQoL, e.g., generic vs. disease-specific tools, or differences in clinical phenotypes. For instance, severe active lupus nephritis

and neuropsychiatric SLE were excluded from the BLISS-52 and BLISS-76 trials, which disallows generalisability of our findings to these subgroups. Additionally, our definitions were applied to SRI-4 responders, i.e., patients who had attained a lower degree of activity relative to non-responders, which may constitute one of the reasons underlying the lack of association between disease activity and patient-reported HRQoL outcomes. Nevertheless, our data suggest that HRQoL outcomes are not solely dependent on clinical and serological features of disease activity, advocating use of patient-reported HRQoL as an integral part of the clinical assessment, as per current recommendations (48).

Patients of Black/African American and White/Caucasian ancestry reported the highest frequencies of adverse outcomes in most HRQoL domains. In logistic regression analysis, White/Caucasian ancestry was associated with adverse role physical, mental health and vitality using SF-36 and with severe fatigue using FACIT-F, independently of disease activity, organ damage and add-on belimumab. Conversely, Asian ancestry was associated with lower frequencies of adverse vitality and severe fatigue. Additionally, Hispanic/Latin American ethnicity was associated with lower frequencies of adverse physical functioning, adverse general health, adverse vitality and severe fatigue. Our findings are in line with observations from different multi-ethnic cohorts showing that patients of White/Caucasian ancestry generally report worse HRQoL than non-White/Caucasians (32), Hispanics (33) or Asians (49), despite a high variability across the studies in terms of study population, clinical features of the participants and selection of comparators. However, in a study by Kiani et al., White/Caucasians reported higher scores in SF-36 physical functioning and role emotional but lower scores in SF-36 vitality than Black/African Americans (46). One explanation for the discrepancies across ancestries and ethnic groups is likely traced to known differences regarding disease prevalence, clinical manifestations, disease activity and acquisition of organ damage (3, 4). Importantly, the determinants of HRQoL are multifactorial, and geographical, economical and sociocultural aspects are also expected to exert considerable influence on patients' HRQoL perception.

Data from both clinical trial and real-life settings suggest that the use of belimumab improves HRQoL along with improvements in disease activity, reduction of glucocorticoid doses, and prevention of severe flares (5, 6, 8, 9). In the present investigation comprising only patients who showed an adequate response to treatment, addition of belimumab 10 mg/kg to standard therapy was associated with lower proportions of adverse physical functioning and severe fatigue compared with standard therapy alone, independently of disease activity and organ damage at the time of the final evaluation. In the SWEFOT trial that compared addition of infliximab with addition of sulfasalazine and hydroxychloroquine in methotrexate-refractory early RA patients, no difference was found regarding proportions of patients achieving a good secondary response to treatment, but patients receiving infliximab reported less cumulative pain and less refractory pain after 21 months on the second-line therapy (50, 51). Although the administration route and, consequently, the visit frequency to the care unit may have exerted a placebo effect in favour of infliximab in SWEFOT, the preventive effects of biological agents against adverse patient-reported outcomes seen in both studies are supportive of molecular trajectories underlying these observations, and provide rationale for further investigation of the effects of biological therapies on HRQoL outcomes.

The *post-hoc* nature of our analysis constituted a major limitation. Furthermore, no data existed regarding epidemiological characteristics and comorbid conditions with a known impact on HRQoL, such as socioeconomical status, social relationships and co-existence of fibromyalgia or depression. We also lacked information about illness perceptions, which are known to impact on HRQoL (52, 53). Finally, disease-specific instruments for assessing HRQoL were not utilised in the BLISS trials; this likely underestimated HRQoL aspects of particular relevance for SLE populations. The aforementioned limitations together with the selected population of the BLISS trials may collectively weaken the external validity of our findings. Nonetheless, strengths of this investigation included the large study population, participation of patients from 32 different countries which allowed us to compare patients of different ancestries and ethnic origins, and the high degree of completeness of the data provided from the CSDR consortium which conferred power on statistical analyses and allowed inclusion of multiple factors in regression models.

In summary, substantial proportions of SLE patients reported adverse HRQoL outcomes despite a documented clinical improvement after a 52-week long therapy, especially in physical aspects. Particularly high proportions were seen within Black/African American and White/Caucasian patients. Notably, addition of belimumab 10 mg/kg to standard therapy exerted a preventive effect against adverse physical functioning and severe fatigue. Our results corroborate that HRQoL diminutions constitute a substantial burden in patients with SLE, and highlight the limitations of current therapeutic strategies. Further investigation of underlying factors is merited, for instance, identification of potential explanations underlying the impact of musculoskeletal, mucocutaneous and cardiovascular damage, toward the development of personalised interventions aiming at improving HRQoL outcomes.

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 human participants were reviewed and approved by Swedish Ethical Review Authority (2019-05498). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AG, VQ, AC, YE, JLa, and IP: study conception and design. AG, VQ, AC, AB, JLi, and IP: acquisition of data. AG, VQ, AC, SE, YE, JLa, and IP: analysis and interpretation of data. All authors were involved in the drafting of the manuscript or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.651249/full#supplementary-material

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